Progress report on new antiepileptic drugs: A summary of the Fourteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XIV).

II. Drugs in More Advanced Clinical Development

*Meir Bialer, ††Svein I. Johannessen, §Matthias J. Koepp, ‡René H. Levy, ***Emilio Perucca, 
†††Torbjörn Tomson and H. Steve White

*Faculty of Medicine, School of Pharmacy and David R. Bloom Center for Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Jerusalem, Israel; †The National Center for Epilepsy, Sandvika, Norway; ‡Department of Pharmacology, Oslo University Hospital, Oslo, Norway; §Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, United Kingdom; ¶Department of Pharmaceutics and Neurological Surgery, University of Washington, Seattle, Washington, U.S.A.; #Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy; **C. Mondino National Neurological Institute, Pavia, Italy; ††Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden; and HDepartment of Pharmacy, School of Pharmacy, University of Washington, Seattle, Washington, U.S.A.

Running title: Eilat Conference Report

Key words: Antiepileptic drugs, Drug development, Epilepsy, Clinical trials

Number of text pages: 34
Number of words (summary): 197
Number of words (main text): 12690
Number of references: 99
Number of Tables: 3
Number of Figures: none

Address correspondence to Meir Bialer, Faculty of Medicine, School of Pharmacy and David R. Bloom Centre for Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Ein Karem, 91120 Jerusalem, Israel. E-mail: bialer@md.huji.ac.il

1
Summary

The Fourteenth Eilat Conference on New Antiepileptic Drugs (AEDs) and Devices (EILAT XIV) took place in Madrid, Spain, on June 13-16, 2018 and was attended by 168 delegates from 28 countries. The Conference provided a forum for professionals involved in basic science, clinical research, regulatory affairs and clinical care to meet and discuss the latest advances related to discovery and development of drugs and devices aimed at improving the management of people with epilepsy. This Progress Report provides a summary of findings on investigational compounds for which data from both preclinical studies and studies in patients were presented. The compounds reviewed include anakinra, cannabidiol, cannabidivarin, fenfluramine, ganaxolone, medium-chain fatty acids, padsevonil and the valproic derivatives valnoctamide and sec-butylpropylacetamide. On June 25, 2018, the U.S. FDA approved a standardized formulation of cannabidiol oral solution for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome, in patients two years of age and older. The report shows that there continues to be a steady flow of potential AEDs progressing to clinical development. Many of these compounds show innovative mechanisms of action, and some have already been tested in placebo-controlled randomized controlled trials, with promising efficacy and safety results.

Key words: Antiepileptic drugs, Drug development, Epilepsy, Clinical Trials
KEY POINTS

• The Eilat Conference on New Antiepileptic Drugs (AEDs) and Devices is a biannual event focused on discussing advances in the development of new treatments for epilepsy.

• The Fourteenth Conference took place in Madrid, Spain, on June 13-16, 2018 and was attended by 168 scientists from 28 countries.

• This article summarizes findings presented at the Conference for 8 compounds (or classes of compounds) for which data from studies in patients are already available.

• Some of these compounds have new mechanisms of action, such as targeting neuroinflammation, interacting with novel receptor types, or combining dual receptor action into a single molecular entity.

• Many of the compounds presented have innovative mechanisms of action and, for some, promising evidence of efficacy has been already provided through randomized controlled trials.
The Fourteenth Eilat Conference on New Antiepileptic Drugs (AEDs) and Devices (EILAT XIV) took place in Madrid, Spain, on June 13-16, 2018 and was attended by 168 delegates from 28 countries. As explained in some detail in an accompanying report, the Conference allowed professionals actively engaged in basic science, clinical research, regulatory affairs and clinical care to meet and discuss key topics related to AED discovery and development. Additionally, the Conference offered the opportunity to review data on a number of novel agents which are currently being investigated as potential epilepsy treatments. Devices used for the detection or treatment of seizures were also discussed, and a summary of the presentations on this topic will be reported in a separate publication.

The accompanying report summarizes the latest findings for 12 different compounds (or classes of compounds) for which preclinical or early phase (Phase I) clinical data were presented. The current report focuses on eight compounds/classes of compounds in more advanced clinical development, for which at least preliminary results from efficacy and safety studies conducted in patients were made available. Specifically, these agents include anakinra, cannabidiol, cannabidivarin, fenfluramine, ganaxolone, medium-chain fatty acids, padsevonil and the valproic derivatives valnoctamide and sec-butylpropylacetamide. For each of these compounds, a concise description of preclinical data, clinical pharmacokinetic data, drug interaction potential and available efficacy and safety results is provided in the sections below.

ANAKINRA

Elaine Wirrell
Child and Adolescent Neurology, Mayo Clinic, Rochester, Minnesota

Introduction and rationale for development

Anakinra, a protein consisting of 153 amino acids with a molecular weight of approximately 17.3 kilodaltons, is a recombinant human interleukin-1 (IL-1) receptor antagonist that is approved for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndromes (CAPS) such as neonatal-onset multisystem inflammatory disease, Muckle-wells syndrome and familial cold autoinflammatory syndrome. Due to its ability to block interleukin-1β (IL-1β), anakinra may represent a novel therapy to attenuate maladaptive neurogenic inflammation and epileptogenesis.

There is abundant evidence for an inflammatory contribution to human epilepsy. Pathological evidence of neurogenic inflammation has been found in human epilepsy surgery specimens with cortical dysplasia and tuberous sclerosis complex, gangliogliomas and dysembrioplastic
neuroepithelial tumours (DNETs)\(^3\) and Rasmussen syndrome.\(^4\) Furthermore, higher seizure frequency correlated with higher numbers of IL-1\(\beta\) and IL-1 receptor type 1 positive neurons. Greater susceptibility to neurogenic inflammation may also play a role in predisposition to febrile seizures, a disorder which affects 3-5% of all children. In a study evaluating polymorphisms in two regions of the IL-1\(\beta\) promoter region in children with and without febrile seizures, Ozen et al.\(^5\) noted significant differences in one of these regions, suggesting that those with febrile seizures may be more susceptible to neurogenic inflammation. In the large Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT) study, plasma cytokines were compared between children with \textbf{prolonged} febrile seizures and those with fever only.\(^6\) Both interleukin-8 (IL-8) and epidermal growth factor (EGF) were significantly higher in the seizure cohort. Furthermore, the native human IL-1 receptor antagonist (IL-1RA)/interleukin-6 (IL-6) ratio was significantly lower in children with \textbf{prolonged} febrile seizures and abnormal T2 hyperintensity in the mesial temporal region on MRI than in those with seizures but normal MRI, suggesting that this ratio may be a marker for acute hippocampal injury.

There is also substantial evidence for a role of neurogenic inflammation in the pathogenesis of febrile infection-related epilepsy syndrome (FIRES), a devastating disorder in which a previously well child presents with refractory status epilepticus following a febrile illness.\(^7\) Mortality is high, and survivors are typically left with intractable, multifocal epilepsy and intellectual disability. No infectious, autoimmune or metabolic etiologies have been found despite extensive evaluation, and it is presumed that this disorder results from a fulminant inflammatory response in the brain. Sakuma et al.\(^8\) compared levels of both plasma and cerebrospinal fluid (CSF) cytokines in 27 cases of FIRES, 14 children with other inflammatory neurological disorders and 18 children with non-inflammatory neurological disorders and found markedly increased pro-inflammatory cytokines and chemokines in the FIRES cohort, particularly in the CSF. A candidate gene analysis comparing polymorphisms of cytokine-related and sodium channel genes in 19 children with FIRES to healthy controls found a significant association in the RN2 allele of IL-1RN and FIRES.\(^9\) This allele results in higher levels of IL-1\(\beta\), and lower levels of IL-1RA, suggesting that those with FIRES may have an underlying genetic predisposition to excessive neurogenic inflammation.

Together with evidence in experimental models, these data provide a rationale for testing anakinra as a potential anti-seizure/antiepileptogenic agent, particularly in syndromes where neurogenic inflammation appears to play an important pathogenetic role, \textbf{such as FIRES}.

\textbf{Pharmacology}

\textit{Anticonvulsant profile}
Anakinra, when administered after bicuculline in the in vitro isolated guinea pig brain rapidly terminated seizures, prevented their recurrence and resolved seizure-associated breakdown of the blood-brain barrier. In addition, anakinra ameliorated memory impairment associated with pentylenetetrazole (PTZ)-induced seizures. Anakinra does not directly modulate neuronal excitability but reduces only the periodic bursting of seizures and may reduce the synchronization associated with the bursting phase.

**Mechanism of action**

Work in rodent models documented a proconvulsant role for IL-1β. In epileptogenesis, both astrocytes and endothelial cells of the blood-brain barrier release IL-1β, which results in ongoing neuronal injury and blood-brain barrier leakage. Conversely, IL-1RA has been shown to have an anticonvulsant role. Work in immature animals has shown that IL-1RA blocks kindling and mitigates augmentation of epileptogenesis enhanced by lipopolysaccharide.

Neurogenic inflammation is evoked by neuronal activity, and contributes to epileptogenesis. The IL-1/Toll Like Receptor 4 system is key to neurogenic inflammation, and the main ligands are IL-1β and high mobility group box 1 (HMGB1). Under normal conditions, HMGB1 is located in the nucleus and regulates gene transcription. After inflammasome activation, HMGB1 translocates to the cytoplasm and activates Toll-like receptor 4, thus exacerbating neurogenic inflammation. These inflammatory mediators directly affect neuronal excitability by rapid post-transcriptional effects on receptors and ion channels, and induce Src kinase dependent phosphorylation of the NR2B subunit of the N-methyl-D-aspartate (NMDA) receptor, which increases calcium influx.

Anakinra is a recombinant human IL-1 receptor antagonist that, unlike the native protein (IL-1RA), is not glycosylated and has an additional N-terminal methionine. The compound blocks the effects of interleukin-1 (IL-1) by competitively inhibiting the binding of interleukin-1α (IL-1α) and IL-1β to the IL-1 receptor.

**Pharmacokinetics**

Anakinra is administered by subcutaneous injection, and has a half-life of approximately 4-6 h in adults. Following subcutaneous injection, its bioavailability is approximately 95%. Anakinra is eliminated renally and thus dosing must be adjusted for renal impairment. In CAPS, the typical starting dose of anakinra is 1-2 mg/kg/day, and the dose may be increased according to clinical response to a maximum of 8 mg/kg/day.

One study evaluated the population pharmacokinetics of anakinra in 87 children and adolescents aged 8 months to 21 years and found that a one compartment model with linear absorption and
elimination described the pharmacokinetics in these patients.\textsuperscript{17} The apparent oral clearance (CL/F) estimated according to the model was 6.24 L/h/70 kg.

**Drug interactions**

There is evidence that increased levels of cytokines (e.g., IL-1) during chronic inflammation may result in reduced cytochrome P450 (CYP) enzymes activity. Theoretically, use of IL-1 receptor antagonists, such as anakinra, in patients with chronic inflammation could normalize CYP activity and modify the serum concentrations of drugs metabolized by these enzymes;\textsuperscript{2} however, this has yet to be demonstrated.

**Efficacy data**

A single case report on the use of anakinra in FIRES has been published,\textsuperscript{18} and an additional two cases presented in abstract form.\textsuperscript{19,20} In the published case, a 32 month old girl was commenced on anakinra 6 mg/kg twice daily (b.i.d.) on day 6 with marked reduction in seizures. Anakinra was then stopped on day 23, but was restarted again on day 54, due to recurrent status epilepticus, with marked improvement. Cytokine analysis showed marked elevations in IL-6 and IL-8 in her CSF but not her serum, suggesting that inflammatory cytokines were being produced in the CSF. After long-term follow-up, she only had occasional seizures and her motor, verbal, and social development was within normal limits for age based on clinical examination alone, although imaging studies showed diffuse brain atrophy. Both cases presented in abstract form started anakinra on day 13 (personal communication from Dr. K Eschbach), and while this was associated with a marked seizure reduction, long-term outcome was still poor.

A small, retrospective case series of four children with intractable epilepsy and comorbid specific polysaccharide antibody deficiency showed seizure reduction with anakinra therapy (marked in 2/4 cases) with exacerbation noted with dose reduction or discontinuation.\textsuperscript{21}

**Tolerability and side effect profile**

The most common adverse effect of anakinra is injection site reaction, and this is usually seen in the first four weeks of treatment.\textsuperscript{2} Other common side effects include headache, nausea, vomiting and pyrexia. Hypersensitivity reactions ranging from rash to anaphylaxis may occur. Anakinra rarely results in neutropenia and/or thrombocytopenia and may increase the risk of infection. Risk of malignancy, particularly lymphoma is uncommon (0.12/100 patient-years). Finally, anakinra may induce immunogenicity, with up to 3% of patients testing positive for neutralizing antibodies after 12 weeks of treatment.

**Planned studies**
A multi-center study of early use of anakinra in children with FIRES is in the planning stages.

CANNABIDIOL

*Volker Knappertz, †Nick Jones, ‡Royston Gray, ‡Amesha Patel, ‡Benjamin J. Whalley, and *Kevan VanLandingham

*Greenwich Biosciences Inc, Carlsbad, California, USA; †Heinrich-Heine University, Düsseldorf, Germany; and ‡GW Research Ltd, Histon, Cambridge, UK.

Molecular structure of cannabidiol here

Introduction and rationale for development

Cannabidiol is a first-in-class AED. It is the second most abundant phytocannabinoid derived from the Cannabis sativa plant which has been found to possess protective effects in preclinical models of epileptiform activity, seizures, and epilepsy. More recently, antiseizure effects of cannabidiol have been demonstrated in clinical trials of childhood onset epilepsies. While the precise mechanisms by which cannabidiol exerts its antiseizure effect in humans remain unknown, cannabidiol lacks appreciable interaction with cannabinoid receptors, which likely also account for its apparent lack of euphoric effects. With 20 years of experience in cannabinoid science, GW Pharmaceuticals has initiated a preclinical and clinical development program investigating the potential therapeutic application of cannabinoids across a wide range of disease states.

GW is currently involved in six Phase II/III studies designed to evaluate the safety and efficacy of a plant-derived liquid pharmaceutical formulation of highly purified cannabidiol (Epidiolex®), as add-on treatment, across four drug-resistant epilepsy conditions: Dravet syndrome, Lennox-Gastaut syndrome, tuberous sclerosis complex, and infantile spasms. On June 25, 2018, Epidiolex® was approved by the U.S. Food and Drug Administration (FDA) for the treatment of seizures associated with Lennox-Gastaut syndrome or Dravet syndrome in patients 2 years of age and older, pending U.S. Drug Enforcement Administration (DEA) rescheduling from schedule 1, which refers to drugs with no currently accepted medical use and a high potential for abuse. It is currently under review by European regulatory authorities for the treatment of drug-resistant seizures associated with Lennox-Gastaut syndrome and Dravet syndrome.

Pharmacology
Activity profile in experimental seizure and epilepsy models

Cannabidiol demonstrated efficacy in hippocampal slice models of epileptiform activity indicative of status epilepticus, significantly reduced the number of seizures in a model of temporal lobe seizures, significantly reduced the incidence of seizure-related mortality in an intracerebroventricular penicillin model of focal seizures, and when co-administered with ethosuximide or valproic acid in an acute PTZ seizure model. It retained its anticonvulsant effect, was well tolerated, and demonstrated additive or synergistic interactions with valproic acid in the latter model.\(^{22}\)

The mouse maximal electroshock (MES) seizure threshold test, a model of generalized seizures, was used to confirm central nervous system (CNS) penetrance and assess antiseizure activity of two human metabolites of cannabidiol; i.e., 7-hydroxy-cannabidiol and 7-carboxy-cannabidiol. Cannabidiol and 7-hydroxy-cannabidiol, but not 7-carboxy-cannabidiol, significantly reduced the incidence of hind limb extension in this model when compared with vehicle.\(^{24}\) Furthermore, the anticonvulsant and tolerability profile of cannabidiol was independently examined in the Epilepsy Therapy Screening Program where effects were verified and further characterized in a battery of well-established rodent seizure models.\(^{25,26}\)

In a pharmacoresistant mouse model of Dravet syndrome, cannabidiol significantly prolonged the median survival of SCN1α heterozygotes and null animals and had no effect on the welfare of wild-type mice. Cannabidiol significantly improved weight gain and delayed the worsening of the neonatal welfare score, natural activity, response to touch, orbital tightening, and body condition score of SCN1α null animals.\(^{27}\) In a lithium-pilocarpine-induced rat model of temporal lobe epilepsy, repeated cannabidiol treatment improved seizures and epilepsy associated co-morbidities in rats. Cannabidiol reduced spontaneous seizures, reversed epilepsy-induced motor dysfunction, did not have any adverse effect on gait of temporal lobe epilepsy animals, and ameliorated epilepsy-induced memory deficits (GW Pharmaceuticals data on file).

Other pharmacological properties

In comparison to valproic acid [125, 250 or 350 mg/kg, intraperitoneally (i.p.)], ethosuximide (90, 175 or 300 mg/kg, i.p.), and phenobarbital (25, 40 or 50 mg/kg, i.p.), cannabidiol (50, 100 or 200 mg/kg, i.p.) did not produce any motor deficits or neurotoxicity in tolerability assays in rats. Repeated cannabidiol treatment, 200 mg/kg/day administered in drinking water for six weeks, reduced epilepsy-induced motor and cognitive deficits in rats (GW Pharmaceuticals data on file).

Mechanism of action
The precise mechanisms by which cannabidiol exerts its anticonvulsant effects remain unknown. Cannabidiol neither directly binds to nor activates CB₁ and CB₂ receptors at physiologically achievable concentrations but is known to reduce neuronal hyperexcitability and inflammation through modulation of intracellular calcium via G protein-coupled receptor 55, transient receptor potential vanilloid type 1 channels and adenosine mediated signaling (GW Pharmaceuticals data on file).²⁸,²⁹

**Toxicology**

In a 2-year carcinogenicity study in rats (HsdBrlHan:WIST), oral dietary administration of cannabidiol (5, 15, 50 mg/kg/day) in the form of a botanical drug substance extract resulted in no effect on tumor formation, no increase in the incidence of neoplasia, no alteration in the time of tumor onset, and no induction of rare tumors. There was, however, a reduced incidence of tumors (pituitary and mammary) generally associated with hormonally mediated neoplasia in aging animals. Cannabidiol was not mutagenic in the *in vitro* Ames test and was not clastogenic in the *in vitro* mammalian cell chromosomal aberration assay or in the *in vivo* rat Comet and bone marrow micronucleus assays (GW Pharmaceuticals data on file).

**Pharmacokinetic and metabolic profile**

After oral administration of ascending single doses of cannabidiol (1500 to 6000 mg) to healthy volunteers, peak plasma concentration (Cₘₐₓ) and area under the plasma concentration versus time curve (AUC) increased with a trend to less than dose-proportionality. Disproportionality was not seen after multiple dosing. For a 2-fold increase in dose over 7 days (750 or 1500 mg/day) there was nearly a doubling in exposure (GW Pharmaceuticals data on file).

Cannabidiol oral bioavailability has been estimated to be 6%.³⁰ When given orally as GW’s pharmaceutical formulation, cannabidiol appears rapidly in plasma with a time to maximum plasma concentration (tₘₐₓ) of 2.5 to 5 h at steady state. Co-administration of cannabidiol with a high-fat/high-calorie meal increased the rate and extent of absorption (5-fold increase in Cₘₐₓ and 4-fold increase in AUC) and reduced the total variability compared with the fasted state in healthy volunteers. Estimations of the terminal half-life of cannabidiol in plasma have ranged from 14 (single dosing) to 60 h (multiple dosing). Single dosing likely leads to underestimates due to low plasma concentrations not allowing an adequate sampling period (GW Pharmaceuticals data on file). A full analysis from GW’s formal single- and multiple-dose human pharmacokinetic study in healthy adult volunteers is expected to be published in late 2018.

CYP2C19 is the major CYP isoform involved in the metabolism of cannabidiol to 7-hydroxy-cannabidiol, an active metabolite which is metabolized further by CYP3A4 to 7-carboxy-
cannabidiol. After multiple dosing with cannabidiol, the 7-hydroxy-cannabidiol metabolite circulates in human plasma at the same order of magnitude as cannabidiol based on AUC. The UDP-glucuronosyltransferase (UGT) isoforms responsible for the Phase II conjugation of cannabidiol are UGT1A7, UGT1A9, and UGT2B7. Cannabidiol is predominantly cleared by metabolism in the liver and gut and excreted in feces, and to a much lesser extent in the urine.\textsuperscript{22}

**Drug interactions**

A Phase I repeated-dose study in healthy volunteers showed a bidirectional interaction between clobazam and cannabidiol leading to increases in exposure to the active metabolites of both compounds.\textsuperscript{31} In that study, there was no important increase of clobazam parent compound exposure when cannabidiol (750 mg b.i.d, 7–14 days) was added to clobazam (5 mg b.i.d., 7–14 days). There was, however, a notable elevation of the active metabolite N-desmethylclobazam of 3.4-fold for both C\textsubscript{max} and AUC, likely mediated by CYP2C19 inhibition. When clobazam was added to cannabidiol, there was an increase in exposure to the active metabolite 7-hydroxy-cannabidiol (C\textsubscript{max}: 1.7-fold; AUC\textsubscript{0-\infty}: 1.5-fold), possibly mediated by inhibition of UGTs.\textsuperscript{31} In 13 children (age 4 to 19 years) with refractory epilepsy taking cannabidiol and clobazam, clobazam levels increased at four weeks by a mean of 60 ± 80% and N-desmethylclobazam levels increased by a mean of 500 ± 300%.\textsuperscript{32}

**Efficacy data**

Three randomized, double-blind, placebo-controlled, multicenter trials have been completed for add-on cannabidiol in the treatment of seizures associated with Dravet syndrome and Lennox-Gastaut syndrome. **Patients were encouraged to take their medication in a consistent manner with respect to food, and there were no specific recommendations for taking cannabidiol with or without food.** The trials demonstrated that GW’s pharmaceutical formulation of purified cannabidiol (≥98% cannabidiol, with trace residual amounts of delta-9-tetrahydrocannabinol with a maximum of 0.1% w/w in active pharmaceutical ingredient, and other plant components) was significantly superior to placebo in reducing seizure frequency over a 14-week treatment period (2-week titration plus 12-week maintenance) in patients with these syndromes. Nearly all (98%) patients who completed the trials entered an ongoing open-label extension study.

In GWPCARE1 [NCT02091375], 120 children and adults with Dravet syndrome (mean age 9.8 years, 52% male) and drug-resistant seizures received either cannabidiol oral solution at a dose of 20 mg/kg/day or placebo given in two equally divided doses, in addition to standard antiepileptic treatment. Patients had a median of 13.0 convulsive seizures (tonic, clonic, tonic-clonic, or atonic) during the 4-week baseline period. The monthly frequency of convulsive seizures decreased by a
median of 39% [interquartile range (IQR), -69.5 to -4.8] for cannabidiol compared with 13% for placebo (IQR, -52.5 to 20.2); with an adjusted median difference of -22.8 (95% CI, -41.1 to -5.4; p = 0.01) between the groups. The median monthly frequency of total seizures of all types was significantly reduced with cannabidiol (p = 0.03). There was no significant reduction (p = 0.88) in non-convulsive seizures (myoclonic, countable focal, other focal, absence).33

In GWPCARE4 [NCT02224690], 171 children and adults with Lennox-Gastaut syndrome (mean age 15.4 years, 51.5% male) received either oral cannabidiol solution at a dose of 20 mg/kg/day or placebo given in two equally divided doses, in addition to standard antiepileptic treatment. Patients had previously discontinued a median of six AEDs, were taking a median of three concomitant AEDs, and had a median of 73.8 drop seizures during the 4-week baseline period. The monthly frequency of drop seizures decreased by a median of 44% (interquartile range; IQR, -69.6 to -1.9) for cannabidiol compared with a median of 22% (IQR, -45.7 to 1.7) for placebo, with an estimated median difference of -17.2 (95% CI -30.3 to -4.1; p = 0.0135) between the groups.34

In GWPCARE3 [NCT02224560], 225 children and adults with Lennox-Gastaut syndrome (mean age 16 years, 57.3% male) received either oral cannabidiol solution at a dose of 10 mg/kg/day, 20 mg/kg/day or placebo given in two equally divided doses. Patients had a median of 85 drop seizures during the 4-week baseline period. The monthly frequency of drop seizures decreased by a median of 37% for 10 mg/kg/day (p = 0.002 versus placebo), 42% for 20 mg/kg/day (p = 0.0005 versus placebo), and 17% for placebo. The estimated median difference in percentage points was -19.2 (95% CI, -31.2 to -7.7, p = 0.002) for the 10 mg/kg/day group and -21.6 (95% CI, -34.8 to -6.7, p = 0.005) for the 20 mg/kg/day group compared with placebo.35 The difference between treatments favored cannabidiol over placebo during the first four weeks of maintenance dosing and persisted throughout treatment (GW Pharmaceuticals data on file).35

In a pooled analysis of the two trials of add-on cannabidiol treatment in patients with Lennox-Gastaut syndrome, more patients or caregivers in the cannabidiol treatment groups reported improvement in overall condition as measured on the Subject/Caregiver Global Impression of Change (S/CGIC) scale than in the placebo groups.36

A recent interim analysis of the ongoing open-label extension trial (GWPCARE5; NCT02224573) of add-on cannabidiol in patients with Dravet syndrome (median time on treatment 39 weeks; 28% with at least 37 weeks) and Lennox-Gastaut syndrome (median time on treatment 38 weeks; 57% with at least 37 weeks) showed that long-term treatment with cannabidiol was generally well tolerated, with a safety profile similar to the 14-week Phase III trials. Long-term cannabidiol was associated with durable reductions in convulsive and total seizure frequency (in Dravet syndrome)
and durable reductions in drop and total seizure frequency (in Lennox-Gastaut syndrome). More than 80% of subjects/caregivers reported improvement in overall condition as measured on the Subject/Caregiver Global Impression of Change scale at 24 and 48 weeks.\textsuperscript{37,38}

Results from an exposure-response analysis of cannabidiol for the treatment of Lennox-Gastaut syndrome suggest that the observed reduction in drop seizures and onset of certain adverse events (AEs) are related to both cannabidiol and 7-hydroxy-cannabidiol exposure. The drop seizure responder rate (patients with $\geq 50\%$ reduction) significantly increased with increasing plasma exposure of cannabidiol and its active metabolite 7-hydroxy-cannabidiol. Positive correlations with plasma exposure (AUC) were also determined for several AEs for both cannabidiol and 7-hydroxy-cannabidiol.\textsuperscript{39}

A treatment responder analysis in patients with Lennox-Gastaut syndrome on and off clobazam showed that add-on cannabidiol resulted in greater seizure reductions versus add-on placebo, regardless of concomitant use of clobazam. AEs were reported more frequently in patients receiving cannabidiol than in those receiving placebo, with more somnolence observed in patients on versus off clobazam.\textsuperscript{34}

**Tolerability and adverse effect profile**

A thorough QT study showed that single therapeutic (750 mg) and supratherapeutic (4500 mg) oral doses of cannabidiol do not affect heart rate and pulse rate or QRS interval duration, QTc, or other electrocardiographic parameters; in addition, there were no treatment-emergent electrocardiographic morphological changes following treatment.\textsuperscript{40}

Although AEs were more frequent in patients receiving cannabidiol than those receiving placebo, they were of mild or moderate severity in the majority of patients and treatment was generally well tolerated. AEs leading to withdrawal occurred in 13% of patients with Dravet syndrome treated with 20 mg/kg/day compared with 2% for placebo.\textsuperscript{33} In patients with Lennox-Gastaut syndrome, AEs leading to withdrawal occurred in 11% treated with 20 mg/kg/day and 1% treated with 10 mg/kg/day, compared with 1% for placebo.\textsuperscript{36} Common AEs reported across the cannabidiol development program are somnolence, decreased appetite, diarrhea, pyrexia, fatigue, lethargy, rash, nasopharyngitis, and pneumonia. Dose-related, reversible elevations of liver transaminase enzyme levels without meeting Hy’s Law criteria were observed with cannabidiol treatment, most commonly in patients receiving valproic acid and predominantly within the first month of treatment.\textsuperscript{37,38}

**Resolution of transaminase elevations of $\geq 5$ times the upper limit of the normal range from the randomized controlled trials and the open-label extension trial occurred with**
discontinuation of cannabidiol in 53% of cases. In 47% of cases, transaminase elevations resolved during continued treatment with cannabidiol, with or without dose reduction of cannabidiol or valproic acid.

Administration of a single oral dose of cannabidiol (750 mg), showed no significant or consistent abuse potential in a highly sensitive population of recreational polydrug users. Higher doses of cannabidiol (1500 mg and 4500 mg), which are above those found to be effective in the Dravet and Lennox Gastaut trials, had detectable subjective effects; however, these were reported significantly less often when compared with the positive controls, alprazolam (2 mg, Schedule IV) and two doses of dronabinol (10 mg and 30 mg, Schedule III), and were not considered clinically significant. At the doses tested, use of cannabidiol was not associated with cognitive or psychomotor impairment, was well tolerated, and produced few abuse-related AEs, suggesting a low abuse potential.

Planned studies

Ongoing studies with cannabidiol include a Phase III dose-ranging study in Dravet syndrome, a Phase III study in tuberous sclerosis complex, and a Phase II/III study in infantile spasms. Eligible patients from these studies, and from the completed randomized trials summarized above, are participating in ongoing open label extension trials. Other indications in epilepsy are being considered for future studies.

CANNABIDIVARIN

*Kevan VanLandingham, †Nick Jones, †Royston Gray, †Clare Horne, †Benjamin J. Whalley, †Mariana Napoles, and *†Volker Knappertz

*Greenwich Biosciences, Inc., Carlsbad, California, USA; †GW Research Ltd, Histon Cambridge, UK; †Heinrich-Heine University, Düsseldorf, Germany

Molecular structure of cannabidivarin here

Introduction and rationale for development

Cannabidivarin is the propyl analog of cannabidiol and is an active constituent derived from the Cannabis sativa plant. Cannabidivarin has shown antiepileptic properties in in vitro models of epileptiform activity and antiseizure properties in in vivo models of seizures. However, the precise mechanisms underlying these effects remain unknown. As part of its cannabinoid development
program, GW Pharmaceuticals and its US subsidiary Greenwich Biosciences are investigating the potential therapeutic applications of cannabidivarin across a range of disease states, including epilepsy.

Pharmacology

Anticonvulsant profile

The anticonvulsant profile of cannabidivarin has been reported previously in some detail. Cannabidivarin exerted an inhibitory effect on epileptiform discharges in vitro in the 4-aminopyridine model and in the magnesium-free model, using extracellular, multi-site electrophysiological recordings from acute rat hippocampal slices. In vivo, cannabidivarin (≥50 mg/kg i.p.) significantly reduced tonic convulsions, and both hindlimb and forelimb extensions in the mouse MES model. Cannabidivarin also reduced the incidence of tonic convulsions in the mouse audiogenic model, with an ED$_{50}$ of 64 mg/kg i.p., and reduced at a dose of 200 mg/kg i.p. the incidence of tonic–clonic seizures in the acute PTZ rat model. Furthermore, in the acute PTZ rat model, cannabidivarin increased the latency to seizure onset and the number of animals that exhibited no sign of seizure activity. When co-administered with either ethosuximide or valproate in the same model, cannabidivarin (200 mg/kg, i.p.) retained its anticonvulsant effect and did not functionally antagonize the anticonvulsant actions of either drug. Cannabidivarin (200 mg/kg, i.p.) alone had no effect against acute pilocarpine-induced temporal lobe seizures, but significantly attenuated these seizures when administered with valproate or phenobarbital at this dose.

Molecular markers of seizures were assessed using the rat PTZ model of acute generalized seizures. Cannabidivarin at a dose of 400 mg/kg per os (p.o.) significantly decreased median seizure severity, increased the latency to seizure onset, and suppressed seizure-related changes in mRNA expression of Fos, Egr1, Arc, Ccl4, and BDNF.

Tests of motor function confirmed that the anticonvulsant effects of cannabidivarin (≤200 mg/kg) in models of acute seizures are due to its anti-ictal properties and not to suppression of motor function. Cannabidivarin (50–200 mg/kg, i.p.) had no significant effects on the static beam and forelimb grip strength tests, in contrast to a therapeutic dose of valproate (125–350 mg/kg, i.p.), which demonstrated a dose-dependent increase in the number of foot slips and the distance traveled on the static beam.

Other pharmacological properties

Cannabidivarin has been reported to inhibit the ability of lysophosphatidylinositol to activate G protein coupled receptor 55 (GPR55), its cognate receptor. Additionally, cannabidivarin is able to
rapidly activate and antagonize the transient receptor potential (TRP) cation channel subfamily V member 1 and 2 (TRPV1, TRPV2), which are both members of the vanilloid receptor family.\(^\text{45}\)

Further work is needed to assess potential similarities and/or differences in effects of cannabidivarin and cannabidiol.

**Mechanism of action**

The mechanism underlying the antiseizure effects of cannabidivarin is unknown and is currently under investigation. Importantly, cannabidivarin lacks appreciable affinity and functional activity at human cannabinoid receptor type 1 and does not bind to human cannabinoid receptor type 2 with significant affinity.\(^\text{42,43,46}\)

**Toxicology**

A large body of preclinical toxicological evidence suggests that cannabidivarin has a wide safety margin between the proposed clinical dose and the dose at which the No-Observed-Adverse-Effect-Level was observed in the nonclinical studies. Although no target organ toxicity has been identified, an adaptive change in liver was noted comprising hepatocyte hypertrophy with an associated change in liver enzymes (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase) (GW Pharmaceuticals data on file).

**Pharmacokinetics and metabolic profile**

Pharmacokinetic studies confirmed that 7-hydroxy-cannabidivarin and 7-carboxy-cannabidivarin are metabolites of cannabidivarin.\(^\text{22}\) However, the clinical relevance of the abundance of metabolites has yet to be established. A single oral dose study in healthy subjects showed that cannabidivarin \(C_{\text{max}}\) and AUC were dose proportional between the 200 and 800 mg dose range.\(^\text{22}\)

**Drug interactions**

Cannabidivarin is being investigated in adults with focal epilepsy. The first part of a Phase II randomized, double-blind, placebo-controlled study of adjunctive cannabidivarin for the treatment of inadequately controlled focal seizures has been completed. Part A [NCT02369471] evaluated the safety and pharmacokinetics of cannabidivarin in the presence of concomitant AEDs and indicated that there were no appreciable differences in the pharmacokinetics and safety profile of cannabidivarin when given concomitantly. These results supported continuing with Part B of the study without additional exclusions of concomitant medications. One hundred sixty-two patients have now completed Part B [NCT02365610] of this study.

**Efficacy data**
A Phase II double blind, randomized, placebo-controlled, two-part study has been conducted to investigate the pharmacokinetics and safety (Part A) and efficacy (Part B) of cannabidivarin as add-on therapy in patients with inadequately controlled focal seizures.

Part B randomized 162 patients to cannabidivarin or placebo (1:1) and included a 4-week baseline period and an 8-week treatment period (2-week titration starting with 400 mg, b.i.d., up to 800 mg, b.i.d. plus 6-week maintenance) followed by a 12-day taper period. The primary endpoint was the change from baseline in focal seizure frequency in patients taking add-on cannabidivarin compared with add-on placebo. The primary endpoint was not met.

**Tolerability and adverse-effect profile**

A Phase I randomized, double-blind, placebo-controlled trial in healthy subjects showed that cannabidivarin was well-tolerated at even the highest tested dose (800 mg once daily over 5 days) and no significant AEs were observed. The overall incidence of AEs was low, with no serious AEs or withdrawals due to AEs. Part A of the Phase II trial showed similar results, with no serious AEs or withdrawals due to AEs. In Part B of the Phase II trial, serious AEs were reported in 3.7% of patients taking cannabidivarin and 1.2% of patients taking placebo. Withdrawals due to AEs were reported in 13.6% of patients taking cannabidivarin and 2.5% of patients taking placebo (GW Pharmaceuticals, data on file).

**Planned studies**

In preclinical models, cannabidivarin is a promising antiseizure agent and has thus far demonstrated a favorable toxicological profile. Its clinical development will include targeting patients with seizure disorders, Rett syndrome, and autism spectrum disorder.

**FENFLURAMINE HYDROCHLORIDE (ZX008)**

Gail Farfel
Zogenix Pharmaceutical, Emeryville, CA, USA

Molecular structure of fenfluramine hydrochloride here

**Introduction and rationale for development**

ZX008 (low-dose fenfluramine HCl) is being developed as an oral solution for the adjunctive treatment of patients with Dravet syndrome. As reported in some detail in the previous EILAT Progress Report, the rationale for the development of fenfluramine in this indication stems from
early observations suggestive of the usefulness of this compound in patients with refractory epilepsy and SCN1A mutations subsequently confirmed to have Dravet syndrome.

**Pharmacology**

The activity profile of fenfluramine in a zebrafish model of Dravet syndrome and the mechanisms of action of fenfluramine, identified to date, have been summarized in the previous EILAT Progress Report. The mechanisms responsible for the efficacy of fenfluramine in Dravet syndrome are under investigation. In a zebrafish model of Dravet syndrome, co-administration of antagonists of 5-HT receptor subtypes with fenfluramine in the mutant larvae suggest that 5-HT1D and 5-HT2C, and possibly 5-HT2A receptor subtypes are involved with the anti-seizure activity. The σ1 receptor has also been implicated, suggesting nonserotonergic mechanisms may also play a role in the anti-seizure effects of fenfluramine.

**Toxicology**

Preclinical toxicology studies of fenfluramine have been summarized previously.

**Pharmacokinetics**

In a recent study in healthy volunteers, fenfluramine was absorbed with a t_{max} of 3.0 h after a single oral dose of ZX008 (0.8 mg/kg) and was eliminated with a terminal half-life of 20.1 h. In another study, administration of ZX008 to healthy volunteers after an overnight fast or 30 min after consumption of a high-fat breakfast resulted in similar exposures to the drug and to its major metabolite, norfenfluramine. Preliminary receptor binding and functional assay experiments suggest that fenfluramine, norfenfluramine, and their enantiomers bind to and have effects at receptors identified in the literature as having a role in epilepsy.

The metabolism of fenfluramine occurs via multiple CYP enzymes, including CYP1A2, CYP2B6, CYP2D6, CYP2C9, CYP2C19 and CYP3A4.

The pharmacokinetics of fenfluramine in patients with Dravet syndrome have not been reported to date.

**Drug interactions**

Results of a study investigating potential drug-drug interactions of fenfluramine with other AEDs were recently presented. Healthy volunteers were treated with a single dose of ZX008 (0.8 mg/kg), a single dose of stiripentol (3500 mg)/clobazam (20 mg)/valproate (25 mg/kg, maximum dose 1500 mg) in combination, and a single dose of ZX008 plus the three-drug combination, with the treatments separated by 17 days. The three-drug combination increased the AUC of
fenfluramine by about 70%, and reduced the AUC of the metabolite by about 40%. In contrast, ZX008 did not have any significant impact on the pharmacokinetic profiles of the three other drugs. When administering these drugs together, a downward dose adjustment of ZX008 may be warranted. It is important to note, however, that this was a single dose study, and that the pharmacokinetic interactions during multiple dosing have not been established.

Efficacy data

Two cohorts including 21 patients with Dravet syndrome have been treated for between one and 29 years with fenfluramine with reports of strong anti-seizure efficacy. In the most recent “prospective” cohort, which began treatment in 2011, patients experienced a median 83% reduction in frequency of major motor seizures (defined as tonic-clonic, tonic, clonic, atonic, and myoclonic seizures lasting >30 sec) while treated with fenfluramine for a median of three years (range, 0.8 to 6.6 years) compared to a 3-month baseline observation period.

Recently the results of the first Phase III double-blind, randomized, placebo controlled trial in 119 patients with Dravet syndrome aged two to 18 years have been reported. Subjects were randomized to placebo, ZX008 0.2 mg/kg/day, or ZX008 0.8 mg/kg/day (1:1:1) and were treated for a total of 14 weeks. Treatments were administered orally in two equally divided doses taken with food approximately 12 hours apart. The study met its primary objective by demonstrating that ZX008 at 0.8 mg/kg/day was superior to placebo in reducing seizure frequency as adjunctive therapy. Compared to placebo, subjects treated with ZX008 0.8 mg/kg/day demonstrated a 63.9% greater reduction in monthly frequency of major motor seizures (defined in this study as hemiclonic, tonic, clonic, tonic-atonic, generalized tonic-clonic, and focal seizures with clear observable motor signs) (p < 0.001). Subjects treated with ZX008 0.2 mg/kg/day experienced a 33.7% greater reduction in monthly major motor seizure frequency compared with placebo (p = 0.019). In addition, 70% of subjects treated with ZX008 0.8 mg/kg/day (p <0.001) and 41% of subjects treated with ZX008 0.2 mg/kg/day (p = 0.001) demonstrated ≥50% reduction in monthly frequency of major motor seizures compared with 7.5% in the placebo group.

Preliminary uncontrolled data suggest that ZX008 may also have anti-seizure effects in patients with Lennox-Gastaut syndrome. In an open-label dose-finding study in 13 patients with Lennox-Gastaut syndrome the frequency of major motor seizures (defined in this study as generalized tonic-clonic, tonic, atonic, and focal seizures with a motor component) declined from a median 60 per month in the baseline period to 22 per month 12 weeks after adding ZX008 at doses from 0.2 to 0.8 mg/kg/day to their treatment regimens. Nine patients continued into an extension study and have...
been treated for three to 15 additional months. At their last study visit, monthly seizure frequency over a total of 8 to 20 months had declined by 58% compared to baseline.56

**Tolerability and adverse effect profile**

The most common treatment-related AEs reported in the two open-label cohorts of patients with Dravet syndrome are mild-to-moderate somnolence, fatigue and anorexia. A similar AE profile was observed in the Lennox-Gastaut study.

In the Phase III Dravet syndrome study, non-cardiac-related treatment-emergent AEs recorded in ≥10% in any treatment group were diarrhea (7.5%, 30.8%, 17.5% for the placebo, 0.2 mg/kg/day, and 0.8 mg/kg/day groups, respectively), weight decrease (0, 12.8%, 5.0%), decreased appetite (5.0%, 20.5%, 37.5%), constipation (0, 2.6%, 10.0%), and lethargy (5.0%, 10.3%, 17.5%). Five subjects in the ZX008 0.8 mg/kg/day group (12.5%) discontinued the study early due to an AE. None of the subjects in the ZX008 0.2 mg/kg/day group and in the placebo group discontinued treatment early due to AEs.

A major concern about the use of fenfluramine is its association with cardiac valvulopathy and pulmonary hypertension when used to treat obesity in adults.57 These cases occurred primarily in patients taking fenfluramine in combination with phentermine.57,58 No incidence of pulmonary hypertension or FDA-defined cardiac valvulopathy has been observed in a cohort of 19 Dravet syndrome patients treated with fenfluramine for up to 29 years at doses ranging from 0.12 to 0.68 mg/kg/day (median=0.27 mg/kg/day) or in the Phase III completed clinical study. All Dravet syndrome patients enrolled in the company-sponsored clinical studies of ZX008 are being monitored with regular echocardiographic examinations. Trace mitral regurgitation or aortic regurgitation are not considered medically meaningful but were captured on the echocardiogram. During the study, five (12.5%) subjects in the placebo group, seven (17.9%) in the 0.2 mg/kg/day, and nine (22.5%) in the 0.8 mg/kg/day group had at least one echocardiogram finding with trace mitral regurgitation and/or aortic regurgitation in the placebo, though no patient met FDA-definition of a clinical valvulopathy.

The efficacy and safety data from the Phase III development program and the ongoing open-label studies of fenfluramine as add-on therapy in Dravet syndrome suggest that a positive benefit to risk profile exists for the use of low-dose fenfluramine in the treatment of uncontrolled seizures in Dravet syndrome.

**Planned studies**
Enrollment has been completed in the pivotal Phase III double-blind, randomized, placebo-controlled studies assessing low dose ZX008 (0.2 or 0.8 mg/kg/day) as add-on treatment for uncontrolled seizures in Dravet syndrome. A long-term open label safety study is still ongoing. Zogenix is currently performing a single Phase III, double-blind, randomized, placebo-controlled study of add-on low dose ZX008 (0.2 or 0.8 mg/kg/day) for the treatment of uncontrolled seizures in Lennox-Gastaut syndrome.

GANAXOLONE

*Lorianne Masuoka, †Michael Saporito, *Jaakko Lappalainen and *Julia Tsai


Molecular structure of ganaxolone here

Introduction and rationale for development

Ganaxolone (3α-hydroxy-3β-methyl-5α-pregn-20-one), a synthetic analog of the progesterone metabolite allopregnanolone, is a positive allosteric modulator of the gamma-aminobutyric acid (GABA)A receptor. Ganaxolone retains the GABA_A receptor modulatory effects of allopregnanolone but does not activate the nuclear (classical) progesterone receptors. Ganaxolone acts at both synaptic and extrasynaptic GABA_A receptors by interacting with binding sites that are different from those involved in the action of benzodiazepines.22 A formulation of ganaxolone suitable for oral dosing has been developed.

The current indications for ganaxolone include programs in post-partum depression and orphan epilepsy indications including refractory status epilepticus and CDKL5 deficiency disorder.

Pharmacology and toxicology

The preclinical pharmacology and toxicology profile of ganaxolone have been summarized in the prior EILAT XIII progress report.22 Seizure protection has been demonstrated in a broad range of animal models, and unlike other GABA_A-receptor modulators, ganaxolone does not show tolerance to anticonvulsant activity. In a rat model of refractory status epilepticus, ganaxolone shows a longer duration of effect than the positive control allopregnanolone, an observation which supports current Phase II studies of ganaxolone in refractory status epilepticus.22 Ganaxolone did not show end organ toxicity in 6-month rat and 12-month dog studies.
Pharmacokinetics

Multiple-dose studies of oral ganaxolone were conducted in healthy volunteers. At steady state, mean $C_{\text{max}}$ and $\text{AUC}_{\text{0-12h}}$ were close to dose proportional at doses up to 600 mg b.i.d., with higher doses producing less than proportional increments in serum drug levels. In general, ganaxolone was rapidly absorbed following oral administration and median $t_{\text{max}}$ values during multiple dosing were attained in 2 h and were independent of dose level. Mean $C_{\text{max}}$ in epilepsy patients and healthy volunteers exceeded 250 ng/mL with oral doses of 800 mg b.i.d. or higher, thereby providing a level of exposure which is expected to be effective for chronic oral treatment of mood disorders and refractory epilepsies such as CDKL5 deficiency disorder (Marinus Pharmaceuticals, data on file). Ganaxolone is eliminated by CYP3A4-mediated metabolism. Its half-life is multiphasic with a dominant phase of 3-6 h.

Drug interactions

The combined data available to date suggest that ganaxolone has low potential to affect the disposition of other drugs. However, the disposition of ganaxolone is influenced by CYP3A4 inducers and inhibitors. The data also suggest that, within the dose range tested in clinical studies, changes in ganaxolone concentrations caused by the presence of enzyme inducing AEDs is unlikely to have a clinically significant effect in clinical response (Marinus Pharmaceuticals, data on file).

Efficacy

Ganaxolone has been studied in a variety of epileptic conditions including West syndrome, focal onset seizures, PCDH19 epilepsy, Lennox Gastaut syndrome and CDKL5 deficiency disorder. Some of these studies have been summarized in previous EILAT Progress Reports.

In an uncontrolled 26-week open label Phase II study of girls with PCDH19 epilepsy, treatment with ganaxolone up to 1800 mg/day (or up to 63 mg/kg/day if less than 30 kg) was associated with a reduction in seizure frequency at 6 months in approximately half the cohort (54%-100% in 4/11, 26%-33% in 2/11). In another uncontrolled 26-week open label study of ganaxolone up to 1800 mg/day (or up to 63 mg/kg/day if less than 30 kg), conducted in eight children with Lennox Gastaut syndrome and severe, treatment-resistant generalized tonic-clonic and/or drop seizures, the median percent reduction in seizures at six months was 32% and the median percent increase in seizure-free days was 33%. In a similar study in seven patients with CDKL5 deficiency disorder, the median percent reduction in seizures at six months was 43% with a median percent increase in seizure-free days of 78%. Ten of the 28 subjects from the three cohorts of this 6-month study enrolled into the
52-week extension study due to good, long-term seizure control, including four of the seven children with CDKL5 deficiency disorder.

In the Phase III study 1042-0603 in patients with focal seizures (ClinicalTrials.gov Identifier: NCT02358538), there was no statistically significant effect of ganaxolone compared to placebo in the primary analysis. A post-hoc analysis, however, showed a statistically significant reduction in seizure frequency for those subjects on ganaxolone who were taking three or more concomitant AEDs compared to those receiving placebo. In this subgroup, ganaxolone was associated with a 20% greater reduction in median seizure frequency than placebo (p=0.02). Although, numerically superior, there was no statistically significant effect of ganaxolone compared to placebo for those subjects taking fewer than three AEDs.

Tolerability and adverse effect profile

In all completed placebo-controlled studies, the most frequently reported AEs in ganaxolone-treated subjects were CNS-related, and included somnolence, dizziness, fatigue, and headache (Table 1). As shown in Table 1, in multiple placebo-controlled studies across multiple indications including epilepsy, few AEs were reported with ganaxolone that occurred at a rate higher than those reported in placebo-treated subjects. These AEs were generally mild and have always been reversible.

Table 1 here

Planned studies

Marinus Pharmaceuticals is currently testing oral ganaxolone in a Phase II long-term extension study in children with PCDH19 epilepsy, CDKL5 deficiency disorder and Lennox Gastaut syndrome. A Phase II study in refractory status epilepticus was initiated in early 2018 with intravenous ganaxolone. A Phase III pivotal study of oral ganaxolone in patients with CDKL5 deficiency disorder will be initiated in 2018.

MEDIUM CHAIN FATTY ACIDS

Matthew C. Walker and Robin S.B. Williams
Introduction and rationale for development

Medium chain fatty acids, including decanoic acid, provide the key constituents of the medium chain triglycerides (MCT) in the MCT ketogenic diet. This diet provides one of the last options for the treatment of patients with refractory epilepsy and has been validated in a clinical trial in childhood epilepsy. Although, often effective, the diet is more commonly used in the treatment of paediatric patients rather than adults, due to the stringent dietary restrictions. The mechanism of the ketogenic diet is broadly considered to be through the hydrolysis of triglycerides to release free fatty acids (decanoic acid and octanoic acid, in the case of the MCT diet), that are metabolised to ketones to provide seizure control. This mechanism has recently been challenged, with the proposal that decanoic acid functions as the therapeutic component of the diet, and that this mechanism can be reproduced by specific related congeners.

Decanoic acid was first suggested as the active component of the diet from research in a simple biomedical model where a range of novel fatty acids was proposed as seizure control treatments. Several of these fats have been subsequently validated in a range of in vitro and in vivo models. Further analysis of decanoic acid showed that it functions to block activity in two acute ex vivo rat hippocampal slice models of epileptiform activity, and this effect was not evident for ketones nor octanoic acid.

Pharmacology

Release of decanoic acid from triglycerides in the gastrointestinal tract enables direct absorption through the gut wall, where it is mostly metabolised in the liver through β-oxidation to produce ketone bodies. However, some fatty acids escape into the circulatory system. In animal models, decanoic acid has been found to penetrate the blood-brain barrier and to be present in brain at 60%–80% of serum levels. In the brain, decanoic acid functions as a non-competitive inhibitor of the α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) receptor, with an IC₅₀ of 0.52±0.02 mM. This is considerably lower than that for octanoic acid (IC₅₀=3.82±0.03 mM).
Decanoic acid (1 mM) blocks PTZ- or low magnesium-induced epileptiform discharges in rat entorhinal cortex-hippocampus slices within 20 min of exposure.\textsuperscript{62,64} The first study to show an antiseizure effect of decanoic acid involved i.p. administration to mice 15 min before seizure induction by subcutaneous injections of picrotoxin. Decanoic acid caused a small but significant delay in the onset to clonic convulsions at 172 mg/kg (from around 480 s without treatment to around 970 s with decanoic acid) whereas fatty acids with longer chain lengths caused greatly increasing onset time, with palmitic acid (16 carbons) delaying convulsions to around 3600 s, and decanoic acid did not increase survival time.\textsuperscript{67} Using the same administration route, this study also suggested that onset to clonic seizures induced by subcutaneous PTZ was not affected by decanoic acid at 172 mg/kg, but survival times increased from around 422 s to 633 s. Although this study suggested a weak effect of decanoic acid on seizure activity, the small effect size and inconsistency between models did not provide strong evidence for an antiseizure effect.

However, two subsequent studies did suggest a role for decanoic acid in seizure control. Using a single bolus oral gavage dose of decanoic acid, seizure thresholds in the 6 Hz model were significantly increased (at 1.7 g/kg and 5.2 g/kg), and a similar increase in the MES threshold was observed at 8.6 g/kg p.o.,\textsuperscript{66} although no effect was observed at decanoic acid doses up to at 8.6 g/kg p.o. following seizure induction with intravenous (i.v.) PTZ. In addition, dietary intake of 35% of calories through decanoic acid (only) triglycerides increased seizure threshold in the 6 Hz model and the latency to first generalised seizure in the flurothyl model.\textsuperscript{68} These effects were not seen following octanoic acid (only) triglyceride dietary intake. Importantly, this study also demonstrated that both decanoic acid and octanoic acid triglycerides provided a common level of ketosis, but only the decanoic acid diet provided seizure control, strongly supporting a role for decanoic acid in seizure control independent of ketone generation. In addition to decanoic acid, a range of congeners, including branched and cyclic derivatives, also show strong seizure control activity.\textsuperscript{62,64,65}

\textit{Other pharmacological properties}

Decanoic acid has been shown to increase mitochondrial proliferation through regulation of a fatty acid receptor, PPAR\textgamma.\textsuperscript{69} In this study, decanoic acid treatment of cultured neural cells was shown to act via PPAR\textgamma to enhance mitochondrial biogenesis and activity of the mitochondrial complex I. This mechanism was not evident for octanoic acid, and is thought to increase ATP availability leading to an increase in seizure threshold and to a reduction in seizure activity following long term treatment.

\textit{Mechanisms of action}
Studies using whole cell patch clamp recordings from CA1 pyramidal neurons have shown that decanoic acid reduces excitatory postsynaptic currents (EPSCs), consistent with an effect on postsynaptic excitatory AMPA receptors. Direct inhibitory activity of decanoic acid against AMPA receptors was then shown using a *Xenopus* oocyte model, where the expression of distinct AMPA receptor subunits (GluA1, GluA2 and GluA3) enabled the detailed analysis of decanoic acid-dependent inhibition in isolated receptors. In *silico* docking analysis suggests decanoic acid binds to AMPA receptors on the M3 helices within the channel region, providing a distinct binding site to that proposed for perampanel – a currently licensed treatment for focal and generalised tonic-clonic seizures through AMPA receptor inhibition.

Decanoic acid directly inhibits AMPA receptors of different subunit combinations, with greatest potency against GluA2/3 (IC$_{50}$ = 0.52 mM), followed by GluA1/2 (IC$_{50}$ = 1.16 mM), and then GluA1 only (IC$_{50}$ = 2.09 mM). This suggests activity against the most common receptor combinations in the mammalian brain. Furthermore, the inhibitory effect of decanoic acid against AMPA receptors is voltage-dependent, where potency against GluA2/3 receptors at -80 mV (IC$_{50}$ of 1.11 mM) is enhanced under more depolarised condition at -40 mV (to IC$_{50}$ of 0.43 mM), suggesting stronger inhibitory activity during prolonged seizure activity.

**Toxicology**

Few reports of decanoic acid toxicology have been published. Unpublished data kindly provided by the Epilepsy Therapy Screening Program suggests no behavioural toxicity in mice at up to 300 mg/kg (i.p.). High concentration single bolus gavage experiments in mice suggest impaired motor performance in the chimney test with a TD$_{50}$ of 17.6 g/kg, and no single dose was shown to significantly impair grip strength.

**Pharmacokinetics**

Several early studies have monitored medium chain fats in peripheral blood from children on the MCT ketogenic diet (reviewed by Augustin et al, 2018). These studies show wide variation in decanoic acid levels (87 – 552 μM, with an average of 157 μM). In contrast, patients had greater levels of octanoic acid, averaging 310 μM, a finding which may reflect the higher content of octanoic acid provided within the MCT supplement. Although octanoic acid is unlikely to inhibit AMPA receptors at therapeutic concentrations, it may function to elevate neuronal decanoic acid levels through preferential oxidation.

**Drug interactions**
Contraindications for the ketogenic diet include defects in fatty acid metabolism and function including fatty acid oxidation, and deficiencies in carnitine-related function, organic translocase, pyruvate carboxylase and hypoglycaemia.

**Efficacy data**

A randomized trial suggested that the classical and the MCT ketogenic diets may show similar efficacy in seizure control, although the classical diet requires a more stringent regimen. In that trial, the classical diet and the MCT diet were administered to a total of 125 children with pharmacoresistant epilepsy, but only 47 patients were evaluable for efficacy at the 12 month assessment. The average percent decrease in seizure frequency compared with baseline was 53% for the evaluable children in the MCT ketogenic diet group (n=25) and 40.8% for those evaluable in the classical ketogenic diet group (n=22). No breakdown of outcome by seizure type was reported.

**Tolerability and adverse effect profile**

Although no data exist regarding decanoic acid-only treatment in patient groups, the MCT ketogenic diet is associated with a range of gastrointestinal-related side effects, such as cramps, bloating, diarrhoea, and vomiting. In addition, it is important to note that AMPA receptors play an important role in synaptic strengthening during long-term potentiation and synaptic plasticity. However, inhibition of AMPA receptor activity has been demonstrated not to impair long-term potentiation or cognition (for review, see Chang et al.) suggesting that dietary decanoic acid is unlikely to adversely affect learning and memory. In contrast, the MCT ketogenic diet has been suggested to be possibly associated with positive effects on cognition.

**Planned studies**

A recently developed modified MCT diet containing high levels of decanoic acid (Betashot) is currently in tolerability clinical trials with both adult and paediatric drug-resistant patients (NCT02825745). This study involves less stringent dietary restrictions than the currently used MCT ketogenic diet.

**PADSEVONIL**

*Konrad J. Werhahn, †David Sciberras, ‡Rafal Kaminski and †Pierandrea Muglia

*UCB Pharma, Monheim am Rhein, Germany and †UCB Pharma, Brussels, Belgium

Molecular structure of padsevonil here

27
**Introduction and rationale for development**

Benzodiazepines, which enhance inhibitory GABAergic neurotransmission, were observed to markedly potentiate the anticonvulsant activity of the synaptic vesicle 2A (SV2A) ligand levetiracetam in a variety of seizure models, with no exacerbation of side effects. These observations led to a rational medicinal chemistry design program to develop a molecule combining presynaptic activity (high affinity binding to SV2) with postsynaptic enhancement of GABAergic inhibition (low-to-moderate affinity binding at the benzodiazepine site of the GABA_A receptor).

Padsevonil (UCB0942) is the first rationally designed AED candidate that inhibits seizure activity by presynaptic modulation of all three SV2 isoforms and postsynaptic enhancement of GABA-mediated inhibition.

**Pharmacology**

*Anticonvulsant profile in animal models*

The antiseizure profile of padsevonil differs considerably from that of selective SV2A ligands. Padsevonil showed substantially higher potency than levetiracetam and brivaracetam across a range of acute seizure models, including several where levetiracetam and brivaracetam show no or limited efficacy (Table 2). Interestingly, padsevonil offered significantly greater protection against seizures than a simple combination of AEDs that target SV2A and GABA_A receptors (levetiracetam or brivaracetam in combination with diazepam) at doses resulting in similar target occupancies. This might be because of padsevonil’s unique SV2A binding mode (see below), the additional interaction with SV2B and SV2C, and the partial agonism at the benzodiazepine receptor.

*Table 2 here*

Amygdala kindling is the most characterized chronic model for focal seizures and is used to further evaluate the activity of AED candidates. Activity in the amygdala kindling model has been shown to be predictive of efficacy against focal to bilateral tonic-clonic seizures in the clinical setting. Padsevonil displayed potent activity in fully kindled mice and rats. It provided complete protection against focal to bilateral tonic-clonic seizures and significantly reduced both seizure severity and afterdischarge duration, indicating activity against local seizure discharge and spread (Table 2).
Adverse effects on the motor performance of mice were evaluated in the rotarod test. Padsevonil displayed a high therapeutic index in the pilocarpine, audiogenic, and 6 Hz models, as well as in the mouse amygdala kindling model, indicating a large margin between doses that provide a therapeutic effect and those that induce side effects.73,75

**Receptor occupancy**

At the dose range providing seizure protection in amygdala kindling models, padsevonil occupancy of SV2A proteins is high, while occupancy of the benzodiazepine site is low. Two positron emission tomography (PET) studies were conducted in humans to evaluate occupancy at the same receptor targets after therapeutically relevant doses.76 Modeling of these PET data confirmed a similar pattern to the preclinical model. For SV2A, there is sustained high level (>90%) receptor occupancy at padsevonil doses of ≥100 mg b.i.d., while for GABA\textsubscript{A}, there is transient low but quantifiable receptor occupancy at padsevonil doses of ≥200 mg b.i.d. (200 mg b.i.d.: 6.4%; 400 mg b.i.d.: 13.4%).76 These PET studies allowed projection of a quantitatively based dosing rationale for future clinical trials of padsevonil, and indicated that the 400 mg b.i.d. dose selected for the proof-of-concept trial achieves desired target occupancies.

**Mechanism of action**

Padsevonil has a unique mechanism of action. Unlike levetiracetam and brivaracetam which selectively bind to SV2A,77 padsevonil binds with high affinity to all three isoforms of the SV2 protein: SV2A (pKi 8.5), SV2B (pKi 7.9), and SV2C (pKi 8.5).78 Some data indicate that SV2B and SV2C might play a role in the pathogenesis of epilepsy and other neurodegenerative diseases.77 The way padsevonil interacts with SV2A also differs from levetiracetam and brivaracetam. With a dissociation half-life of 29 minutes, padsevonil has slower kinetics at SV2A than levetiracetam and brivaracetam (too fast to measure at 37°C), indicating that it engages with SV2A proteins for longer.78 Furthermore, padsevonil has approximately 100 to 1000 times higher binding affinity for SV2A than brivaracetam or levetiracetam, respectively (pKi 8.5 versus 6.6 and 5.2).78 Postsynaptically, padsevonil binds with moderate affinity to the benzodiazepine binding site of the GABA\textsubscript{A} receptor, where it acts as a partial agonist.78,79 Drug tolerance is an important clinical concern, which limits chronic use of benzodiazepines for epilepsy treatment.80,81 Agents with lower intrinsic efficacy at the benzodiazepine receptor could be associated with a lower likelihood of tolerance, and padsevonil may display a reduced potential for tolerance induction, as previously demonstrated for other partial GABA\textsubscript{A} agonists in animal models.79,81 Padsevonil maintained anticonvulsant activity after repeated administration in mice, while treatment with diazepam under the same experimental conditions was associated with a significant loss of activity (UCB Pharma,
data on file). These data suggest minimal potential for tolerance development with padsevonil, consistent with its moderate affinity and partial agonism at the benzodiazepine site.

**Pharmacokinetics and metabolic profile**

In two Phase I ascending-dose trials (single dose up to 490 mg, multiple dose up to 400 mg b.i.d.) in healthy volunteers (n=6 and n=10 per dose, respectively), the pharmacokinetic profile of padsevonil demonstrated high inter-subject variability (>50% in AUC). After single oral doses ranging between 10 to 490 mg, exposure to padsevonil (C\textsubscript{max} and AUC) increased linearly with dose. Concentration-time profiles demonstrated a short time to peak and a biphasic disposition with a median terminal half-life of ~6–7 h.

After a single dose, padsevonil CL/F was ~70 L/h and independent of dose within the 30 to 490 mg dose range. After multiple dosing (100–400 mg b.i.d.), the clearance of padsevonil decreased, resulting in an increase in exposure (1.5–2-fold higher than after a single dose) and steady state was reached after 2–3 days of dosing. Renal excretion of padsevonil was low (<0.06%). Pharmacokinetic data supported a twice daily dosing regimen and tolerability data indicated 400 mg b.i.d. as the maximum tolerated dose.

**Drug interactions**

Because the metabolism of padsevonil is mediated by CYP isoforms, the potential for drug interactions with enzyme-inducing/inhibiting drugs is being thoroughly evaluated.

**Efficacy data**

A randomized, double-blind, placebo-controlled Phase II trial (EP0069, NCT02495844) enrolled adult patients (≥18 years) with ≥4 observable focal seizures per week (includes focal aware with motor symptoms, focal impaired awareness, and focal to bilateral tonic-clonic seizures), who had failed to achieve seizure control with ≥4 AED regimens of adequate dose and duration and were on a stable dose of ≥1 AED. Concomitant treatment with drugs known to induce CYP3A4 enzymes was prohibited.

After a 2-week prospective baseline, patients were randomized (1:1) to receive padsevonil or placebo concomitantly with their current, stable AED regimen and entered a 3-week double-blind inpatient period. In the padsevonil arm, treatment was initiated at 100 mg/day and doses were up-titrated to 400 mg bid (maximum tolerated dose) over 1 week, followed by a 2-week inpatient maintenance. In the placebo arm, 2-week maintenance was followed by up-titration to 400 mg b.i.d. padsevonil in week 3. All patients continued on padsevonil in an 8-week open-label outpatient period. The primary efficacy outcome was the 75% responder rate (≥75% reduction in observable
focal seizure frequency) from 2-week baseline to 2-week inpatient period (last 2 weeks for padsevonil versus first 2 weeks on placebo). The first secondary efficacy outcome was the median reduction in seizure frequency. A total of 55 patients were randomized (padsevonil: 28; placebo: 27). Patients had a median duration of epilepsy of 24.2 years and a median baseline seizure frequency of 8.24 seizures per week, which is more than three times the frequency generally observed in AED clinical trial programs. Overall, 75% of patients had ≥8 prior AEDs (including those at baseline). The majority of patients were taking two or more concomitant AEDs (1 AED: 9.1%; 2 AEDs: 30.9%; 3 AEDs: 32.7%; ≥4 AEDs: 27.3%), most commonly levetiracetam (36.4%), lacosamide (30.9%), and oxcarbazepine (30.9%). During the 2-week inpatient maintenance, 8 of 26 patients (30.8%) on padsevonil versus 3 of 27 patients (11.1%) on placebo had a ≥75% reduction in focal seizure frequency from the baseline period (odds ratio: 4.14; p = 0.0679). The median percent reduction in weekly focal seizure frequency from baseline to the 2-week inpatient period was 53.7% with padsevonil versus 12.5% with placebo. The median difference was 34.0% (95% confidence interval: 3.0, 67.5; p = 0.026 [not adjusted for multiplicity]). Two patients on padsevonil and one on placebo were seizure-free during the inpatient period. Overall, 53 of 55 patients continued to the outpatient open-label padsevonil treatment. During the last four weeks of outpatient padsevonil treatment, 16 of 51 patients (31.4%) were ≥75% responders, demonstrating maintenance of seizure control during the outpatient period. The median percent reduction in weekly focal seizure was 55.2% (n=51). No patients were seizure-free for the entire trial period. Fifty patients completed the trial.

**Tolerability and adverse effect profile**

In two Phase I trials in healthy volunteers, padsevonil was generally well tolerated at doses up to 400 mg b.i.d. Transient, self-limiting CNS AEs (fatigue, somnolence, dizziness, disturbance in attention) occurred with increasing frequency at higher doses but attenuated with repeated doses. One serious AE of delirium occurred 2 days after the final padsevonil dose and was attributed to padsevonil. One healthy volunteer withdrew due to fatigue. At the highest doses (≥400 mg/day), transient reductions were seen in memory, alertness, psychomotor performance, and vigilance that lessened with repeated dosing.

In the proof-of-concept trial, the most common treatment emergent AEs (TEAEs) with padsevonil were CNS-related, consistent with its pharmacology. Fifty of 55 patients (90.9%) reported TEAEs, most commonly somnolence (25 [45.5%]), dizziness (24 [43.6%]), headache (14 [25.5%]), and fatigue (13 [23.6%]). Two patients (3.6%) had serious TEAEs (status epilepticus [n=1];
impaired judgment, delirium, dysphoria \([n=1]\), 18 patients (32.7\%) had TEAEs that required a dose change, and one patient (1.8\%) discontinued due to TEAEs (dysphoria and mood swings).\(^8\) There was no evidence for any consistent effect on laboratory parameters, vital signs, weight, and electrocardiogram evaluations.\(^8\)

**Planned studies**

The results of padsevonil initial investigation and the clinically meaningful data justify further clinical development. A long-term open-label extension to the proof-of-concept trial (EP0073, NCT02625090) and ongoing Phase II/III trials (EP0091, NCT03373383; EP0093, NCT03370120) are evaluating the efficacy and long-term safety of adjunctive padsevonil in patients with drug-resistant focal seizures.

**VALNOCTAMIDE AND SEC-BUTYLPROPYLACETAMIDE:SECOND GENERATION DRUGS TO VALPROIC ACID**

*Meir Bialer and †John H. McDonough

*Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel and †Nerve Agent Countermeasures, Medical Toxicology Division, US Army Medical Research Institute of Chemical Defense, Maryland, USA

Molecular structure of valnoctamide and sec-butylpropylacetamide here

**Introduction**

Valpromide is the corresponding amide of valproic acid, valnoctamide is a chiral constitutional isomer of valpromide, and sec-butylpropylacetamide is a one-carbon homologue of valnoctamide. In contrast to valpromide, which is a prodrug of valproic acid, valnoctamide and sec-butylpropylacetamide act as drugs on their own with minimal biotransformation to their corresponding acids. Racemic sec-butylpropylacetamide and racemic valnoctamide possess a unique broad-spectrum antiseizure profile in animal models, with \(ED_{50}\) values 3-20 times more potent than those of valproic acid.\(^22,59,84\) Sec-butylpropylacetamide and valnoctamide possess two stereogenic carbons in their chemical structure (denoted above with the asterisk *) and exhibit stereoselective pharmacokinetics in humans (valnoctamide) and rats and dogs (valnoctamide and sec-butylpropylacetamide).\(^22,59,85\)
Since the previous EILAT Progress Report, new results have been acquired with both valnoctamide and sec-butylpropylacetamide.

**Pharmacology**

*Anticonvulsant profile in animal models*

The activity of racemic-valnoctamide and racemic-sec-butylpropylacetamide has been assessed in a model of status epilepticus (SE) in which seizures were induced by tetramethylenedisulfotetramine (TETS), a highly lethal neurotoxic rodenticide that acts as a non-competitive GABA\(_A\) receptor antagonist. For comparison, valproic acid was also evaluated in the same model. Severe TETS intoxication in humans is often associated with refractory convulsive SE. Mice were pretreated with a single dose of riluzole (10 mg/kg, i.p.) and 10 min later received a lethal dose of TETS (0.2 mg/kg, i.p.). Riluzole does not inhibit TETS-induced SE but does protect against the rapidly lethal effects of TETS in mice, providing a model of persistent seizure activity. Behavioral seizure activity was assessed by visual observation and the latency to cessation of SE was recorded. Animals were monitored for 7 days after seizure termination. Latency to cessation of SE was defined as the interval between the first behavioral myoclonic twitch and termination of seizure activity. Sec-butylpropylacetamide, valnoctamide and valproic acid were administered ip at 40 min after the first myoclonic twitch. Sec-butylpropylacetamide at doses of 54 and 100 mg/kg terminated SE within ~4 and ~2 min, respectively, and protected 65% and 100% of animals from mortality for >7 days. Valnoctamide at doses 50 and 100 mg/kg terminated SE within, ~7 and ~2 min, respectively, and protected 62.5 and 90% of animals from mortality. Both sec-butylpropylacetamide and valnoctamide produced sedation in treated animals, which was especially pronounced at the dose of 100 mg/kg. Valproic acid (100 mg/kg) terminated TETS SE transiently in 80% of animals and only 20% animals survived. A high valproic acid dose (200 mg/kg) terminated SE within ~8.8 min and protected 80% of animals from mortality. Vehicle treatment failed to terminate TETS-SE, resulting in delayed mortality (within 2h) in 57-80% of the animals depending the percentage of the vehicle components (water, alcohol and propylene glycol) and its injected volume. In conclusion, both sec-butylpropylacetamide and valnoctamide effectively terminate TETS-induced behavioral SE and protect animals from mortality and are more potent and more rapidly acting than valproic acid.

In separate studies, sec-butylpropylacetamide and valnoctamide were tested, in comparison with phenobarbital, for their ability to antagonize organophosphate nerve agent-induced seizures in juvenile rats and to evaluate their potential to treat benzodiazepine-resistant-SE. The rationale for conducting these studies is that children are likely to be among the casualties in a civilian nerve agent exposure, and since this population is particularly susceptible to seizures when compared to...
adults, the activity of anticonvulsants used to treat nerve agent-induced seizures needs to be studied early in life. Although models of nerve agent-induced seizures to test antiseizure activity in adult animals have been around for years, one of the first models to conduct these tests during the first weeks postnatally was developed only recently (Ardinger et al., personal communication).

Female and male postnatal day (PND) 21 and 28 and PND 70 adult control rats were implanted with EEG headpieces and exposed to seizure-inducing doses of the organophosphate nerve agents sarin or VX. Five minutes after seizure onset, animals were treated by the i.p. route with sec-butylpropylacetamide, valnoctamide or phenobarbital, three drugs that enhance GABAergic signaling. The up-down method was used to determine the anticonvulsant ED₅₀ of each of the three drugs. In the up-down method an initial testing dose is chosen, usually based on previous work, and then a succession of doses in 0.20 – 0.25 log units above and below this starting dose are chosen at fixed steps between doses. The first animal is tested at the initial dose, and if this dose terminates the seizure, the next test animal is tested at the next lower dose, whereas if the initial test dose does not terminate the seizure the next test animal is tested at the next higher dose. The rule is: if the seizure is terminated - go down a dose for the next test animal; if the seizure is not terminated - go up a dose in the next test animal. Testing proceeded in this fashion until four reversals occurred. The results of these experiments are shown Table 3. In general, there were little differences between sexes within a given age group. The most notable differences were at PND 28, with males requiring higher doses of sec-butylpropylacetamide than females in both the VX and the sarin models. For sec-butylpropylacetamide, ED₅₀ values were relatively homogeneous across age groups in the sarin model, while higher doses were required at PND 28 and PND 70 males relative to PND 21 males in the VX model.

Table 3 here

Toxicology

Mitochondrial liver toxicity

Liver toxicity is an established adverse effect of valproic acid, which is particularly problematic for patients with mitochondrial disorders. The comparative mitochondrial toxicity of valproic acid, valnoctamide, sec-butylpropylacetamide and their corresponding acids (and valproic acid analogues) was determined by studying their effect on respiration rate of isolated rat brain and rat liver mitochondria. Specific effects on mitochondrial pyruvate transport were evaluated by swelling experiments. While the pyruvate and 2-oxoglutarate oxidation rates of rat brain mitochondria were
nearly unaffected by valproic acid, rat liver mitochondrial pyruvate and 2-oxoglutarate oxidation was severely impaired by valproic acid concentrations above 100 µM. Among the reactions involved in pyruvate oxidation, pyruvate transport and dehydrogenation steps were not affected by valproic acid, while α-lipoamide dehydrogenase was strongly inhibited (IC₅₀=80 µM). A similar inhibition of α-lipoamide dehydrogenase (IC₅₀=100 µM) was also caused by valproic acid’s one-carbon homolog sec-butylpropylacetic acid (sec-butylpropylacetamide’s corresponding acid) and to a lesser extent (IC₅₀=300 µM) by valproic acid’s constitutional isomer valnoctic acid (valnoctamide’s corresponding acid), while the corresponding amides of the above three acids (valpromide, sec-butylpropylacetamide and valnoctamide) showed only small effects (IC₅₀ >1 mM).

In conclusion, the active intrinsic inhibitors of pyruvate and 2-oxoglutarate oxidation are most likely to be the Coenzyme A conjugates of valproic acid and its acid analogues sec-butylpropylacetate and valnoctic acid, which affect selectively α-lipoamide dehydrogenase in liver. The valproic acid amide derivatives valnoctamide and sec-butylpropylacetamide showed low inhibitory effects on mitochondrial oxidative phosphorylation in liver, which might result in these compounds having reduced hepatotoxicity risk in patients with epilepsy associated with mitochondrial disorders.⁹³

**Efficacy and adverse effect profile of valnoctamide in patients with acute mania**

Women of child-bearing age with bipolar disorder face a dilemma when pregnant, as most mood stabilizers have teratogenic potential.⁹⁴,⁹⁵ Intense research has been aimed at designing non-teratogenic CNS-active valproic acid derivatives retaining antiepileptic and mood stabilizing properties.⁹⁶,⁹⁷ In a head-to-head comparison, valnoctamide unlike valproic acid, was found to be non-teratogenic in mice, rats and rabbits.²²,⁵⁹ These promising results led to the conduction of a double-blind randomized trial to assess the efficacy and safety of valnoctamide monotherapy in comparison to placebo and to risperidone, used as an active control, in the treatment of patients with an acute manic episode.

Unlike the preclinical candidate sec-butylpropylacetamide, valnoctamide was commercially available in Europe as an anxiolytic agent and underwent an earlier clinical trial in bipolar disorder, because this was the preferred indication of the funding institution (The Stanley Medical Research Institute).⁹⁸,⁹⁹ This three-week parallel group trial, for which preliminary results were reported in a previous ElLAT Progress Report, was conducted in 173 patients in an acute manic episode, who were randomized to receive valnoctamide 1500 mg/day given three times daily (t.i.d., n=71), risperidone 6 mg/day given t.i.d (n=32), or matching placebo (n=70). The primary outcome measure was the change in Young Mania Rating Scale (YMRS) score. The Clinical
Global Impression Scale for Bipolar Disorder (CGI-BP) was also utilized, as well as the Positive and Negative Syndrome Scale (PNASS) to evaluate psychosis. Samples for the determination of plasma valnoctamide concentrations were collected at the end-of-study visit. Valnoctamide did not differ significantly from placebo on any of the study endpoints. However, in the per-protocol analysis changes in total YMRS scores showed a trend of significance (p = 0.17) in favor of valnoctamide. Risperidone also failed to show superiority to placebo for YMRS score changes compared to baseline (p = 0.32). Mixed models for repeated measures showed that risperidone produced significantly more improvement than placebo in the overall CGI-BP (p = 0.036), and the severity scale for mania (p = 0.021). No significant correlation was observed between plasma valnoctamide levels and change in symptoms severity (r = 0.045, p = 0.790), however, interpretation of this finding should take into account the fact that valnoctamide levels were measured at various times after dosing. The Kaplan-Meier survival curve revealed higher discontinuation rates, mainly due to lack of efficacy, in the valnoctamide group compared to the other study groups (p = 0.026).

The overall frequency of AEs was 32.9% in the placebo group, 36.6% in the valnoctamide group, and 56.3% in the risperidone group. A Cox regression model analysis showed that the risperidone group discontinued treatment due to AEs sooner than in the placebo group (p = 0.01). The same was found for the valnoctamide group compared with the placebo group, but the difference just failed to reach statistical significance level (p = 0.052).

The study indicated that valnoctamide 1500 mg/day as monotherapy was well tolerated but lacked efficacy in patients with acute mania. These results differ from those reported by Bersudsky et al. who found that dual therapy with valnoctamide and risperidone was superior to risperidone monotherapy in more severely-ill patients (mean YMRS of 34, compared with 29 in the latest study).

These results may suggest that valnoctamide, at least in combination with risperidone, might be efficacious in more severely affected population, particularly because it is known that in general more severely ill patients show greater therapeutic response to antimanic drugs. The limitations of the latest study are further highlighted by the fact that in the same study risperidone itself failed to do better than placebo in improving the primary outcome measure, even though it should be acknowledged that the sample size for the risperidone group was smaller. Overall, further studies on the therapeutic potential of valnoctamide as an antimanic medication appear to be justified, particularly in the light of its good tolerability and its lack of teratogenic activity in animal models.
which is an incentive to assess valnoctamide for its potential efficacy in epilepsy as a second generation to valproic acid.96,97

CONCLUSIONS

Review of the data presented at the EILAT XIV Conference, summarized in this Report and in an accompanying article, indicates that major efforts in preclinical and clinical research are continuing in order to develop novel AEDs. The overarching aim is to discover treatments with superior efficacy and safety compared with existing agents, especially for the management of patients with difficult-to-treat epilepsies. These efforts build to a large extent on advances which have been made in understanding the mechanisms of epileptogenesis and ictogenesis, as well as the mechanisms of action of existing treatments. The methodological approaches used in the discovery and development of these compounds shows an increasing utilization of sophisticated preclinical models, including those designed to mimic pharmacoresistance, those that permit evaluation of potential antiepileptogenic effects, and those that replicate specific genetic defects found to be causative of epilepsy. In terms of clinical targets, an interesting observation is the trend to focus on orphan epilepsy syndromes, particularly severe epileptic encephalopathies with onset in early age.

In the case of compounds for which at least preliminary clinical efficacy data were presented, the rationale for development shows remarkable diversity. Some molecules (e.g. decanoic acid) are being developed based on initial evidence derived from dietary treatments, while for others, such as cannabidiol for Dravet syndrome and Lennox-Gastaut syndrome, or fenfluramine for Dravet syndrome, the incentive to conduct placebo-controlled randomized controlled trials originated largely from evidence generated by early uncontrolled observations. The rationale for developing valproic acid derivatives and padsevonil was to improve activity profiles through structure modification, which in the case of padsevonil also aimed at exploiting potential synergism between the properties of different drug classes. The rationale for developing ganaxolone relates to research on neurosteroids as modulators of neuronal excitability, while in the case of anakinra initial exploratory use in selected patients was triggered by research on the role of neuroinflammation in the pathogenesis of some epilepsies. Further studies with each of these agents are continuing, and it is likely that at least some of them will be approved for routine clinical use in the near future.

ACKNOWLEDGEMENTS

Edward Weselcouch, funded by Zogenix Inc, Inc provided professional medical writing assistance to preparation of the ZX008 section on fenfluramine hydrochloride (ZX008).
The authors of the padsevonil section thank the patients and their caregivers in addition to the investigators and their teams who contributed to these studies. They also acknowledge Barbara Pelgrims, PhD (UCB Pharma, Brussels, Belgium) for managing the development of the summary, and Michaela Fuchs, PhD (Evidence Scientific Solutions, Horsham, United Kingdom) for writing assistance, which was funded by UCB Pharma.

The authors of the valnoctamide and sec-butylpropylacetamide section acknowledge the support provided by an Inter-Agency Agreement between NIH/NINDS (Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2) to the pediatric anticonvulsant project. The views expressed in the section are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol of the study was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The contributions of Dr. Liana Matson, Dr. Stephanie Miller-Smith and Kari Haines are gratefully acknowledged.

DISCLOSURES ON CONFLICT-OF-INTEREST

Manuscript’s authors

Meir Bialer received in the last three years speaker’s or consultancy fees from Alkaloid, Arkin Holdings, Boehringer Inglheim, Bial, Teva, Shire, Upsher-Smith and Vidac Pharma. He received research support from the Israel Ministry of Defense and Rafa Pharmaceuticals. Svein I. Johannessen received consulting fees from GW Pharma. Matthias J. Koepp served on scientific advisory boards of Bial, GE Healthcare, Livanova, and Novartis, received honoraria from Bial, Eisai, GSK, Novartis and UCB Pharma, and funding support from the Epilepsy Research UK, FP7, Henry Smith Foundation, MRC, National Lottery Charity Board, NIHR, Waterloo Foundation and Wellcome Trust. Emilio Perucca received research funds from the European Union, the Italian Medicines Agency, the Italian Ministry of Health and the Italian Ministry for Education, University and Research, and speaker’s or consultancy fees from Eisai, GW Pharma, Livanova, Medichem, Mylan, Sandoz, Sanofi, Sun Pharma, Takeda, and UCB Pharma. Rene Levy received in the last three years consulting fees from Biocodex, Goodmans, and Xenon-Pharma. Torbjörn Tomson received speaker honoraria to his institution from Eisai, UCB, Sandoz, and Livanova honoraria to his institution for advisory boards from UCB and Eisai, and received research support from Stockholm County Council, CURE, GSK, UCB, Eisai, Bial and Novartis. H. Steve White reports
serving in the last 12 months as a consultant to Bial Pharmaceutics and Greenwich Biosciences and research advisor to Citizens United for Research in Epilepsy (CURE). Dr. White also reports receiving research funding from the US Department of Defense.

**Summaries’ authors**

Anakinra: Elaine Wirrell is an investigator for GW Pharma and Zogenix with related fees being paid to her Institution. She received personal remuneration from Sunovion and Biomarin for participation in advisory boards.

Cannabidiol: Drs. Knappertz and VanLandingham are employees of Greenwich Biosciences Inc. Drs. Jones, Gray, Whalley, and Patel are employees of GW Research Ltd. Medical writing support was provided to the authors by Keira Kim, an employee of Greenwich Biosciences Inc.

Cannabidivarin: Drs. VanLandingham and Knappertz are employees of Greenwich Biosciences Inc and Drs. Jones, Gray, Horne, Whalley, and Napoles are employees of GW Research Ltd Medical writing support for the cannabidivarin section was provided to the authors by Keira Kim, an employee of Greenwich Biosciences Inc.

Fenfluramine hydrochloride (ZX008): Gail Farfel is an employee of Zogenix, Inc. and reports stock ownership in the company.

Ganaxolone: Lorianne Masuoka, Julia Tsai and Jaakko Lappalainen are full-time employees of Marinus. Michael Saporito is a consultant to Marinus.

Medium chain fatty acids: Mathew Walker and Robin Williams have received research funding from Vitaflo Ltd, have received Consultancy and/or Speakers’ fees from UCB pharma, and hold a patent (WO 2012069790, WO 2013186570) related to this work.

Padsevonil: Konrad J. Werhahn, David Sciberras, Rafal Kaminski, and Pierandrea Muglia are employees of UCB Pharma.

Valnoctamide and sec-butylpropylacetamide: John H. McDonough has no conflict of interest to disclose. Meir Bialer has received in the last three years speakers or consultancy fees from Alkaloid, Arkin Holdings, Boehringer Inglheim, Teva, Shire, Upsher-Smith and Vidac Pharma. He received research support from the Israel Ministry of Defense and Rafa Pharmaceuticals.

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.
REFERENCES


