Progress report on new antiepileptic drugs: A summary of the Sixteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XVI).
I. Drugs in Preclinical and Early Clinical Development

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ABSTRACT

The Eilat Conferences have provided a forum for discussion of novel treatments of epilepsy among basic and clinical scientists, clinicians, and representatives from regulatory agencies as well as from the pharmaceutical industry for three decades. Initially with focus on pharmacological treatments, the Eilat Conferences now also include sessions dedicated to devices for treatment and monitoring. The Sixteenth Eilat Conference on Antiepileptic Drugs and Devices (EILAT XVI) was held in Madrid, Spain on May 22-25, 2022 attended by 157 delegates from 26 countries participants. As in previous Eilat Conferences, the core of EILAT XVI consisted of a sequence of sessions where compounds under development were presented and discussed. This progress report summarizes preclinical and, when available, phase 1 clinical data on five different investigational compounds in preclinical or early clinical development, namely GAO-3-02, GRT-X, NBI-921352 (formerly XEN901), OV329, and XEN496 (a pediatric granular formulation of retigabine/ezogabine). Overall, the data presented in this report illustrate novel strategies for developing antiseizure medications (ASMs), including an interest in novel molecular targets, and a trend to pursue potential new treatments for rare and previously neglected severe epilepsy syndromes.

Key words:

Antiseizure medications, antiepileptic drugs, drug development, epilepsy, GAO-3-02, GRT-X, NBI-921352, OV329, XEN496

KEY POINTS

- The Sixteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XVI) was held in Madrid, Spain, on May 22-25, 2022
- This progress report summarizes data on five investigational compounds in preclinical or early clinical development
- Preclinical and, when available, phase 1 data are presented for GAO-3-02, GRT-X, NBI-921352, OV329, and XEN496
- The data illustrate novel ASM development strategies, including an interest in new molecular targets and a trend to focus on new treatments for rare and severe epilepsy syndromes

1 INTRODUCTION

For three decades, the biannual Eilat Conferences have been a forum for discussion of novel treatments of epilepsy. Initiated in 1992 with a focus on new antiseizure medications (ASMs), the program of the Eilat Conferences expanded in 2016 to include sessions dedicated to devices for epilepsy treatment and monitoring. The Sixteenth Eilat Conference on Antiepileptic Drugs and Devices (EILAT XVI) was held in Madrid, Spain on May 22-25, 2022, and was attended by 157 participants from 26 countries, with approximately equal representation from industry and academia. A Satellite Symposium on current and novel treatments for Dravet syndrome, sponsored by the Spanish Dravet Syndrome Foundation, was hosted on the opening day. The program started with a special session on antiepileptogenic and disease-modifying therapies, whereas devices for seizure detection and interventions were discussed on May 25.

As in previous Eilat Conferences, the core of the EILAT XVI program consisted of sessions where compounds under development were presented and discussed. In total 12 compounds were presented in these sessions. This article provides summaries for five compounds that are in preclinical or early (phase 1) clinical development, namely GAO 3-02, GRT-X, NBI-921352 (formerly XEN901), OV329, and XEN496 (a novel pediatric granular formulation of retigabine/ezogabine). Summaries for the remaining seven treatments in more advanced stages of clinical development are presented in an accompanying article.[1] While all novel pharmacological treatments that were discussed during the EILAT XVI Conference are presented in these two progress reports, the articles do not claim to provide an exhaustive overview of all ASMs under development. As an indication of the broad range of potential treatments being currently in development, Table 1 provides a list of investigational agents presented at the EILAT XVI Conference and at the U.S. Epilepsy Foundation Pipeline Conference[2], which took place in Santa Clara, California, on June 4-5, 2022.

2 GAO-3-02

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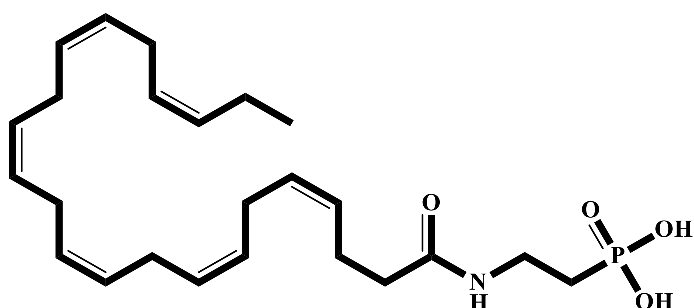

GAO-3-02

2.1 Introduction and rationale for development

GAO-3-02 is a biomimetic derivative of the endogenous compound N-docosahexanoylethanolamide (synaptamide). GAO-3-02 was initially designed using predictive biochemistry in order to maintain the pharmacological properties of synaptamide, known to target G-protein-coupled receptor 110 (GPR110) and cannabinoid type 2 (CB2) receptors[3,4], while changing some physico-chemical properties of the molecule to enhance solubility in biological fluids and stability against enzymatic hydrolysis.

2.2 Pharmacology

2.2.1 Activity profile in experimental models of seizures and epilepsy

Our previous work, described in the EILAT XV progress report[5], showed activity of GAO-3-02 on cognition and seizures. We demonstrated that treatment with GAO-3-02 at a dose of 2 mg/kg intraperitoneally (i.p.) from day 0 to day 7 following pilocarpine-induced status epilepticus (SE), prevented, in a sustained manner, the alteration of long-term potentiation during epileptogenesis, and spatial learning impairment after epilepsy onset. We also showed in a single population of fully amygdala-kindled rats that administration of GAO-3-02 at single doses of 5, 10 and 50 mg/kg i.p., given 3 to 5 days apart, resulted in a significant, but

delayed reduction in seizure severity, with the maximum effect observed at the 10 mg/kg dose. In the same rats, repeated administration over 3 weeks of increasing doses of GAO-3-02 (all rats were treated with 5 mg/kg i.p. the first week, 10 mg/kg i.p. the second week and 20 mg/kg i.p. the third week) did not further decrease seizure severity compared with single-dose administration. However, chronic administration did increase the proportion of seizure-free rats. Although not dose-related, the seizure freedom rate was 46% for the whole population of rats. Additionally, 31% of all animals were still free from seizures 56 days after treatment cessation. Overall, these preliminary results showed that GAO-3-02 exhibits beneficial effects on cognition following SE in rats, as well as on seizures in fully amygdala-kindled rats.

In early experiments in 5-week-old Swiss mice (20-25 g), GAO-3-02 (up to 20 mg/kg, i.p) was not effective against seizures induced by pentylenetetrazole (PTZ, 80 mg/kg s.c.) [5]. Since then, we investigated whether a higher dose could have antiseizure effects in the PTZ model. Specifically, we assessed the effects of acute (single injection) and subacute (one daily injection for 3 consecutive days) treatment with of GAO-3-02 (100 mg/kg i.p) on PTZ-induced seizure latency and severity, as well as mortality. In the acute treatment experiments, GAO-3-02 was administered 3 h before PTZ. In the subacute treatment protocol, the last injection of GAO-3-02 was administered 18-20 h prior to PTZ. In both sets of experiments, the results in the GAO-3-02-treated mice were compared with those of vehicle-injected mice (controls).

In the acute treatment experiments, GAO-3-02 (100 mg/kg i.p.) had no protective effect against PTZ-induced seizures or mortality. Specifically, generalized convulsive seizures induced by PTZ were observed in 71% of GAO-3-02 treated mice (n=14) versus 64% of vehicle-treated mice (n=14, $P > 0.99$, Fisher's exact test). Latency to first generalized convulsive seizure was $1{,}027 \pm 149$ sec in GAO-3-02-treated mice versus $1{,}010 \pm 180$ sec in control mice ($P = 0.94$, *t*-test). Mortality rate was 21% in GAO-3-02 treated mice versus 14% in controls ($P > 0.99$, Fisher's exact test).

In contrast, subacute treatment with the same dose of GAO-3-02 (100 mg/kg i.p. daily for 3 days) induced a clear-cut protective effect against PTZ-induced seizures. Specifically, the proportion of mice protected against generalized convulsive seizures increased from 8% in controls (n=13) to 50% in GAO-3-02 treated mice (n=12, $P = 0.03$, Fisher's exact test). Latency to first generalized convulsive seizure increased by 92%, from 608 ± 81 sec in controls to $1{,}170 \pm 191$ sec in GAO-3-02-treated mice ($P = 0.01$, *t*-test). Mortality rate did not

differ between treatment groups (0% in GAO-3-02 treated mice versus 15% in controls, $P = 0.48$, Fisher's exact test).

2.2.2 Mechanisms of action

Cannabinoid type 2 (CB2) receptors and G protein-coupled receptors 110 (GPR110) are known to be endogenous targets of synaptamide.[3,6] The fact that GAO-3-02 has structural similarity with synaptamide suggests that it may act via these receptors. Accordingly, we investigated as a first step the interaction of GAO-3-02 with the CB2 receptor, biophysically and functionally.

CB2 is a G-protein coupled receptor that couples primarily to Gi/o proteins to modulate an array of signaling pathways. Characterization of CB2 receptor activation focuses mainly on inhibition of adenylyl cyclase, extracellular signal–regulated kinase 1/2 (ERK1/2) activation, pathways involving arrestin, protein kinase B (Akt), ceramide, and ion channel modulation.[6] A wide range of *in vitro* and *in vivo* studies demonstrated that CB2 receptors are involved in the control of neuroinflammation[8,9] and neurogenesis[10] , both of which can be implicated in epileptogenesis, seizures and cognitive impairment.[11,12]

Using micro-scale thermophoresis, we found that dissociation constant (Kd) values for GAO-3-02 binding to human and murine CB2 receptors were in low nanomolar concentrations (5.64 nM and 1.26 nM, respectively). The orthosteric radiolabeled agonist WIN55212-2 was not displaced by GAO-3-02. However, *in vitro* functional activation of CB2 receptors by GAO-3-02 was observed in CB2 receptor overexpressing Chinese Hamster Ovary (CHO) cells in the presence and absence of bovine serum albumin, with half-maximal rescue concentrations (RC_{50}) of 283 nM and 32 nM respectively, suggesting allosteric binding.

Activation of GPR110 by synaptamide leads to neuritogenesis and synaptogenesis in developing neurons, and to attenuation of inflammation through increased cyclic AMP/protein kinase A (cAMP/PKA) signaling.[4,6] Having in mind that GAO-3-02 has been designed as a synaptamide biomimetic derivative, GPR110 is hypothesized to be functionally activated by GAO-3-02 too. We are currently developing assays (binding and functional) to confirm this hypothesis.

2.3 Toxicology

The long-term effects of GAO-3-02 on learning and memory, spontaneous locomotor, anxiety-like behavior and synaptic plasticity was investigated in mice using behavioral and electrophysiological tests. GAO-3-02 was first dissolved in tap water and given ad libitum for 4 months (from the 10th to the 13th month of age), corresponding to a dose of 50 mg/kg/day. From the 14th month, animals received further oral administration by gavage (50 mg/kg twice a week) until 18 months. Control animals were given vehicle solution (cremophor - ethanol – water, 1:1:18, v/v/v) instead of GAO-3-02.

At 15 months of age, learning and memory of mice were evaluated in the Morris water maze test. Escape latency decreased with increasing number of training days, as expected. No statistical differences were observed between GAO-3-02 treated mice and controls during training, indicating that GAO-3-02 did not negatively affect learning and memory. At 16 months of age, spontaneous locomotor activities and anxiety-like behavior of mice were evaluated using the open field and the elevated zero maze tests. In neither of those tests there were significant differences in total and 5-minute locomotor distances between GAO-3-02 treated mice and controls ($P > 0.05$). There were also no differences between GAO-3-02 treated mice and controls ($P > 0.05$) for time spent in the inner zone in open filed and the time spent in the open arms in the elevated zero maze, suggesting that long-term exposure to GAO-3-02 did not exacerbate anxiety-like behavior.

Induction of long-term potentiation, the cellular and molecular mechanism underlying memory formation, was assessed in the hippocampal CA1 region of vehicle and GAO-3-02 groups at months 15-18. We found that slices from mice pretreated with GAO-3-02 exhibited a significant induction of long-term potentiation of a magnitude similar to that observed in control slices ($174.26 \pm 10.3\%$ for GAO-3-02-treated mice versus $186.34 \pm 7.7\%$ for vehicle-treated control mice, $t = 45\text{-}50$ min, $P = 0.25$), indicating that chronic treatment with GAO-3-02 does not affect induction of hippocampal long-term potentiation.

Off target pharmacology assessment was performed by screening *in vitro* the functional activation of 47 targets (G-protein-coupled receptors, ion channels, kinases, nuclear receptors, transporters, and other non-kinase enzymes). GAO-3-02 was found to bind to CB2 and adrenergic beta-2 (ADRB2) receptors but not CB1 receptors. At the moment, there is no reason or data to suggest that GAO-3-02 binding to ADRB2 receptors is functionally linked to its mechanism of action; nor does binding to CB2 and ADRB2 receptors raise any safety concerns. Further, drug induced genotoxicity was examined by non-Good Laboratory Practice

(non-GLP) Ames and CHO micronucleus tests and did not show potential induction of mutagenesis/chromosomal aberrations.

2.4 Pharmacokinetics and metabolic profile

GAO-3-02 is still in preclinical development and no human pharmacokinetic data are available. The introduction of a polar head (i.e., ethanolamine phosphate) in its chemical structure was aimed at protecting the active molecule from gastrointestinal, liver and blood-blood-barrier hydrolysis. *In vitro* experiments confirmed that GAO-3-02 is not sensitive to hydrolysis by alkaline phosphatase and fatty acid amide hydrolase. Because of the role of fatty acid amide hydrolase in the catabolism of fatty acid amide derivatives, it is important to note that GAO-3-02 was shown not to be an inhibitor of this enzyme. *In vitro* experiments in human enterocytes demonstrated stability of GAO-3-02 over a 120 min time period, indicating the absence of metabolism in this system. The two pKa values of GAO-3-02 (pKa1: 2.43; pKa2: 7.43) indicate that the compound is water insoluble within the stomach, but highly water soluble within the intestinal tract. Therefore, absorption of GAO-3-02 is expected to occur from the intestine but not from the stomach. In fact, *in vitro* CaCo2 experiments effectively demonstrated the ability of intestinal cells to absorb GAO-3-02.

2.5 Drug interactions

Formal drug interaction studies with GAO-3-02 have not been conducted yet.

2.6 Efficacy and adverse effects

No clinical studies with GAO-3-02 have been conducted to date.

2.7 Planned studies

We plan to evaluate the antiseizure activity of GAO-3-02 activity in the 6-Hz model, the intra-hippocampal kainic acid mouse model of mesial temporal lobe epilepsy (MTLE), and in a rat model of absence epilepsy (the WAG/Rij rat). We also plan to continue exploration of GAO-3-02 action as an allosteric agonist/allosteric modulator of the CB2 receptor, and as a GPR110 ligand. Ongoing comparative *in vitro* and *in vivo* studies of distribution, metabolism

and clearance of GAO-3-02 performed in different rodent and non-rodent species, will enable the final choice of two species to be used in further toxicology studies, including the Investigational New Drug (IND)-enabling package.

2.8 Disclosures

All authors of this section contributed equally to the studies presented.

3 GRT-X

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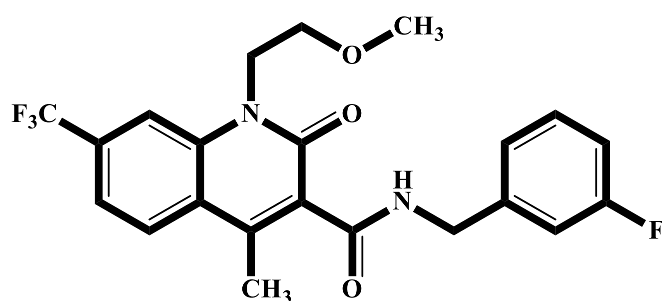

GRT-X

3.1 Introduction and rationale for development

GRT-X (N-[(3-fluorophenyl)-methyl]-1-(2-methoxyethyl)-4-methyl-2-oxo-(7-trifluoromethyl)-1H-quinoline-3-carboxylic acid amide) was synthesized during a search for novel analgesics that act as activators of voltage-gated Kv7 channels.[13] In a receptor and ion channel screen, the mitochondrial translocator protein 18 kDa (TSPO) receptor was identified as the only other target of GRT-X in addition to Kv7 potassium channels.[14,15] Activators of

Kv7 channels such as retigabine exert antiseizure and analgesic activities.[16] TSPO activators have been shown to have substantial efficacy in animal models of neurodegeneration, neurotrauma, pain, and anxiety.[17,18,19] Thus, a drug that activates both TSPO and Kv7 potassium channels would be highly interesting for the treatment and prevention of epilepsy, which is often associated with neurodegeneration, anxiety, and other psychiatric comorbidities.[20] This prompted us to compare the antiseizure efficacy of GRT-X with that of retigabine in a variety of rodent models of epileptic seizures.[14] Furthermore, the tolerability of the two compounds was compared in mice and rats.

3.2 Pharmacology

3.2.1 Mechanism of action

Whole-cell patch-clamp recordings in the voltage-clamp mode obtained from recombinant CHO-K1 cells demonstrated that GRT-X activated neuronal hKv7.2/3, hKv7.4, and hKv7.5 channels with a higher potency than retigabine. In an assay of displacement of the selective TSPO ligand [^{3}H]-PK11195, GRT-X bound to rat heart membranes with high affinity as indicated by a half-maximal inhibitory concentration (IC_{50}) of 0.07 µM, while retigabine was ineffective. *In vivo*, oral treatment with GRT-X (10 mg/kg) significantly increased brain levels of neurosteroids, including 3α,5α-tetrahydroprogesterone (3α,5α-THP, allopregnanolone) and 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC), implying that binding of GRT-X to TSPO stimulates the intramitochondrial transport of cholesterol and increases neurosteroidogenesis. Median percent increases in brain steroid levels by GRT-X were 627% (pregnenolone), 107% (progesterone), 116% (5-α-dihydroprogesterone), 132% (3α,5α-THP), 4911% (deoxycorticosterone), 1333% (5α-deoxycorticosterone), and 1040% (3α,5α-THDOC), respectively.

The selectivity of GRT-X was investigated in broad target profiling studies *in vitro,* which covered more than 140 targets of different pharmacological classes (e.g. ion channels, G protein-coupled receptors, transporters, kinases and other enzymes). Except for Kv7 potassium channels and TSPO, GRT-X exerted no relevant interactions with these targets.

3.2.2 Activity profile in experimental seizure models

GRT-X and retigabine were administered orally at numerous doses in six rodent models,

namely the maximal electroshock seizure (MES) test in rats, the electroconvulsive seizure (ECS) threshold test in rats, the subcutaneous (s.c.) PTZ seizure test in rats, the timed intravenous (i.v.) PTZ seizure threshold test in rats, the audiogenic seizure test in DBA/2 mice, and the 6-Hz seizure model in mice. Potency comparisons were based on both doses and peak plasma concentrations. Overall, GRT-X was more effective than retigabine in three of the six seizure models, the most important difference being the high efficacy in the 6-Hz (32 mA) seizure model in mice (Table 2). Based on drug plasma levels, GRT-X was at least 30 times more potent than retigabine in the latter model.

3.2.3 Other pharmacological properties

In a rat model of diabetic neuropathy, mechanical hyperalgesia was dose-dependently inhibited by GRT-X.[15] After severe crush lesion of cervical spinal nerves in rats, GRT-X promoted survival, speeded up regrowth of sensory and motoneurons, and accelerated recovery of behavioral and neuronal responses to heat, cold, mechanical and electrical stimuli. These properties may reduce the likelihood of acute pain becoming chronic, and even potentially relieve established chronic neuropathic pain. Interestingly, GRT-X was more effective in models of neuropathic pain than the selective Kv7.2/3 activator ICA27243 and the TSPO activator etifoxine.[1]5

In order to test the dual mode of action of GRT-X in diabetic rats, anti-hyperalgesic effects of GRT-X were compared when given alone or in combination with the selective Kv7 antagonist XE991 or with the selective alpha-reductase inhibitor finasteride to prevent increased synthesis of anti-hyperalgesic neurosteroids mediating the effects of TSPO activation[15]. Both pretreatments *partially* reduced the anti-hyperalgesic activity of GRT-X. Neither XE991 nor finasteride had their own effect on mechanical thresholds. When similar mechanistic experiments were repeated with the Kv7 activator retigabine, no such cross-target activity was observed. These results indicate that indeed functional engagement of both targets, Kv7 channels and TSPO, contribute to the pharmacological effects of GRT-X.

3.3 Toxicology

Exploratory safety pharmacology assays were performed in rats *in vivo* to evaluate the effect of GRT-X on the central nervous system (CNS).[15] Effects of GRT-X were examined in the rotarod test at doses of 3.0-100 mg/kg per os (p.o.), the open-field (10 and 30 mg/kg p.o.), and

the beam-walk test (3.0-30 mg/kg p.o.). No-Observed-Effect-Levels (NOEL) for GRT-X in the rotarod and open-field tests were 10 mg/kg, and equal to or above 30 mg/kg p.o. in the beam-walk test. In a satellite group of rats, GRT-X plasma concentrations after doses of 10-100 mg/kg (p.o., gavage) were assayed using selective liquid chromatography (LC)-mass spectrometry (MS)/MS. GRT-X showed an acceptable CNS safety profile with plasma exposures at the NOEL doses in rats at least twice as high as those determined after oral administration of evidently anti-hyperalgesic and neuroregenerative doses of GRT-X (effective dose 50 or ED_{50} = 0.53 mg/kg in the model of hyperalgesia after diabetic neuropathy, and effective dose of 5 mg/kg in the nerve lesion model, respectively). For comparison, ED_{50} values in the seizure models are reported in Table 2. A similar safety profile as in rats was also obtained in mice.[14] In a conditioned place preference paradigm in the rat, GRT-X lacked a rewarding effect, suggesting that this compound may be devoid of abuse potential.[15]

3.4 Pharmacokinetics and metabolic profile

Plasma protein binding of GRT-X is high with a fraction bound of 97-99% and 92-96% in humans and rats, respectively. The rat blood-to-plasma ratio (0.78) indicates that the erythrocyte concentration is 2-fold lower than the plasma concentration.

After i.v. injection of 1 mg/kg to rats, GRT-X mean terminal half-life was 2.3 h, and total plasma clearance was 11.6 mL/min/kg. The volume of distribution of 2.2 L/kg in rats suggests that GRT-X permeates into most tissues rapidly despite the relative high extent of binding to plasma proteins. The oral bioavailability of GRT-X in rats was 73%. Comparison of GRT-X distribution to plasma and brain after oral dosing of 10 mg/kg to male Sprague-Dawley rats demonstrated that the concentration-time course in rat brain mirrors that in plasma without any delay, and the brain-to-plasma AUC (area under the drug concentration- time curve) ratio was 4.6.

After oral administration of 2-10 mg/kg GRT-X to rodents, maximal concentrations (C_{max}) of GRT-X in plasma are reached within 5-30 min after dosing, indicating that the compound is very rapidly absorbed from the gut. This is in line with the very high transcellular permeability of GRT-X in both directions across Caco-2 cell monolayers. In MDR1-MDCK cells, a high apical to basolateral permeability was determined. The efflux ratios of GRT-X in the transport assay suggest that GRT-X is a very weak substrate for human P-glycoprotein, in

line with the high brain/plasma ratio. These findings mean that GRT-X is readily available within the CNS, and not evidently restricted by efflux transporters such as P-glycoprotein at the blood-brain barrier.[15]

No pharmacokinetic data are available for humans.

3.5 Drug interactions

Not known.

3.6 Efficacy data and adverse effects

No data are available in humans. Preclinical data have been reported above.

3.7 Planned studies

The further development of GRT-X is on hold.

4 NBI-921352 (XEN901)

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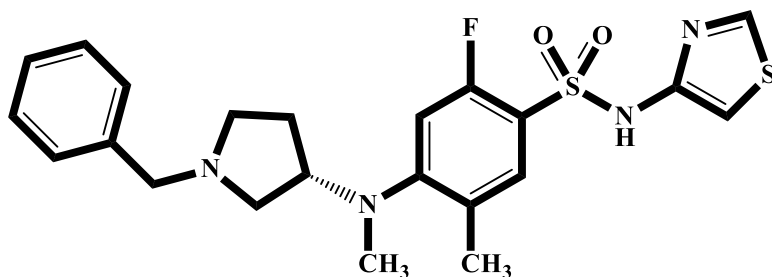

NBI-921352 (XEN901)

4.1 Introduction and rationale for development

NBI-921352 (formerly XEN901) is a novel, small-molecule, and selective Nav 1.6 sodium channel inhibitor being developed for the treatment of epilepsy, including *SCN8A* developmental and epileptic encephalopathy (*SCN8A*-DEE) in pediatric patients and focal seizures in adult patients. Preclinical and clinical data have been previously published in the EILAT XIV and XV progress reports.[5,21]

The *SCN8A* gene encodes the Nav 1.6 sodium channel, which is highly expressed in excitatory neurons in the CNS. Gain-of-function mutations in the *SCN8A* gene can result in *SCN8A*-DEE, a severe condition with onset usually occurring in infancy.[22] Seizures associated with *SCN8A*-DEE are typically extremely drug-resistant, although ASMs that act by blocking voltage-gated sodium channels have shown a certain degree of efficacy.[23] However, the sodium channel blocking ASMs currently in use do not selectively target sodium channel isoforms, potentially limiting their efficacy and safety. Blocking sodium channel isoforms primarily expressed in inhibitory interneurons (e.g., Nav 1.1) would be expected to have proconvulsant effects whereas targeting isoforms primarily expressed in excitatory neurons (e.g., Nav 1.2, Nav 1.6) is predicted to have antiseizure effects.[24,25,26] A drug that selectively targets Nav 1.6 would thus likely retain (or improve) antiseizure efficacy and have improved safety compared to non-specific sodium channel blocking ASMs. Additionally, a specific Nav 1.6 inhibitor could potentially address the specific pathophysiologic underpinnings of *SCN8A*-DEE.

4.2 Pharmacology

4.2.1 Activity profile in animal models of seizures and epilepsy

NBI-921352 has been shown to have antiseizure effects in the MES model in rats and mice, and in a modified 6-Hz assay in transgenic mice expressing a gain-of-function mutation of *SCN8A*.[5,21,27]

4.2.2 Mechanism of action

NBI-921352 has 130-fold selectivity for Nav 1.6 compared to other Nav isoforms (Nav 1.1 – Nav 1.7) and was selective for >50 targets in radioligand binding assays. The IC_{50} of NBI-921352 at the human Nav 1.6 channel is 51 nmol/L.[5,21,27]

4.3 Toxicology

NBI-921352 has been evaluated in a comprehensive nonclinical development program consisting of safety pharmacology, pharmacokinetic, repeat-dose, genotoxicity, and juvenile toxicity studies. The No-Observed-Adverse-Effect Level (NOAEL) for NBI-921352 administered p.o. in chronic GLP toxicology studies was 75 and 150 mg/kg/day in adult male and female rats, respectively (26-week study), and was 6 mg/kg/day in adult dogs (39-week study). The safety profile of NBI-921352 administered p.o. in juvenile dogs was comparable to that in adults, with no adverse findings observed at any of the doses tested in either dose-finding (dose range: 1 to 12 mg/kg/day) or 4-week GLP toxicology studies (dose range: 8 to 16 mg/kg/day). NBI-921352 also showed no genetic toxicity in a battery of GLP assays and no unexpected pharmacologic effects in the central nervous, respiratory, or cardiovascular systems that would impede further clinical development.[5,21]

4.4 Pharmacokinetics and metabolic profile

Oral NBI-921352 had a median time to peak plasma concentration (t_{max}) between 1 and 1.5 h for single doses ranging from 5 to 80 mg in a phase 1 study and a median t_{max} of 1 h for a single 100 mg dose in a second phase 1 study. For single ascending doses ranging from 5 to 80 mg, C_{max} and plasma AUC values generally increased proportionally to dose and

elimination half-life was between 7.5 and 10.6 h. For multiple ascending doses up to 75 mg/day once daily and up to 45 mg twice daily (b.i.d), steady-state was achieved after 2 to 4 days and b.i.d. dosing was shown to reduce variability in plasma NBI-921352 levels (i.e., peak-to-trough fluctuation index) compared to once-daily dosing. For the pediatric (i.e., granule) formulation of NBI-921352 (50 mg), the presence of food delayed t_{max} by approximately 2 h, reduced C_{max} by 40%, and had no effect on plasma AUC. Overall, the pediatric formulation was shown to be bioequivalent to the adult (i.e., immediate-release tablet) formulation.[5,21]

In vitro studies have shown that NBI-921352 is primarily metabolized by the cytochrome P450 (CYP) enzymes CYP3A4, CYP2D6, and CYP2C9. The full metabolic profile of NBI-921352 is currently under investigation.[5,21]

4.5 Drug interactions

In a phase 1 study, phenytoin at a dose of 100 mg three times daily (t.i.d.) for 10 days had no significant effect on overall NBI-921352 plasma exposure after a single 100 mg dose.[5,21]

4.6 Efficacy data

No efficacy studies have been completed in patients with epilepsy, although some data have been obtained from studies of healthy subjects. In a phase 1 study involving transcranial magnetic stimulation (TMS), 5 to 6 days of NBI-921352 administration (50 or 75 mg once daily) resulted in numerically larger active and resting motor thresholds, as well as a decreased amplitude of TMS-evoked potential at 180 ms, compared to placebo. These changes are consistent with the hypothesis that NBI-921352 may reduce cortical and corticospinal excitability.[5,21]

4.7 Adverse effects

NBI-921352 showed a favorable safety profile in two phase 1 studies with doses up to 100 mg/day once daily (or up to 45 mg b.i.d.) for up to 7 days. No serious or severe treatment emergent adverse events (TEAEs) or TEAE-related discontinuations were reported in either trial and TEAEs in all participants receiving NBI-921352 were generally mild.[5,21]

4.8 Planned studies

Recruitment is ongoing for a randomized, double-blind, placebo-controlled phase 2 trial (NCT04873869) to evaluate the efficacy, safety, tolerability, and pharmacokinetics of adjunctive NBI-921352 therapy in pediatric participants with *SCN8A*-DEE. A total of 52 male and female participants (aged 2 to 21 years) will be enrolled and randomized 1:1 to either NBI-921352 or placebo. Participants will be required to have a diagnosis of *SCN8A*-DEE as confirmed by a diagnosis confirmation panel, which involves both genetic (i.e., a pathogenic gain-of-function mutation in *SCN8A*) and clinical findings (i.e., seizure onset prior to 18 months of age and developmental delay). An initial sentinel cohort of 8 participants will be followed for safety and tolerability and evaluated for observed pharmacokinetics relative to predicted plasma exposures. After a review of the sentinel cohort data by an external and independent data monitoring committee, the remaining participants will be enrolled. Participants randomized to NBI-921352 will receive increasing doses based on weight for 6 weeks, followed by 10 weeks of treatment at their final tolerated dose. For those participants who do not enroll in a separate extension study, there will then be 2 weeks of treatment with decreasing doses and 4 weeks of treatment-free follow-up. Those randomized to placebo will receive matching pills to maintain the treatment blind (Figure 1). The primary efficacy endpoint will be percent change from baseline in 28-day seizure frequency for countable motor seizures (i.e., generalized tonic-clonic, tonic, atonic, or focal seizures with a noticeable motor component) during the treatment period. Secondary efficacy endpoints will be Clinical Global Impression of Change (CGIC), Clinical Global Impression of Severity (CGIS), Parent/Caregiver Global Impression of Change (GIC), and Parent/Caregiver Global Impression of Severity (GIS).

Recruitment is also ongoing for a randomized, double-blind, placebo-controlled phase 2 dose-finding study (NCT05159908) to evaluate the safety, tolerability, pharmacokinetics, and efficacy of adjunctive NBI-921352 treatment in adult participants with focal seizures. Approximately 100 male and female participants (aged 18 to 65 years) with focal seizures will be enrolled. Participants will be randomized to receive either NBI-921352 on one of three dosing schedules or placebo for 13 weeks (Figure 2). The primary endpoints will be the number of participants with serious TEAEs, number of participants with TEAE-related discontinuations, and the average plasma concentration of NBI-921352 (pre-dose to 8 h post-dose). Secondary endpoints will be percent change from baseline in monthly focal seizure

frequency during the treatment and maintenance periods, CGIC scores at week 11, and the percent of participants with ≥50% reduction in monthly focal seizure frequency during the treatment period.

5 OV329

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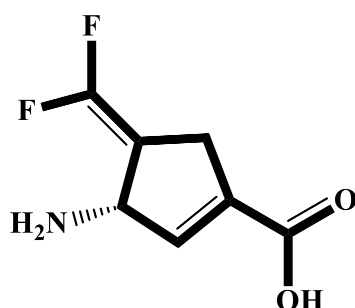

5.1 | Introduction and rationale for development

OV329 is a γ-aminobutyric acid (GABA)-aminotransferase (AT) inhibitor that is being developed for rare epilepsies, including seizures associated with tuberous sclerosis complex (TSC). Currently, two drugs (everolimus and cannabidiol) are approved for the treatment of adult and pediatric patients with seizures associated with TSC. In addition, vigabatrin is commonly used for the treatment of infantile spasms which are sometimes associated with TSC. However, over 60% of TSC patients continue to have refractory seizures[28] and the safety of vigabatrin, in particular ocular toxicity[29], remains a concern. OV329 has a similar mechanism of action to vigabatrin, which functions by reducing the activity of GABA-AT, thus increasing the levels of synaptic GABA, the primary inhibitory neurotransmitter in the brain.[30] OV329 has the potential to control seizures in people with drug-resistant epilepsy by increasing inhibition of abnormally hyperexcitable neurons. Non-clinical studies have suggested that OV329 is active in multiple seizure models, is more potent than vigabatrin at inactivating GABA-AT and may have the potential for an increased therapeutic index and safety margin against visual field deficits as compared to vigabatrin.

5.2 Pharmacology

5.2.1 Activity profile in animal models of seizure and epilepsy

OV329 is the most potent GABA-AT inhibitor known to date, being approximately 1220 times more efficient than vigabatrin for inactivation of GABA-AT.[30] OV329 has shown antiseizure effects in multiple distinct rodent seizure or epilepsy models. OV329 shows antiseizure activity in acute models such as the i.v PTZ seizure threshold test in rats[31] and the N-methyl-D-aspartate (NMDA) mouse model of infantile spasms.[32] In the i.v PTZ seizure threshold test model, a single dose of OV329 (5, 20 and 40 mg/kg i.p.) was given 6 h before PTZ infusion. OV329 at 40 mg/kg resulted in increased seizure threshold, with lower doses having no significant effects.[31] Models characterized by gradual epileptogenesis developing over weeks were also sensitive to the antiseizure effects of OV329. In these subacute models, including the amygdala-kindled rat model[31] and the intra-hippocampal kainite mouse model of MTLE[33], a single high dose of OV329 produced near complete protection from seizure activity. In the amygdala-kindled chronic model in rats, OV329 increased the seizure threshold after single i.p. injections of 30 and 40 mg/kg.[31] In the intra-hippocampal kainate mouse model of MTLE, OV329 was administered p.o. (0.01, 0.1, 1.0 or 10 mg/kg) or i.p. (10 mg/kg) as a single dose given 4 weeks following kainate injection and the development of epileptogenesis.[33] OV329 significantly suppressed the number of focal seizures in this model at 10 mg/kg with a trend toward improvement during the last hour of seizure assessments at the 1.0 mg/kg dose. As part of the Epilepsy Therapy Screening Program, OV329 was not found to be active in the MES or 6-Hz psychomotor seizure tests.

The findings summarized above provide support for the development of OV329 as a candidate for the treatment of drug-resistant seizures.

5.2.2 Mechanism(s) of action

OV329 is a selective and potent inactivator of GABA-AT, the enzyme responsible for the catabolism of GABA to succinic semialdehyde. Assessment of time-dependent reactivation of GABA-AT after treatment (in vitro) with OV329 indicates the inactivation includes both an irreversible and a reversible component.[30] The decrease in GABA-AT leads to increases in synaptic GABA levels, thus inhibiting neuronal hyperexcitability (Figure 3). The kinetics and magnitude of changes in GABA-AT and GABA following single and repeat doses of OV329 are currently being evaluated in vivo.

5.3 Toxicology

Due to ocular toxicity sensitivities associated with this class of therapeutics, safety assessment for changes in retinal function and structure were evaluated in Sprague-Dawley rats and Balb/c mice. Initial results indicate that OV329 is well tolerated up to 3 mg/kg/day for 45 days for all assessed ocular endpoints (fundoscopy, electroretinography, optical coherence tomography and histological assessments). In addition, OV329 was assessed in single-dose and repeat dose (10-day dosing) toxicity studies in Sprague-Dawley rats and Beagle dogs. In rats, OV329 doses up to 15 mg/kg in males and 10 mg/kg in females were well tolerated. In male and female dogs, OV329 was well tolerated with doses up to 1.0 mg/kg. OV329 was not genotoxic in the Ames assay. No CNS or cardiovascular safety liabilities were identified in an Irwin study in mice, or an *in vitro* human Ether-à-go-go-Related Gene (hERG) assay. OV329 is currently being evaluated in 28-day, repeat-dose GLP toxicology studies in rats and dogs to further define the spectrum of off-target effects and establish a NOAEL to enable first-in-human studies.

5.4 Pharmacokinetics and metabolic profile

Following oral administration in rats and dogs, t_{max} was 15-30 min, and the elimination half-life was approximately 75-90 min. Dogs had higher plasma exposure (AUC basis) than rats for equivalent oral doses. *In vitro* studies in hepatocytes showed that the clearance of OV329 was significantly greater in rats than dogs, with estimated intrinsic clearance (CL_{int}) values of 24.3 (rat), 13.1 (human), and 8.97 (dog) mL/min/kg. OV329 was minimally bound (4 to 20%) to proteins in rat, dog, and human plasma at 37°C.

5.5 Drug interactions

No clinical pharmacokinetic drug interaction studies have been conducted to date. *In vitro* studies indicate that OV329 is not a substrate or inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4. OV329 induces mRNA expression of CYP1A2 and CYP3A4 (approximately 5 and 3-fold, respectively; classified as "moderate inducer"). The enzyme activity of CYP2B6 was increased by approximately 4-fold with no increase in mRNA expression.

5.6 Efficacy data and adverse effects

No clinical studies with OV329 have been conducted to date. IND-enabling studies are in progress.

5.7 | Planned Studies

Phase 1 studies in healthy volunteers are anticipated.

6 XEN496

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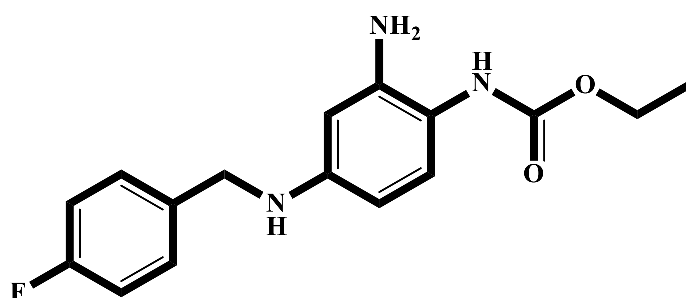

Retigabine (XEN496)

6.1 Introduction and rationale for development

XEN496 is a novel, granular ("sprinkle"), immediate-release pediatric formulation of retigabine (also known as ezogabine) being developed for the treatment of seizures associated with *KCNQ2* developmental and epileptic encephalopathy (*KCNQ2*-DEE). *KCNQ2*-DEE is a rare, severe neurodevelopmental disorder that typically presents in the first weeks of life with multiple, daily seizures caused by loss of Kv7.2-mediated potassium current. Retigabine, a Kv7 potassium channel modulator, was previously approved for the adjunctive treatment of focal and focal to bilateral tonic-clonic seizures in adults. Retigabine was withdrawn from the global market in 2017 for commercial reasons. Before its withdrawal, based on the mechanism of action, compounded or crushed retigabine tablets were used off-label in infants

and young children with *KCNQ2*-DEE, with reported improvements in seizure control and development.[34,35]

XEN496 was formulated specifically for administration to children, including newborns. It is packaged in sprinkle capsules of varying fill weights to allow for ease of body weight-based dosing without the need for extemporaneous compounding. XEN496 may be dispersed in breast milk, infant formula, or soft foods prior to dosing. In addition to having a neutral taste and mouth feel, the minimal non-specific binding of XEN496 makes it compatible with both feeding bottle plastics and pediatric nasogastric feeding tubes.[36]

6.2 Pharmacology

6.2.1 Activity profile in experimental models of seizures and epilepsy

Studies of retigabine using *KCNQ2* loss-of-function knock-in mouse models of kainic acid-induced seizures have been described previously in the EILAT XV progress report.[5] In these models retigabine was shown to significantly attenuate kainic acid-induced seizures, and to do so more markedly than phenobarbital.

6.2.2 Other pharmacological properties

Due to the expression of KCNQ channels in the urinary bladder and gall bladder, retigabine has been associated with urinary retention in rats and mice, but not in dogs or monkeys. The sensitivity of rodents to inhibition of micturition is due to their inability to exert control over bladder musculature. KCNQ channels are also expressed in the vascular (arterial) smooth muscle. Cardiovascular studies in dogs and pigs have suggested that retigabine administration could result in hypotension due to the inhibition of vascular smooth muscle contraction, indicating that retigabine has the potential to reduce blood pressure.

6.2.3 Mechanisms of action

The KCNQ (Kv7) family consists of 5 genes, *KCNQ1* through *5* that are highly expressed in the CNS, peripheral nervous system, heart, and smooth muscle. Kv7.2/7.3 heterotetramers are critical for maintaining neuronal M current. In *KCNQ2*-DEE, loss of function mutations in the Kv7.2/7.3 channels impairs M current and increases neuronal excitability. This can lead to

multiple, daily, treatment-resistant seizures which may present as early as the first week of life. *In vitro* studies have demonstrated the ability of retigabine to stabilize resting neuronal membrane potentials by activating Kv7.2/7.3 channels and restoring the M current.[37] Additionally, retigabine has been shown to modulate GABAergic neurotransmission *in vitro*.[37]

6.3 Toxicology

As summarized in the EILAT XV progress report[5], prior to its original approval, retigabine had been evaluated in a comprehensive battery of *in vitro* and *in vivo* genotoxicity and safety pharmacology studies, reproductive and developmental toxicology studies, and carcinogenicity studies in mice and rats, as well as juvenile toxicity studies in rats.

6.4 Pharmacokinetics and metabolic profile

The pharmacokinetic profile of retigabine has been thoroughly characterized prior to its initial approval, as summarized previously.[5]

In a rat pharmacokinetic study, XEN496 was compared head-to-head with crushed retigabine tablets dissolved in an aqueous carboxymethyl cellulose suspension selected to match the viscosity of infant formula, and to mimic the off-label use reported in pediatric patients. The study found the pharmacokinetic parameters of XEN496 to be equivalent to the crushed retigabine tablets.[36] The lack of an effect of food on the systemic exposure of retigabine after administration of the tablet formulation has previously been characterized.[38]

The pharmacokinetic profile of XEN496 in humans, including the effect of food, has been evaluated in 24 healthy adults in a phase 1, single center, open-label, randomized, single dose, 2-way crossover study, in which participants were administered the contents of one 400 mg XEN496 sachet (as a suspension in 120 mL water) in both the fed and fasted states.[39] Absorption of XEN496 was relatively rapid under both fed and fasted conditions with median t_{max} of 3 and 2 h, respectively. Thereafter, plasma concentrations declined in a mono-exponential manner, with a mean half-life of 7.2 h in the fed and 8.8 h in the fasted state.

In humans, retigabine is metabolized not through CYPs, but extensively through N-acetylation and subsequent N-glucuronidation. A similar disposition pattern and half-life were observed for the primary circulating N-acetyl metabolite of retigabine. Eating a

standardized high-fat, high-calorie meal 30 minutes before XEN496 administration slightly delayed t_{max} of both XEN496 and N-acetyl-retigabine by 1 h compared to the fasted state. While food had no effect on C_{max} or systemic plasma exposure of N-acetyl-retigabine, the fed state did slightly decrease XEN496 C_{max} but had no effect on systemic plasma exposure (Figure 4).

Because the tablet formulation of retigabine is no longer commercially available, results from this pharmacokinetic study with XEN496 were compared with historical data from a similarly designed single-dose, 2-way crossover study evaluating the effects of food on the pharmacokinetic profile of the 400 mg retigabine tablet.[40] While total systemic plasma exposure was equivalent in this comparison for both parent compound and primary metabolite under both fed and fasted conditions, the fed state decreased C_{max} of XEN496 by 32%, but increased C_{max} of retigabine by 38% following administration of the 400 mg tablet formulation. This observation may have implications for safety and tolerability of XEN496, as decreased C_{max} may be beneficial in terms of managing CNS side effects. Modest differences were observed in C_{max} for the two formulations under the fasted state but may not be clinically meaningful since pediatric patients typically are not dosed under fasted conditions.[39] The data also suggested that XEN496 may have a better dissolution profile, resulting in a more uniform absorption and a smoother pharmacokinetic profile compared to that of the adult tablet formulation.

6.5 Drug interactions

As described in detail in the EILAT XV progress report[5], retigabine showed little or no potential to inhibit or induce the major CYP enzymes. A population pharmacokinetic analysis using pooled data from phase 1-3 trials showed no significant impact of retigabine on the pharmacokinetics of other frequently used ASMs.[41] Conversely, carbamazepine and phenytoin have been shown to reduce retigabine plasma AUC by approximately one third.[5]

6.6 Efficacy data

At this time, studies evaluating the efficacy of XEN496 in the target patient population have not completed. Retigabine has been extensively studied prior to its initial approval as an adjunctive treatment for focal seizures in adults.[42,43]

6.7 Adverse effects

The initial approval of retigabine was based on a safety database comprising 29 completed phase 1 studies, five completed phase 2 studies, two completed phase 3 studies, and six long-term, open-label extension (OLE) studies investigating its use as an adjunctive treatment for adults with focal seizures, as well as a compassionate use program and two studies in other indications. As such, the adverse effects associated with retigabine have been extensively characterized. Across the combined phase 2/3 studies, TEAEs leading to discontinuation were reported by 437 of 1365 (32%) retigabine-treated patients.[43] The most common TEAEs leading to treatment withdrawal were dizziness (74/1365, 5%), somnolence (60/1365, 4%), confusional state (49/1365, 4%), fatigue (45/1365, 3%), coordination abnormal (27/1365, 2%), and disturbance of attention (26/1365, 2%). As described in the EILAT XV progress report[5], blue discoloration of the skin, lips, nails, and additional tissues has been observed in approximately 10% of patients in long-term clinical trials with retigabine. Long-term treatment has also been associated with retinal pigmentary abnormalities, butvision loss has not been reported.In the phase 1 study of the effect of food on pharmacokinetic parameters of XEN496 following a single 400 mg dose, the most common TEAEs were dizziness (52% under fasted conditions and 27% under fed conditions), oral hypoesthesia (26% under fasted conditions and 14% under fed conditions), and fatigue (22% under fasted conditions and 32% under fed conditions). Most TEAEs were considered mild in intensity and resolved by the end of the study, with slightly higher incidences under fasted conditions compared to fed conditions. No clinically significant physical or neurological examination findings were observed and mean clinical laboratory evaluations, vital signs, and electrocardiogram (ECG) values were generally within the reference range.

Only two subjects (8%) in the phase 1 study experienced TEAEs that were considered severe and possibly related to drug administration. One subject experienced syncope following a blood draw approximately 1 h after drug administration and another subject experienced depressed mood 3 days after receiving XEN496. Both severe TEAEs were experienced in the fasted condition.

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6.8 Planned studies

Enrollment is ongoing for a phase 3, multicenter, placebo-controlled, randomized trial to assess the efficacy of adjunctive XEN496 in infants and children with *KCNQ2*-DEE aged from 1 month to less than 6 years (NCT04639310). After randomization, patients receive either XEN496 or placebo over a 24-day dose titration period up to a top dose of 21 mg/kg/day, followed by a 12-week maintenance period. The primary efficacy endpoint is the percent change in monthly motor seizure frequency from baseline to the end of the double-blind 12-week treatment period. In addition to seizure outcomes, Global Impression of Change and Severity scores are being assessed, along with evaluation of quality of life and neurocognitive development and behavior, measured using the Bayley Scales of Infant and Toddler Development (BSID-III). Patients completing the phase 3 trial may be eligible to enroll in an OLE study (NCT04912856), during which long-term changes in seizures and other features of the complex disease phenotype will be further evaluated.

7 CONCLUSIONS

The five compounds in preclinical or early clinical development presented in this article demonstrate the current diversity in drug development strategies, in molecular drug targets, as well as in target patient populations. While GAO-3-02 is a biomimetic derivative of an endogeneous compound targeting G-protein-coupled receptors, GRT-X and XEN 496 act on specific potassium channels (with GRT-X also acting on TSPO), NBI-921352 is a selective inhibitor of the Nav 1.6 sodium channel, and OV329 is a GABA-AT inhibitor. Three of the compounds are developed for the treatment of specific rare epilepsies, including seizures associated with TSC (OV329), and two different DEEs, namely *SCN8A*-DEE (NBI-921352) and *KCNQ2*-DEE (XEN496). The range of targeted syndromes illustrates a current trend to develop novel pharmacological treatments which are based on improved understanding of the molecular mechanisms underlying some rare and severe epilepsies.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTION

The primary authors of this article planned and coordinated the preparation of this manuscript, and identified the compounds to be presented based on a review of the literature, congress reports, review of ClinicalTrial.gov website, and consultation of personal records. They also selected presenters and authors of the summary reports in consultation with the companies or institutions responsible for development of the identified compounds, reviewed and edited the summary reports, and compiled the abstract, introduction and conclusions. The sections summarizing data for each of the compounds presented were prepared by the authors of the summary reports, who approved the edited version of each section.

ETHICAL PUBLICATION STATEMENT

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.

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Table 1. List of agents currently in development for the treatment of seizures and epilepsy presented in the main program of the Sixteenth Eilat Conference on Antiepileptic Drugs and Devices (EILAT XVI, Madrid, Spain, May 22-25, 2022) and the 2022 Epilepsy Foundation Pipeline Conference (Santa Clara, California, June 4-5, 2022).

Compound	Putative mechanism of action	Development phase	Presented at EILAT XVI	Presented at Pipeline 2022
ABI-009	mTOR inhibitor	Clinical	No	Yes
BL-001	Microbiome-biased therapy mimicking the effects of the ketogenic diet	Preclinical	No	Yes
Bumetanide/bumetanide derivatives	Inhibitors of Na-K-2Cl-co-transporter (NKCC) type 2	Clinical/ preclinical	Yes	No
Coda chemogenetic receptor/activator drug	Chemogenetic activation of engineered chimeric ligand gated chloride channels	Preclinical	No	Yes
Darigabat	Subtype-selective $GABA_A$ receptor PAM	Clinical	Yes	Yes
ENX-101	Subtype-selective $GABA_A$ receptor PAM	Clinical	No	Yes
ETX-020155	Neurosteroid ($GABA_A$ receptor PAM)	Clinical	No	Yes
Ganaxolone	Neurosteroid ($GABA_A$ receptor PAM)	Clinical	Yes	Yes
GAO-3-02	Activator of CB2 and, possibly, GPR110 receptors	Preclinical	Yes	No
GRT-X	Activator of TSPO and Kv7 potassium channels	Preclinical	Yes	No
Lorcaserin	Selective $5\text{-}HT_{2C}$ receptor agonist	Clinical	Yes	No
LP352	$5\text{-}HT_{2C}$ receptor superagonist	Clinical	No	Yes
LPCN2101	Neurosteroid ($GABA_A$ receptor PAM)	Preclinical (?)	No	Yes
NBI-921352 (formerly XEN 901)	Selective Nav1.6 sodium channel blocker	Clinical	Yes	Yes
NRTX-1001 (formerly NTX101)	Regenerative GABA-releasing neural cell therapy	Preclinical	No*	Yes
OV329	GABA-aminotransferase inhibitor	Preclinical	Yes	Yes
PRAX-562	Persistent sodium current inhibitor	Clinical	No	Yes

Radiprodil	NR2B negative allosteric modulator	Clinical	No	Yes
Rozanolixisumab	Humanized anti-FcRn Imonoclonal antibody	Clinical	No	Yes
Soticlestat (TAK935)	Selective cholesterol 24-hydroxylase inhibitor	Clinical	Yes	No
STK-001	ASO acting as selective modulator of productive *SCN1A* mRNA levels	Clinical	Yes	Yes
XEN496	Activator of Kv7.2/7.3 potassium channels	Clinical	Yes	No
XEN 1101	Selective KCNQ2/3 (Kv7.2/7.3) potassium channel opener	Clinical	Yes	Yes

* Presented at EILAT XV (ref. 5).

Abbreviations: ASO, antisense oligonucleotide; CB2 receptor, cannabinoid type 2 receptor; FcRn, neonatal Fc receptor; GABA, γ-aminobutyric G; $GABA_A$ receptor, GABA type A receptor; GPR110, G-protein-coupled receptor 110 receptor; (GPR110) and cannabinoid type 2 (CB2) receptors 5-HT_{2C} receptor, serotonin type 2c receptor; mTOR, mammalian target of rapamycin; NR2B, N-methyl D-aspartate (NMDA) recept.or subtype 2B; PAM, positive allosteric modulator, TSPO, mitochondrial translocator protein

Table 2. Antiseizure effective dose 50 (ED_{50}) values of GRT-X and retigabine in rat and mouse seizure models. For details, see Bloms-Funke et al. [13] MES, maximal electroshock seizure test; PTZ, pentylenetetrazole seizure test, NE = not effective.

Seizure model	ED_{50} (mg/kg p.o.)		Potency of GRT-X vs. retigabine
	Retigabine	GRT-X	
MES, rat	3.9	3.7	~1
s.c. PTZ, clonic seizures, rat	23	~70	0.33
s.c. PTZ, tonic seizures, rat	~10	~37	~0.3
s.c. PTZ, mortality, rat	<10	<10	~1
DBA/2 mice, wild running	NE (>10 mg/kg)	4.8	>2
DBA/2 mice, clonic seizures	NE (>10 mg/kg)	3.5	>2.9
DBA/2 mice, tonic seizures	~2	<1	>2
DBA/2 mice, mortality	~2	<1	>2
6-Hz (32 mA), focal seizures, mouse	NE (>100 mg/kg)	13	>7.7

Figure Legends

Figure 1. Study design of a double-blind, placebo (PBO)-controlled phase 2 trial to evaluate the efficacy, safety, tolerability, and pharmacokinetics of NBI-921352 as adjunctive therapy in participants with SCN8A-DEE.

Figure 2. Study design of a double-blind, placebo (PBO)-controlled phase 2 dose-finding study to evaluate the efficacy, safety, tolerability, and pharmacokinetics of NBI-921352 as adjunctive therapy in adult participants with focal seizures.

Figure 3. Schematic representation of the mechanism of action of OV329.

Figure 4. Plasma XEN496 and N-acetyl-retigabine (NAMR) concentration versus time curves under fed and fasted conditions (data represent means ± SD of 21 subjects).