Progress report on new antiepileptic drugs: A summary of the Sixteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XVI). II. Drugs in more advanced clinical development

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ABSTRACT

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 delegates from 26 countries representing basic and clinical science, regulatory agencies as well as pharmaceutical industries. One day of the Conference was dedicated to sessions presenting and discussing investigational compounds under development for the treatment of seizures and epilepsy. The current progress report summarizes recent findings and current knowledge for seven of these compounds in more advanced clinical development for which both novel preclinical or patient data are available. These compounds include bumetanide and its derivatives, darigabat, ganaxolone, lorcaserin, soticlestat, STK-001, and XEN1101. Of these, ganaxolone has been approved by the U.S. Food and Drug Administration (FDA) in March 2022 for the treatment of seizures associated with cyclin-dependent kinase-like 5 deficiency disorder in patients 2 years of age and older.

Key words:

Antiepileptic drugs, antiseizure medications, drug development, epilepsy

KEY POINTS

- This progress report summarizes preclinical and clinical data on seven different investigational compounds
- The compounds discussed include bumetanide and its derivatives, darigabat, ganaxolone, lorcaserin, soticlestat, STK-001, and XEN1101
- These summaries illustrate differences in developmental strategies, from repurposing to specific drug design, as well as diversity in indication targets
- Ganaxolone was recently approved by the U.S. Food and Drug Administration (FDA) for the treatment of seizures associated with cyclin-dependent kinase-like 5 deficiency disorder in patients aged 2 years and older.

1 INTRODUCTION

The Eilat Conferences have been a forum for discussion of novel treatments for epilepsy since 1992. 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. One day of the Conference was dedicated to the presentation and discussion of investigational compounds under development. Potential suitable compounds for these sessions were identified by members of the Eilat Organizing Committee reviewing all information available to them.

Of the 12 novel potential antiseizure medications (ASMs) presented during the EILAT XVI sessions on New Drugs in Development, five were compounds in preclinical or early clinical (phase 1) development and are presented in an accompanying article.[1] The current article provides summaries for the seven investigational drugs which are in more advanced clinical development. These include bumetanide and its derivatives, darigabat, ganaxolone, lorcaserin, soticlestat, STK-001, and XEN1101.

2 BUMETANIDE AND ITS DERIVATIVES

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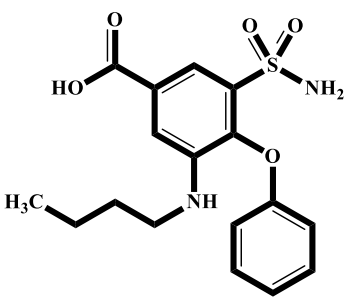
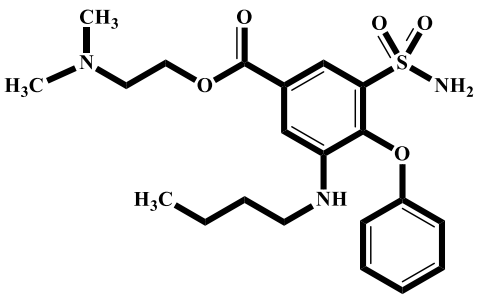
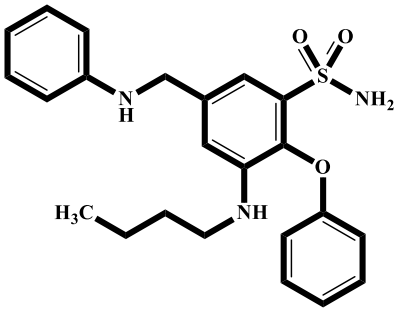


Bumetanide



DIMAEB (BUM5)



Bumepamine (BUM13)

2.1 Introduction and rationale for development

Bumetanide (3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid), is a potent ("high ceiling"), fast-acting loop diuretic that is widely used for the treatment of edema associated

with congestive heart failure, hepatic, pulmonary, and renal diseases, both in adults and children including term and preterm infants. [2,3] The diuretic effect of bumetanide is due to inhibition of sodium reabsorption in the ascending limb of the loop of Henle by blocking the Na-K-2Cl-cotransporter (NKCC)-2. Bumetanide also blocks NKCC1, which plays a major role in regulating intracellular Cl^- concentration in many tissues, including the brain.[4] Alterations in cerebral cellular chloride homeostasis, in which NKCC1 is involved, can play a role in the pathogenesis of a number of brain disorders, such as neonatal seizures, epilepsy, autism spectrum disorder (ASD), and ischemic and traumatic brain injury.[5-8] This has generated interest in using bumetanide for the experimental or off-label treatment of such disorders. However, the value of bumetanide for the prevention or treatment of brain disorders is limited by its poor penetration across the blood brain barrrier.[9,10] As a result, clinically approved oral or parenteral doses of bumetanide yield brain concentrations far below those required to inhibit NKCC1 in the brain.[4]

In the EILAT XIII progress report[11], we described several strategies explored by our group to improve the effectiveness of bumetanide and its derivatives for the treatment of central nervous system (CNS) disorders. Here, we will shortly summarize novel studies that were published since then. We will concentrate on bumetanide and two derivatives that we developed: the bumetanide prodrug DIMAEB (the N,N-dimethylaminoethylester of bumetanide or "BUM5") and the bumetanide side-chain derivative bumepamine (3-butylamino-2-phenoxy-5-phenylaminomethyl-benzenesulfonamide or "BUM13"). Furthermore, we performed a series of studies with other clinically approved loop diuretics to identify compounds that potently block NKCC1 and penetrate the brain more effectively than bumetanide.

2.2 Pharmacology

2.2.1 Activity profile in animal models of seizures and epilepsy

The effects of bumetanide in animal models of seizures and epilepsy as well as in preclinical models of other brain disorders have been reviewed recently.[4,8,9,12-14] In most seizure models, bumetanide is ineffective when administered alone but may increase the antiseizure potency of established ASMs such as phenobarbital. In our hands, however, the ability of bumetanide to increase the potency of phenobarbital in adult seizure models was not robust; in most seizure or epilepsy models that we used, no significant effects were observed.[4] This was

different with the bumetanide prodrug DIMAEB and the side-chain derivative bumepamine, which both markedly increased the effect of phenobarbital in seizure models in adult rodents.[15-17] However, unexpectedly, in contrast to bumetanide, bumepamine did not directly inhibit NKCC1.[18]

More recently, we used a novel rat model of birth asphyxia-induced neonatal seizures that was developed by Kai Kaila's group in Helsinki.[19] In this model, both low (0.3 mg/kg) and high (10 mg/kg) doses of bumetanide did not exert any significant effect when administered alone or in combination with phenobarbital.[20,21] In contrast, both DIMAEB and bumepamine significantly increased the antiseizure effect of phenobarbital.[21] In an *in vitro* study using serum and brain homogenates from neonatal rats, we demonstrated that DIMAEB is rapidly cleaved to bumetanide[22], as previously demonstrated for serum and brain of adult rodents.[15] We also showed that neonatal serum of healthy term infants is capable of cleaving DIMAEB to bumetanide[22], indicating that esterase activity is already high enough at 1-2 days after birth, which would be a prerequisite for using this bumetanide prodrug in neonates.

In another series of experiments, we investigated various other loop diuretics previously not known to inhibit NKCC1. We found that (i) azosemide is a more potent inhibitor of human NKCC1 than bumetanide and various other loop diuretics[23]; (ii) both azosemide and its close analog torasemide increase the antiseizure potency of phenobarbital in a mouse model[24]; but (iii) neither azosemide nor torasemide reaches higher relative brain levels than bumetanide.[25] The latter finding is due to the fact that all three loop diuretics are actively transported out of the brain, which reduces their brain-to-plasma ratio.[10,25]

The groups of Thomas Erker in Vienna, Austria, and Laura Cancedda in Genoa, Italy, reported several other potentially interesting bumetanide derivatives[14,26], but it remains to be established whether these compounds exhibit advantages versus bumetanide in the treatment of brain disorders. Additional NKCC1 inhibitors that are not structurally related to bumetanide and resulted from high-throughput screening were reported by Roche[27] and Roy et al.[28], but experimental data on these compounds are not yet available.

2.2.2 Other pharmacological properties

Beneficial as well as detrimental effects of bumetanide have been reported for a large variety of preclinical models of brain disorders.[4,8,13,14] Often, these effects occurred at low systemic bumetanide doses that do not lead to NKCC1-inhibitory brain concentrations, suggesting that

the effects were either related to NKCC1-expressing cellular targets of these drugs outside the brain parenchyma or to molecular off-target effects.[4] Only a few preclinical studies tested bumetanide derivatives in animal models of brain disorders other than seizures or epilepsy.

2.2.3 Mechanism(s) of action

Bumetanide and its derivatives are often used for inhibition of NKCC1. However, all compounds that have been used for this goal also inhibit NKCC2 at similar concentrations. It was long thought that NKCC2 is solely expressed in the kidney, but more recent studies have shown that it is expressed in several other tissues, including the gastrointestinal tract, pancreas, and the rat and human inner ear, most likely acting together with NKCC1 in the regulation of endolymph volume. [4] In addition to NKCC1 and NKCC2, numerous "off-target" effects of bumetanide have been reported that may add to the pharmacology of this drug. [4] Such off-target effects, which, at least in part, may be also mediated by bumetanide metabolites, include inhibition of carbonic anhydrases, modulation of multiple transporters involved in uptake or efflux of bumetanide, and modulation of GPR35 (a G protein-coupled receptor that is expressed in numerous cell types within and outside the CNS) and the alpha3-subunit of the γ-aminobutyric acid type A ($GABA_A$) receptor. [4] Overall, these data suggest that the pharmacology of NKCC inhibitors such as bumetanide may be much more complex than previously thought.

2.3 Toxicology

Bumetanide is a potent diuretic, which, if given in excessive amounts, can lead to a profound diuresis with water and electrolyte depletion, and development of hypokalemia. Similar to other loop diuretics such as furosemide, bumetanide has been shown to produce ototoxicity in various animal species. Ototoxicity has been also reported after high doses in humans, including infants. Furthermore, bumetanide may potentiate the ototoxic effects of aminoglycosides, resulting in temporary or permanent hearing loss. In two recent clinical trials on the effect of bumetanide on neonatal seizures, 12.5% of the neonates developed hearing loss upon treatment with bumetanide.[29,30] The mechanisms of bumetanide's ototoxic effect have been described in detail in the EILAT XIII progress report.[11]

When testing the activity of the bumetanide derivatives described above, we focused primarily on diuretic potency and the resulting hypokalemia as undesired effects. It is our aim

to identify derivatives with greater brain penetration and lower diuretic potency compared to bumetanide. Both DIMAEB and bumepamine have significantly lower diuretic activity than the parent compound.[15,17]

2.4 Pharmacokinetic and metabolic profile

Bumetanide has a well-characterized pharmacokinetic profile.[11] Its half-life is 0.8–1.5 h in adults, and it is longer in infants (about 6 h, with a range of up to 15 h). The apparent volume of distribution is only 0.12-0.15 l/kg, reflecting its poor tissue distribution. Bumetanide is rapidly metabolized in different species including humans by oxidation of the N-butyl side chain. About 60% of a bumetanide dose is eliminated unchanged in humans and dogs, while rats excrete less than 10%o the dose as unchanged drug. Six urinary metabolites have been identified, including N-desbutyl-bumetanide and the δ-, γ-, and β-hydroxybutyl-metabolites. These metabolites are devoid of significant diuretic activity. However, all of the identified metabolites of bumetanide retain the sulfamoyl moiety, which may mediate effects on targets other than NKCCs, e,g. inhibition of carbonic anhydrase.[4]

The pharmacokinetics of the bumetanide derivatives described above have been assessed only in rodents.[15,17,25]

2.5 Drug interactions

Except for life-threatening conditions where no alternative treatments are available, parenteral administration of bumetanide in patients to whom aminoglycoside antibiotics are also being given should be avoided (see section 2.3), particularly in the presence of impaired renal function. Probenecid should not be administered concurrently with bumetanide, because probenecid reduces the diuretic effect of bumetanide most likely by inhibiting its renal tubular secretion. Bumetanide may potentiate the effect of various antihypertensive agents, necessitating a reduction in the dosage of these drugs. Indomethacin blunts the increases in urine volume and sodium excretion seen during bumetanide treatment, so concurrent therapy is not recommended.

Bumetanide reduces the total (renal) clearance of lithium, leading to a high risk of lithium toxicity.

2.6 Efficacy data

There is little evidence for bumetanide being effective as an antiseizure agent in adults with epilepsy, apart from a couple of studies.[31,32] In contrast, one phase 1/2 trial and one phase 2 trial of bumetanide as an adjunct to phenobarbital in newborns with neonatal seizures was recently published.[29,30] While an Open Label Exploratory Dose Finding and Pharmacokinetic Clinical Trial of Bumetanide for the Treatment of Neonatal Seizure Using Medication Off-patent (NEMO) reported by Pressler et al.[29] was terminated early due to ototoxicity and lack of efficacy, the trial by Soul et al.[30] reported a significantly greater reduction in seizure burden, as assessed by continuous electroencephalogram (EEG) monitoring, with the combination of phenobarbital plus bumetanide (0.1, 0.2 or 0.3 mg/kg) versus phenobarbital alone. However, in the latter trial, drug efficacy was only analyzed as an exploratory endpoint, and there was no predefined primary efficacy endpoint in the study design. Soul et al.[30] concluded that definitive proof of bumetanide's efficacy awaits an appropriately powered phase 3 trial, which we would emphatically advise against because of the many reasons, including bumetanide's ototoxic potential.[4,33]

With respect to bumetanide's potential for the treatment of brain disorders, the experience acquired from a series of clinical trials in patients with ASD is of particular interest.[34-36] While data from initial pilot and phase 2 trials looked promising, two large phase 3 trials assessing bumetanide in the treatment of several hundred children and adolescents with moderate-to-severe ASD showed no effectiveness, leading to early termination of the trials.[37] Similarly to trials on neonatal seizures, the clinical studies on bumetanide in autism were based on the false assumption that systemically administered bumetanide exerts specific and effective actions on GABAergic signaling *in vivo*.[4] However, these negative findings were predictable based on what is known about bumetanide's extremely poor properties as a CNS drug.

None of the bumetanide derivatives described here has been tested in humans.

2.7 Adverse effects

In addition to ototoxicity, the adverse effects of bumetanide are linked to its potent diuretic action and include fluid loss, hypotension, tachycardia, and electrolyte disturbances, including hypochloraemia, hypokalaemia, hyponatremia, hypophosphatemia, and hypocalcemia as well as hyperglycemia.[2]

2.8 Future perspectives

Since we started our experiments on bumetanide and its derivatives about 15 years ago, the original idea of targeting NKCC1 in the brain to specifically reduce the intracellular chloride concentration in damaged principal neurons with depolarizing or excitatory $GABA_A$ receptor responses[38] turned out to be unworkable.[4] In addition to the insurmountable pharmacokinetic problems with bumetanide and most of its derivatives, there has been a steep increase in our knowledge of the expression patterns and functions of the ubiquitous NKCC1 transporter. We now know that the neuronal expression of NKCC1 in the brain is a very small fraction, several orders of magnitude lower than the overall expression of this transporter in the brain.[4] Most of the NKCC1 expression is found on different types of glial cells and at the choroid plexus. Consequently, the effects of global inhibition of NKCC1 in the brain under various conditions cannot be attributed to changes in neuronal functions. Even if a brain permeable NKCC1 blocker were available, it would act on NKCC1 in all kinds of cells, including neurons and glia. This makes the effects of NKCC1 blockers highly unpredictable, leading to potentially detrimental and/or beneficial actions depending for instance on age- and disease-related alterations in the cellular NKCC1 expression patterns and functionality. Thus, the basic concept regarding the antiseizure actions of bumetanide is long outdated.[4] We do not question that bumetanide and other NKCC1 inhibitors may exert preclinical effects in various brain disorder models, even when administered at low and clinically relevant doses, but these effects may be related to NKCC1-expressing cellular targets of these drugs outside the brain parenchyma (e.g., blood-brain barrier, choroid plexus, endocrine, and immune system), as well as molecular off-target effects.[4] Even if a next-generation of brain permeable NKCC1-selective inhibitors would be designed, which may be facilitated by ongoing and future work on molecular modeling on high-resolution cryo-electron microscopy-based structures of NKCC1 in various species[4,39], in our opinion it will not be possible to develop compounds that only act on neuronal NKCC1. Thus, although each drug discovery effort potentially contributes to our understanding of the biology of NKCC1, the complexity of this target is probably too high to allow any meaningful development of novel compounds (whether brain-blood barrier permeant or not) for the clinical treatment of brain diseases.

2.9 Acknowledgments

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3 DARIGABAT (CVL-865)

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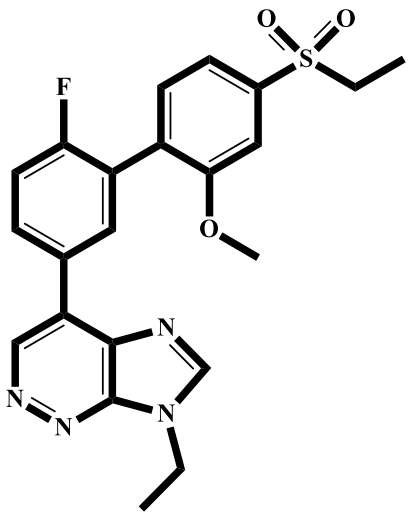

Darigabat (CVL-865)

3.1 Introduction and rationale for development

For more than two decades, the search has been underway for compounds that modulate the benzodiazepine binding site of $GABA_A$ receptors but with a much-reduced liability to adverse effects such as sedation, loss of efficacy, addiction, dependence, and withdrawal symptoms. The elucidation that benzodiazepines exert their actions as positive allosteric modulators (PAMs) of different subtypes of $GABA_A$ receptors together with the advancement of molecular cloning techniques evidenced that specific pharmacological actions of benzodiazepines are attributed to specific $GABA_A$ receptor subtypes.[40,41] This demarcation of

the subtypes has led to a rigorous effort to develop subtype-selective $GABA_A$ receptor PAMs for the chronic treatment of epilepsy and other CNS disorders.

Darigabat, formerly known as CVL-865, was rationally designed to selectively enhance the inhibitory effect of GABA at α2/3/5 subunit-containing $GABA_A$ receptors to suppress aberrant overexcitation that underlies epileptic activity. Because darigabat has minimal interaction with the α1 subunit, which mediates the induction of sedative effects, it is expected to achieve high receptor occupancy without inducing potentially dose-limiting sedation. The nonproprietary name for this compound, darigabat, which contains the stem *-gabat,* acknowledges its novel mechanism of action which is distinct from both the benzodiazepine and neurosteroid classes of drugs.

3.2 Pharmacology

3.2.1 Activity profile in experimental models of seizures and epilepsy

The robust activity of darigabat in widely used and translationally relevant preclinical models of epilepsy such as the amygdala kindling model, the pentylenetetrazole (PTZ) model, and the genetic absence epilepsy rat from Strasbourg (GAERS) model have been reported in the EILAT XV progress report.[42] The data from those models suggest that darigabat may have broad-spectrum efficacy across different seizure types.

More recently, darigabat has been profiled in the mouse model of mesial temporal lobe epilepsy (MTLE), a model which displays differential sensitivity to ASMs and has a utility in the identification of new treatments for drug-resistant forms of focal epilepsy.[43] The MTLE mouse model is generated by unilateral intrahippocampal injection of a single low dose (1 nmole) of kainic acid in adult mice, and subsequent epileptic activity is recorded following implantation of a bipolar electrode under general anaesthesia. After a period of epileptogenesis (approximately 4 weeks), spontaneous and recurrent hippocampal paroxysmal discharges (focal seizures) can be recorded using intracerebral electroencephalography. The number and cumulated duration of hippocampal paroxysmal discharges were recorded following administration of vehicle per os (p.o.), darigabat (0.3-10 mg/kg, p.o.), and the positive control diazepam (2 mg/kg, intra-peritoneally, i.p.). Darigabat dose-dependently reduced the expression of hippocampal paroxysmal discharges, demonstrating comparable efficacy to diazepam at doses of 3 and 10 mg/kg. The data demonstrate that selective

enhancement of the inhibitory effect of GABA via darigabat suppresses the aberrant overexcitation that underlies epileptic activity in a model of treatment-resistant focal seizures.

3.2.2 Mechanism of action

Neuronal signaling via the $GABA_A$ receptor plays a critical role in a wide range of processes within the CNS. $GABA_A$ receptors are heteropentamers assembled from the 19 members (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3) of the $GABA_A$ family, with the most abundant subtypes comprising α, β, and γ subunits in a 2:2:1 stoichiometry.[40] Receptor activation results in an increased membrane chloride conductance, which typically causes an influx of chloride ions and results in membrane hyperpolarisation which dampens neuronal excitability (Figure 1).

All the overt effects of benzodiazepines (sedative, anxiolytic, anticonvulsant, muscle relaxant, addictive, and amnestic effects) are mediated by $GABA_A$ receptors. These broad pharmacological effects result in wide ranging clinical utility of benzodiazepines, including induction of presurgical sedation, myorelaxation, and the treatment of anxiety, pain, and seizures. However, this diverse pharmacology is also associated with significant side effects that limit their clinical utility in some of these populations, even at low receptor occupancy. Molecular studies assessing the contribution of those subunits to the *in vivo* pharmacology of benzodiazepines have shown that α1 subunits are responsible for sedative effects, α1/2 subunits for anticonvulsant effects, α2/3 subunits for anxiolysis and α2/3/5 subunits for analgesia.[44,45] As such, there has been a rigorous effort to develop subtype-selective PAMs for epilepsy and other CNS disorders.

The *in vitro* profile of darigabat has been summarized previously.[42] Briefly, darigabat has a high affinity for $GABA_A$ receptors containing an α1, α2, α3 or α5 subunit but has no affinity for $GABA_A$ receptors containing α4 or 6 subunits as these receptors do not possess a benzodiazepine binding site. Functionally, darigabat shows low (<20%) activity at $GABA_A$ receptors containing α1 subunits, and greater positive allosteric modulation (90-140%) at receptors containing α2/3/5 subunits.[45] While the *in vitro* activity of darigabat is lower than that of benzodiazepines such as diazepam (% α2 modulation of 134% vs 293% for diazepam),[44] the reduction or removal of α1-mediated side effects can be compensated for by achieving higher levels of occupancy at α2-containing $GABA_A$ receptors. Both preclinical and early clinical data support this assumption, with robust measures of pharmacodynamic activity observed in nonclinical models, in healthy volunteers, and in the photosensitive

epilepsy model in patients with epilepsy at non-sedating doses achieving high receptor occupancy.[44-46] Furthermore, the relatively low intrinsic activity of darigabat compared to benzodiazepines could reduce the potential for inducing tolerance, in agreement with results reported for other subtype-selective PAMs in preclinical models.[47]

3.3 Toxicology

Preclinical toxicology studies of darigabat have been summarized in the EILAT XV progress report.[42] In addition, chronic toxicology studies in rats and dogs are now complete and support long-term clinical studies at a dose of 25 mg twice daily (b.i.d.). Of note from these studies, abrupt discontinuation of darigabat administration after 6- or 9-month dosing in rats and dogs respectively did not result in severe withdrawal symptoms such as seizures. Conversely, relatively short-term (2-week) administration of the benzodiazepines diazepam and lorazepam in dogs followed by abrupt discontinuation has been reported to result in a severe abstinence syndrome indicative of physical dependence.[48] Whilst these observations are encouraging, specific non-clinical studies to examine abuse potential and withdrawal effects of darigabat are yet to be conducted.

3.4 Pharmacokinetic and metabolic profile

As reported previously[42], studies conducted in healthy subjects have shown that darigabat is absorbed rapidly from the gastrointestinal tract, with peak plasma concentrations (C_{max}) being observed at about 1-2 h after dosing. Darigabat is eliminated with a mean half-life of approximately 11 h after multiple dosing. Based on studies conducted with human liver microsomes, darigabat undergoes extensive cytocrome P450 3A4 (CYP3A4) -mediated metabolism. No active metabolites have been identified.

A positron emission tomography (PET) study conducted in humans using [^{11}C]-flumazenil as a ligand has enabled an understanding of the relationship between plasma concentrations (and dose) of darigabat and receptor occupancy.[45] Accordingly, administration of approximately 15 mg/day darigabat is predicted to achieve >50% occupancy, and approximately 50 mg/day to achieve >80% occupancy.

3.5 Drug interactions

The potential for drug interactions of darigabat with co-administered enzyme inducers and inhibitors has not yet been established.

3.6 Efficacy data

The efficacy results of a proof-of-principle clinical trial in the photosensitivity model have been summarized previously.[42,46] In this model, which is predictive of clinical efficacy in patients with epilepsy for a range of antiseizure mechanisms,[49] single-dose oral administration of 17.5 mg darigabat (approxmately 60% receptor occupancy) and 52.5 mg darigabat (approxmately 80% receptor occupancy) were associated with a marked and statistically significant reduction in the photoparoxysmal response compared to placebo, which was similar in degree to the positive control lorazepam (2 mg).

3.7 Adverse effects

We have previously summarised the tolerability and adverse event profile of darigabat across six phase 1 trials (136 healthy participants) and three phase 2 trials (74 patients with chronic low back pain, 72 with generalized anxiety disorder, 7 with photosensitive epilepsy).[42] In these trials, darigabat was generally well tolerated. Dizziness and somnolence were the most commonly reported treatment-emergent adverse events (TEAEs).

3.8 Planned studies

A multicenter, randomized, double-blind, placebo-controlled, adjunctive-therapy, parallel-group, phase 2 proof-of-concept trial is ongoing to assess the efficacy, safety, and tolerability of darigabat (maintenance doses of 7.5 mg b.i.d. and 25 mg b.i.d.) in 150 adults with drug-resistant focal epilepsy (ClinicalTrials.gov: NCT04244175, **Error! Reference source not found.** 2). The key inclusion criteria include: (a) men and women aged 18 to 75 years with a diagnosis of epilepsy with focal aware, focal impaired awareness or focal to bilateral tonic-clonic seizures for at least two years; (b) drug resistance, defined as persistence of seizures despite use of at least two prior appropriate ASMs[50]; (c) treatment with at least one but no more than three ASMs ; and (d) a history of an average of four or more spontaneous and observable seizures per 28-day period for at least three months.

The COVID-19 pandemic introduced unprecedented complexities in conducting clinical trial assessments via traditional participant visits to clinical sites at specified time points. To ensure continuity of trial participant care, quality data collection, and prevention of lost trial data, modifications to allow remote data capture were successfully implemented. This included the introduction of health care providers to perform blood procurement and assessment of vital signs at the participants' homes, direct to patient shipment of study medication, remote electrocardiogram (ECG) collection, and allowance for scales to be collected by telemedicine when COVID-19-related issues prevented a clinical site visit. An open-label extension (OLE) study (CVL-865-SZ-002, ClinicalTrials.gov: NCT04686786) will evaluate efficacy and long-term safety for patients completing the proof-of-concept trial.

4 GANAXOLONE

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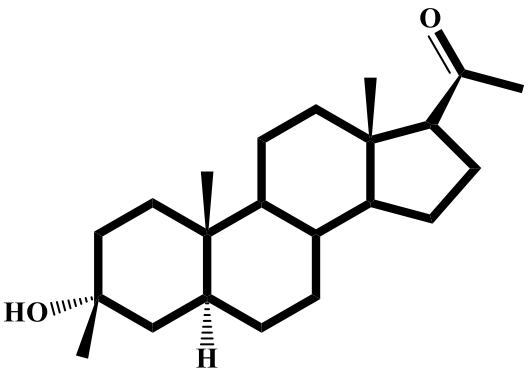

Ganaxolone

4.1 Introduction and rationale for development

Ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is an analogue of the endogenous neurosteroid, allopregnanolone.[51] In previous EILAT progress reports, we reported on preliminary evidence showing ganaxolone's potential efficacy in several rare pediatric epilepsies, including cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD), protocadherin 19 (PCDH19)-related epilepsy, and refractory status epilepticus (SE).[11,42,52] In this progress report, we focus on the current status of ganaxolone's development for CDD and tuberous sclerosis complex (TSC).

4.2 Pharmacology

Ganaxolone is a PAM of $GABA_A$ receptors at both synaptic and extrasynaptic sites. Synaptic $GABA_A$ receptors become functionally inactive and resistant to benzodiazepines after they are internalized during prolonged seizures.[53] Conversely, ganaxolone-responsive extrasynaptic $GABA_A$ receptors are not internalized, nor do they become functionally inactive with prolonged seizure activity. Given the differential response characteristics between extrasynaptic and synaptic $GABA_A$ receptors, modulation of extrasynaptic receptors represents an attractive mechanism for the treatment of SE and, potentially, chronic epilepsies.[54]

Additional information on the pharmacology of ganaxolone has been provided in the EILAT XIII and EILAT XIV progress reports.[11,52]

4.3 Toxicology

A summary of toxicology data for ganaxolone has been provided in the EILAT XIII and EILAT XIV progress reports.[11,52]

4.4 Pharmacokinetics and drug interactions

Information on the pharmacokinetics and drug interaction potential of ganaxolone is summarized in the EILAT XV progress report.[42]

4.5 Efficacy data

4.5.1 CDKL5 Deficiency Disorder (CDD)

CDD is a rare, X-linked, epileptic encephalopathy with an estimated incidence of 1 : 40,000 live births.[55,56] Clinical characteristics commonly include early-onset refractory epilepsy, hypotonia, cortical visual dysfunction, severe neurodevelopmental impairment, and sleep disturbances. Seizures associated with CDD are often refractory to treatment with existing ASMs, and improvements may be short-lived.[57]

The MARIGOLD Study (ClinicalTrials.gov: NCT03572933) is a phase 3, randomized, double-blind, placebo-controlled trial that evaluated ganaxolone in patients with refractory

epilepsy associated with CDD.[58] An OLE of this trial is ongoing. The primary objective of the double-blind phase was to assess the efficacy and safety of ganaxolone compared with placebo as adjunctive therapy for the treatment of major motor seizures (defined as bilateral tonic, generalized tonic-clonic, bilateral clonic, atonic, or focal to bilateral tonic-clonic) in children and young adults with genetically confirmed CDD. Ganaxolone was dosed three times daily (t.i.d.) at a maintenance dose of up to 63 mg/kg/day or 1,800 mg/day maximum. The primary endpoint was percentage reduction in 28-day major motor seizure frequency during the 17-week double-blind phase relative to the 6-week baseline phase. The key secondary endpoints were proportion of study participants with a ≥50% reduction in major motor seizure frequency and Clinical Global Impression Scale-Improvement (CGI-I) administered by the clinician and caregiver. The study also evaluated several exploratory endpoints.

A total of 101 patients (79% female) were randomized, 50 to ganaxolone and 51 to placebo. Study participants had a median age of 6 years and had received a median of 7 prior ASMs. Although all patients received at least one dose of a study drug, seizure frequency for one patient randomised to ganaxolone was not recorded at baseline and thus the primary endpoint was investigated in a sample of 100 patients. For the primary endpoint, study participants treated with ganaxolone experienced a median 30.7% reduction in major motor seizure frequency compared to a 6.9% reduction for study participants treated with placebo ($p = 0.004$, Wilcoxon rank sum test). For the key secondary endpoints, ganaxolone demonstrated a directional improvement in the proportion of study participants with ≥50% reduction in major motor seizure frequency as well as directional improvements in both the clinician's and caregiver's CGI-I scores, but these did not achieve statistical significance.[58]

Ganaxolone was generally well tolerated (<5% discontinuation rate in ganaxolone-treated participants). TEAEs occurred in 86% and 88.2% of participants randomized to ganaxolone and placebo, respectively. Most frequent TEAEs reported more commonly with ganaxolone than placebo were somnolence, pyrexia, and upper respiratory tract infection. Serious TEAEs occurred in 12.0% (n=6) and 9.8% (n=5) of ganaxolone- and placebo-treated participants, respectively. Ganaxolone has gained U.S. Food and Drug Administration (FDA) approval for the treatment of seizures associated with CDD in March 2022.

4.5.2 Tuberous Sclerosis Complex (TSC)

TSC, caused by pathogenic variants in *TSC1* or *TSC2* genes, is associated with malformations and benign tumors in the brain and other organs. Over 80% of patients with TSC have epilepsy (mostly focal seizures) and are often drug-resistant (approximately 60%).[59-61] Here, we report results from an open-label, phase 2 explorative study of adjunctive therapy with ganaxolone in patients with TSC-associated refractory epilepsy (ClinicalTrials.gov: NCT04285346). The primary endpoint included change from baseline in (a) focal motor seizures without impairment of consciousness or awareness ; (b) focal seizures with impairment of consciousness or awareness; (c) focal seizures evolving to bilateral tonic-clonic seizures, and (d) generalized motor seizures including tonic-clonic, bilateral tonic, bilateral clonic, or atonic/drop seizures. After a 4-week titration period, study participants underwent 8 weeks of maintenance treatment with ganaxolone, up to 63 mg/kg/day or a maximum of 1,800 mg/day on a t.i.d. dosing regimen. Participants/caregivers tracked the frequency of TSC-associated seizures using diaries during the 4-week baseline and 12-week treatment phases. As mentioned above, the primary endpoint was the median percent change from baseline in the frequency of TSC-associated seizures during the 12-week treatment period. The percentage of study participants who achieved ≥50% reduction in seizure frequency was a secondary endpoint. Post hoc analyses included assessment of the percent change from baseline in focal seizure frequency, the percentages of patients who achieved a ≥50% reduction in TSC-associated seizure frequency in concomitant cannabidiol and everolimus subgroups, and the percent changes from baseline in TSC-associated seizure frequency in patients who did and did not report somnolence-related TEAEs (which included somnolence, sedation, fatigue, and lethargy).

A total of 23 patients were enrolled in the study (median age 11.0 years, range: 2-32 years). The median TSC-associated seizure frequency at baseline was 36.6 (Interquartile Range, IQR: 22.8 to 69.0). Median reduction (95% Confidence Interval, CI) in TSC-associated seizures per 28 days was 16.6% (56.4% -14.9%) during the 12-week treatment period compared with baseline (Intention To Treat ; ITT, n=23). The median reduction in frequency of focal seizures (n=19) was 25.2%. The proportion of study participants achieving a ≥50% reduction in TSC-associated seizures (responder rate) was 30.4%, with responder rates of 25.0% and 36.4% in participants taking concomitant cannabidiol (n=12) or everolimus (n=11), respectively.

A total of 20 (87.0%) participants experienced TEAEs, most of which were mild or moderate in severity. The most-commonly reported TEAEs were somnolence, fatigue, and sedation. Three serious TEAEs of seizure, aspiration, and angioedema occurred in one participant each.

The seizure was considered treatment-related. No deaths occurred during the study. Participants who did not experience somnolence-related TEAEs (n=6) demonstrated a median 27.9% reduction in TSC-associated seizure frequency compared to a 16.6% median reduction observed in those who did report somnolence-related TEAEs (n=17).

In this highly refractory TSC-associated epilepsy patient population, in which most patients were taking second-generation concomitant ASMs, adjunctive ganaxolone treatment was associated with a modest median percent reduction in TSC-associated seizure frequency. Approximately one-third of patients in the study experienced ≥50% seizure reduction with 12-weeks of adjunctive ganaxolone. Limited data suggest a possible connection between safety and seizure outcome, as evidenced by the differences in rates of reduction in seizure frequency in patients who did versus did not experience somnolence-related TEAEs. A global, double-blind phase 3 study of ganaxolone in seizures associated with TSC is planned.

4.6 Adverse effects (aggregated safety data)

To date, over 1900 study subjects have received at least one dose of ganaxolone (data cutoff: April 21, 2021, data on file, Marinus Pharmaceuticals, Inc.). In placebo-controlled studies, there were 1844 subjects who received either placebo (n=743) or ganaxolone (n=1101). The frequency of TEAEs was 62.9% (693/1101 subjects) for ganaxolone and 53.8% (400/743 subjects) for placebo. The rate of serious TEAEs was similar between ganaxolone and placebo-treated subjects: 2.8% (31/1101) and 3.8% (28/743), respectively. The only serious TEAE reported in more than 2 subjects in the ganaxolone group was seizure (0.5% ganaxolone and 0.7% placebo). The most frequently reported TEAEs (>5% of subjects) occurring in a higher proportion of ganaxolone-treated subjects compared to placebo were somnolence (22.4% ganaxolone, 8.1% placebo), dizziness (12.6% ganaxolone, 3.9% placebo), and fatigue (9.3% ganaxolone, 4.8% placebo). CNS-related events appeared to be dose related. There was no discernable safety signal related to bone marrow suppression, bone demineralization, nephrolithiasis, cardiac valvulopathy, or liver function. There have been no significant changes noted in body weight and no clinically significant trends in ECG parameters or vital signs.

4.7 Ongoing and planned studies

A multicenter, double-blind, placebo-controlled study of 124 subjects with refractory SE who have failed at least two intravenous (i.v.) ASMs (the RAISE study, ClinicalTrials.gov: NCT04391569) is ongoing. Eligible patients are randomized to i.v. ganaxolone (administered as a bolus dose followed by a 36-h continuous infusion, followed by a 12-h taper) or placebo. Standard-of-care treatment for SE is maintained throughout the study. The co-primary endpoints are (a) proportion of participants with SE cessation within 30 minutes of i.v. ganaxolone initiation without medications for the acute treatment of SE, and (b) proportion of participants with no progression to i.v. anesthesia for 36 h following i.v. ganaxolone initiation. Secondary endpoints include lack of SE recurrence between 72 hours and four weeks following study drug initiation, and health care utilization and disability assessments.

The RAISE II trial, supporting the potential European registration of i.v. ganaxolone for refractory SE, is currently planned. The RAISE II trial is a randomized, placebo-controlled trial that differs from the RAISE trial in the U.S. In the RAISE II trial, ganaxolone can be initiated earlier, following failure of one or more i.v. ASMs, and study drug is initiated concurrently with a standard-of-care i.v. ASM.

The phase 2 RESET trial of adjunctive ganaxolone in established SE is planned. The study consists of an open-label, dose finding phase that will enroll patients in whom SE has not resolved following adequate benzodiazepine dosing, with i.v. ganaxolone initiated concurrently with the initial second-line i.v. ASM. The study will enroll up to 40 participants and uses a sequential Bayesian optimal interval design to determine the optimal dose of i.v. ganaxolone (bolus followed by 4 to 12-h infusion) and the required duration of infusion.

5 LORCASERIN

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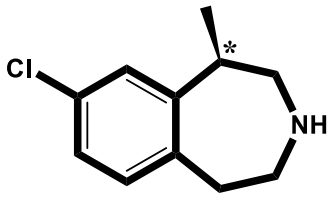

Lorcaserin

5.1 Introduction and rationale for development

Lorcaserin is a selective serotonin receptor type 2C (5-HT_{2C}) agonist that was originally developed and marketed as a weight-loss medication[62], but was voluntarily withdrawn from the market globally in 2020 (see section 5.7).[63] Lorcaserin has been subsequently investigated as a potential ASM for the treatment of seizures associated with Dravet syndrome, based on evidence that stimulation of 5-HT_{2C} receptors has antiseizure effects.[64-66]

The mechanism by which 5-HT_{2C} receptor agonists inhibit seizure activity are not well understood, but research suggests that the activation of 5-HT_{2C} on GABAergic interneurons might play a role.[64] The clinical efficacy of fenfluramine, an agonist of multiple serotonin receptors, in patients with Dravet syndrome supports the therapeutic rationale of 5-HT_{2C} receptor stimulation in the management of seizures in patients with this syndrome.[67] Accordingly, lorcaserin is currently in phase 3 development for the treatment of seizures associated with Dravet syndrome.

5.2 Pharmacology

52.1 Activity in experimental models of seizures and epilepsy

The antiseizure activity of lorcaserin has been shown in several models of seizures.[68] In the GAERS, a well-established rat model of absence seizures, lorcaserin suppressed seizures in a dose-dependent manner.[66] Antiseizure effects of lorcaserin have been also reported in a zebrafish model of Dravet syndrome, in which stimulation of 5-HT_{2C} receptors resulted in decreased seizure-like activity.[65,69]

5.2.2 Mechanism of action

It is suggested that GABA-mediated synaptic inhibition may play a role in the antiseizure effects seen with 5-HT_{2C} receptor agonists, but the exact mechanism by which these agonists inhibit seizure propagation is not well understood. A study in Sprague–Dawley rats suggested that activation of 5-HT_{2C} receptors expressed on GABAergic interneurons in the dorsal raphe nucleus (DRN) leads to the dose-related inhibition of DRN 5-HT neuron firing.[64] The authors concluded that 5-HT_{2C} feedback might provide potential targets for drug therapies of neuropsychiatric disorders. Further research to investigate the exact mechanisms of action of lorcaserin for the treatment of seizures is therefore warranted.

Lorcaserin was designed to selectively activate 5-HT_{2C} receptors, without significant activation of 5-HT_{2A} and 5-HT_{2B} receptors, or interaction with 5-HT transporters.[62,70] *In vivo* animal studies demonstrated that behaviors consistent with 5-HT_{2A} receptor agonism were only seen with >10-fold higher doses of lorcaserin compared with doses that induced behaviors typical to 5-HT_{2C} receptor activation.[62] Selectivity towards 5-HT_{2C} over 5-HT_{2A} and 5-HT_{2B} receptors provides a potential advantage for lorcaserin over non-selective 5-HT_2 agonists because stimulation of 5-HT_{2A} receptors has been associated with hallucinogenic activity and stimulation of 5-HT_{2B} receptors with cardiovascular toxicity.[62] In a study assessing the effect of the peripheral administration of lorcaserin on different rat brain regions, lorcaserin moderately inhibited a subpopulation of dopaminergic neurons in ventral tegmental area, but had no effect in substantia nigra pars compacta (at doses of 5–640 μg/kg, i.v.) or the extracellular levels of dopamine in the nucleus accumbens and striatum (at doses of 0.3, 3 mg/kg i.p.)[71], which differentiates lorcaserin from drugs of abuse.

5.3 Toxicology

The toxicity of oral lorcaserin has been evaluated in general toxicity, reproductive toxicity, genotoxicity, and cardiovascular toxicity studies conducted in mice, rats, and non-human primates.[62] In single-dose studies in rats, the maximum tolerated dose was 500 mg/kg p.o. due to mortality at 1000 mg/kg; the maximum tolerated dose in monkeys was 100 mg/kg p.o. in a 10-day study.[72] In a 2-year repeated-dose carcinogenicity study in mice, the no-observed-adverse-effect-level (NOAEL) was 50 mg/kg p.o., which is 4- to 7-times higher than the plasma exposure in humans at the dose of 10 mg b.i.d.[73] In general toxicity studies in rats, the NOAEL was 5 mg/kg/day, which is 1.6- to 3-times greater than the plasma exposure in humans at the dose of 10 mg b.i.d. In a 52-week general toxicity study in monkeys, the NOAEL was 2 mg/kg/day, which is approximately 0.8-times the plasma exposure in humans at the dose of 10 mg b.i.d.[72] There appeared to be no adverse findings of relevance to humans in repeated-dose animal studies at doses resulting in plasma exposures comparable with those observed in humans treated with therapeutic doses for weight management indication.[62] Lorcaserin administered during the period of embryofetal organogenesis in rats and rabbits showed no evidence of teratogenicity at plasma exposures up to 44- and 19-times the plasma exposure observed in humans treated with therapeutic doses for weight management indication, respectively.[72] Lorcaserin also had no mutagenic effects in *in vitro* and *in vivo* assays.[72] In *in vitro* studies in isolated canine Purkinje fibers, lorcaserin prolonged action

potential duration at 90% (APD_{90}) at 30 μM (6.96 μg/mL), but had no effect on APD_{60} at 3, 10, and 30 μM, therefore suggesting minimal clinical significance.[72] No differences in the histology of cardiac valves or adjacent cardiac tissue were observed between control- and lorcaserin-treated animals in toxicity and carcinogenicity studies in mice, rats, and non-human primates over the treatment duration of up to 2 years.[62]

The carcinogenic potential of lorcaserin was assessed in two-year carcinogenicity studies in mice and rats.[74] There were no treatment-related increases in the incidence of any tumor in mice at doses that produced plasma exposure in males and females of 8- and 4-times the daily human clinical dose (10 mg b.i.d.), respectively.[74] In female rats, an increased incidence of mammary adenocarcinoma was observed at 100 mg/kg, which was associated with plasma exposures that were 87-times the daily human clinical dose. The incidence of mammary fibroadenoma was increased in female rats at all doses (10, 30, or 100 mg/kg) with no safety margin to the human clinical dose.[74] In male rats, treatment-related neoplastic changes were observed in the subcutis (fibroadenoma, Schwannoma), the skin (squamous cell carcinoma), mammary gland (adenocarcinoma and fibroadenoma), and the brain (astrocytoma) at ≥30 mg/kg (plasma exposure 17-times human clinical dose).[74]

5.4 Pharmacokinetic and metabolic profile

The pharmacokinetic properties of lorcaserin, including the effects of age, sex, renal function, and hepatic function, have been evaluated.[62,73,75] Following oral administration in healthy subjects, C_{max} is reached approximately 1.5–2 h after dosing. Lorcaserin has a plasma half-life of approximately 11–12 h.[62,75] In a multiple-dose study, steady-state plasma lorcaserin concentrations were reached within 5 days after starting b.i.d. dosing.[62,76]

Lorcaserin is metabolized in the liver by multiple enzymatic pathways and metabolites are excreted primarily in the urine. Lorcaserin's major circulating and urinary metabolites, the sulfamate (M1) and the N-carbamoyl glucuronide (M5) derivatives, respectively, do not appear to have pharmacological activity.[68,73,75] There was no apparent effect of sex or race on lorcaserin plasma exposure in healthy and overweight and obese adults.[62,73] In a subgroup of patients from phase 3 trials in obese or overweight adults, body weight was a significant covariate on the apparent clearance and apparent volume of distribution of lorcaserin: patients in the highest body weight quartile had 27% lower mean plasma exposures than patients in the lower body weight quartiles.[73] Data from a pharmacokinetic study (ClinicalTrials.gov:

NCT02398669) of single-dose lorcaserin 10 mg in obese children (N=10; aged 6–11 years) showed that lorcaserin C_{max} and area under the plasma drug concentration-time curve (AUC) tended to increase with decreasing body weight (Eisai Inc., data on file). The pharmacokinetic data in the overall pediatric population are limited, and thus further evaluation to characterize pharmacokinetics in children, including those with Dravet syndrome, is required to confirm if weight-based dosing is needed. In patients with varying degrees of impaired renal function from mild to end-stage renal disease (N=32), lorcaserin C_{max} and AUC after a single 10 mg dose were not meaningfully affected by renal function, but exposure to the metabolites M1 and M5 was increased in the presence of impaired renal function.[73] To this end, lorcaserin is not recommended for use in patients with severely impaired renal function or end-stage renal disease, and should be used with caution in patients with moderate renal impairment.[75] In patients with mild or moderate hepatic impairment, mean lorcaserin AUC values after a single 19 mg dose were increased by approximately 24% and 30%, respectively, compared with healthy controls, but the difference was not considered clinically significant and did not warrant dose adjustment.[73,75] The effect of severe hepatic impairment on lorcaserin pharmacokinetics was not evaluated.[75]

5.5 Drug interactions

Based on results from *in vitro* characterization and drug–drug interaction clinical studies, lorcaserin was found to be a mild-to-moderate inhibitor of CYP2D6.[73] Therefore, concomitant administration of lorcaserin may increase plasma exposure (AUC) of CYP2D6 substrates.

No formal analyses have been performed on the effects of other agents on lorcaserin pharmacokinetics. However, as lorcaserin is metabolized by multiple enzymatic pathways involving CYPs as well as sulfotransferases (SUL), uridine-5'-diphospho-glucuronosyltransferases (UGT) and flavin-containing monooxygenase (FMO) enzymes, concomitant administration of agents which inhibit drug metabolizing enzymes is predicted to have minimal impact on lorcaserin exposure.[68,75]

5.6 Efficacy data

Results of two small real-world studies suggested potential efficacy of lorcaserin in managing seizures associated with Dravet syndrome.[65,77] A first study reported reduction in total number of seizures in five patients with Dravet syndrome (mean age 11.8 years; range 7–18 years)

receiving off-label lorcaserin (0.19–0.32 mg/kg/day), three of whom had seizure-free days or weeks.[65] Another study retrospectively assessed the response to lorcaserin in patients (age at treatment onset, 3–40 years; mean 16.4 years) with treatment-resistant epilepsies (n=35; mean overall dose 15.7 mg/day; mean dose for children: 13.7 mg/day) including 20 individuals with Dravet syndrome.[77] In the patients with Dravet syndrome, lorcaserin treatment was associated with a 43% reduction in the frequency of motor seizures. It was unclear, however, if this group of patients included the five patients with Dravet syndrome reported previously by Griffin et al.[65] The efficacy of lorcaserin for Dravet syndrome is currently being assessed in a phase 3 study and an extended access program (see section 5.8).

5.7 Adverse effects

Evidence on the safety of lorcaserin is available from the three phase 3, randomized, double-blind, placebo-controlled studies conducted in overweight and obese patients with or without diabetes mellitus (ClinicalTrials.gov: NCT00395135, NCT00603291 and NCT00603902).[78] In the pooled analysis of two phase 3 trials in non-diabetic patients treated with lorcaserin 10 mg b.i.d. (N=3195), the most commonly observed TEAEs were headache, dizziness, fatigue, nausea, dry mouth, and constipation.[78] Single TEAEs that might be associated with serotonin excess were reported in 1.7% of patients treated with lorcaserin and 0.6% of patients receiving placebo. The incidence of TEAEs related to depression (according to Standardized MeDRA Queries of narrow terms) was similar with lorcaserin 10 mg b.i.d. versus placebo (2.5% vs 2.4%, respectively); however, discontinuations due to depression-related TEAEs were more frequent with lorcaserin than with placebo (1.3% versus 0.8%, respectively). Euphoria was reported in six (0.2%) patients treated with lorcaserin compared with one (0.03%) patient receiving placebo. In a phase 3 trial in diabetic patients, the most common TEAEs were headache, hypoglycemia, back pain, cough, and fatigue.

A post-marketing study (CAMELLIA-TIMI, ClinicalTrials.gov: NCT02019264) was conducted in 12,000 overweight and obese patients with cardiovascular disease and/or multiple cardiovascular risk factors to evaluate the effects of lorcaserin (10 mg b.i.d.) on major adverse cardiovascular events.[79] No significant difference was observed in major adverse cardiovascular events between lorcaserin and placebo after a median follow-up of 3.3 years. It was concluded that long-term use of lorcaserin does not increase the incidence of major adverse cardiovascular events in overweight/obese patients with cardiovascular risk factors. Results also showed that the incidences of serious TEAEs were similar between the

lorcaserin and placebo groups, although drug-related TEAEs leading to discontinuation were more frequent with lorcaserin than with placebo (7.2% versus 3.7%, respectively). Events of suicidal ideation and behavior were reported by 0.4% and 0.2% of patients who received lorcaserin and placebo, respectively. Psychiatric disorders were reported for 12.4% and 10.6% of patients with lorcaserin and placebo, respectively, with the most common being depression, insomnia, and anxiety (Eisai Inc., data on file). No significant differences between lorcaserin 10 mg b.i.d. and placebo were observed in an echocardiographic sub-study in which FDA-defined valvulopathy occurred in 1.8% and 1.3% of patients receiving lorcaserin and placebo, respectively, at 1 year.[79]

Lorcaserin was voluntarily withdrawn from the market globally in 2020 after a request from the FDA, based on the observation of a numerical imbalance in the number of patients diagnosed with malignancies in the lorcaserin arm (7.7%) versus the placebo arm (7.1%) in CAMELLIA-TIMI.[80] The study was not designed nor powered to assess the difference in malignancy rates between treatment arms. However, the FDA concluded that the potential risks of lorcaserin outweigh its benefits for the weight loss indication for which it had been approved.[80]

There are limited safety data on lorcaserin in patients with epilepsy or Dravet syndrome. In a retrospective chart review of 35 patients with severe childhood-onset epilepsies, 20 of whom had Dravet syndrome, the most common TEAEs (reported by >10% of patients) were decreased appetite, decreased attentiveness, and weight loss.[77] Further studies are warranted to evaluate the safety and tolerability of lorcaserin in patients with Dravet syndrome.

5.8 Planned studies

Currently, the efficacy and safety of lorcaserin as adjunctive treatment for Dravet syndrome is being assessed in two ongoing clinical trials in North America. MOMENTUM 1 (E2023-A001-304, ClinicalTrials.gov: NCT04572243) is a phase 3, multicenter, double-blind, randomized, parallel-group, placebo-controlled study in patients aged ≥2 years with a diagnosis of Dravet syndrome and ≥4 convulsive seizures during the 4-week baseline period. The study comprises a 14-week double-blind treatment period and a 12-week OLE phase. The primary objective is to demonstrate the superiority of lorcaserin versus placebo in terms of change from baseline in convulsive seizure frequency per 28 days.

MOMENTUM 2 (E2023-A001-405, ClinicalTrials.gov: NCT04457687) is an open-label extended access program for lorcaserin in patients aged ≥2 years with Dravet syndrome and other refractory epilepsies who initiated lorcaserin treatment before the market withdrawal announcement, or who have completed the MOMENTUM 1 study (Dravet syndrome only), and may benefit from continued lorcaserin treatment based on the investigator's judgement. For eligible patients, clinical data will be obtained from medical records as part of a retrospective chart review.

6 SOTICLESTAT (TAK-935)

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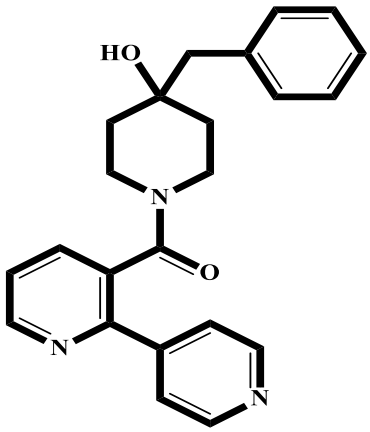

Soticlestat (TAK-935)

6.1 Introduction and rationale for development

Soticlestat (TAK-935), a first-in-class ASM, is a selective inhibitor of cholesterol 24-hydroxylase (CH24H; also known as CYP46A1) that is currently in development for the treatment of Dravet syndrome and Lennox–Gastaut syndrome. CH24H is the primary enzyme responsible for the catabolism of cholesterol to 24(S)-hydroxycholesterol (24HC) in the brain. Because aberrant cholesterol metabolism is implicated in neurological disorders that often cause seizures[81-83], CH24H inhibition with soticlestat represents a promising strategy for the treatment of developmental and epileptic encephalopathies (DEEs) such as Dravet syndrome and Lennox–Gastaut syndrome.

Recent preclinical studies have provided evidence of target engagement, with radiolabeled soticlestat binding specifically to CH24H in murine brain sections, as well as in rhesus macaques *in vivo*, as demonstrated using PET.[82] Central target engagement was also confirmed in a phase 1 PET study in healthy volunteers (ClinicalTrials.gov: NCT02497235). In a multiple-rising-dose phase 1 study in healthy volunteers, soticlestat dose-dependently reduced plasma 24HC levels, indicating pharmacodynamic activity, and was generally well tolerated.[84]

6.2 Pharmacology

6.2.1 Activity profile in animal models of seizures and epilepsy

The therapeutic potential of soticlestat was previously identified in a transgenic mouse model carrying mutated human amyloid precursor protein and presenilin, and expressing an epileptic phenotype.[83] Subsequently, the anticonvulsive properties of soticlestat were characterized in several rodent models of epilepsy that have long been used to identify ASMs, with efficacy being demonstrated in Frings audiogenic seizures and kindling development models (manuscript under review). The antiepileptogenic potential of soticlestat was demonstrated in a mouse model of MTLE.[85] Overall, these data suggest that soticlestat has therapeutic potential to modify the process of seizure generation.

In *Scn1a*$^{+/-}$ mice (a model of Dravet syndrome), soticlestat reduced seizure burden, protected against hyperthermia-induced seizures, and completely prevented sudden unexpected death in epilepsy (SUDEP) (Figure 3A). No generalized tonic-clonic seizure events in soticlestat-treated mice advanced to the most severe stages that include tonic hindlimb extension (Figure 3B), which is indicative of brainstem invasion and correlated with increased SUDEP risk.[86]

6.2.2 Mechanism(s) of action

CH24H is the primary enzyme responsible for cholesterol catabolism in the brain. Upon central nervous system injury, CH24H is induced in reactive astrocytes and microglia, triggering increased catabolism of cholesterol, with downstream effects that contribute to the pathophysiology of an epileptic condition.[83]

Soticlestat binds specifically to CH24H and reduces brain 24HC levels.[82,83] CH24H inhibition with soticlestat also lowers plasma 24HC levels in healthy adults and patients with DEEs,

making 24HC a potential biomarker for pharmacodynamic activity and central target engagement.[87] Reduction of brain 24HC is hypothesized to reduce glutamatergic signaling via multiple mechanisms. By inhibiting catabolism of cholesterol to 24HC, soticlestat maintains the integrity of plasma-membrane lipid rafts required for glutamate reuptake, reducing extracellular glutamate levels and attenuating excessive glutamatergic signaling.[83] 24HC is also known for various neuromodulatory activities such as positive allosteric modulation of N-methyl-D-aspartate (NMDA) receptors and an inflammatory signaling, meaning that inhibition of CH24H could potentially reduce glutamatergic signaling through multi-modal mechanisms.[83] Taken together with the aforementioned pharmacology profile, these findings support the notion that soticlestat controls seizures through mechanisms that differ from currently available ASMs. As such, it could provide a novel therapeutic option for epileptic disorders that are not adequately controlled by existing treatments.

6.3 Toxicology

The standard chronic toxicology package has been completed to allow soticlestat dosing in humans, including pediatric populations.

6.4 Pharmacokinetic and metabolic profile

Soticlestat pharmacokinetics were characterized in phase 1 studies in healthy volunteers, within dose ranges of 15–1350 mg for single ascending doses and 100–600 mg/day for multiple ascending doses, resulting in rapid absorption, negligible renal excretion of the unchanged form, and rapid elimination.[84,88] Systemic exposure increased in a manner that was greater than dose proportional over the dose ranges evaluated but was not affected by formulation or administration with food. The mean terminal half-life was 0.8–7.2 h across doses.[88] Similar pharmacokinetic properties were observed in healthy Japanese volunteers with administration of soticlestat 200–1200 mg (single rising doses) and 100–300 mg b.i.d. (multiple daily doses) (ClinicalTrials.gov: NCT04461483), with mean half-lives ranging from 5.1 to 8.7 h following a single dose and from 2.6 to 3.6 h following multiple doses. In a phase 1 study to assess absorption, distribution, metabolism and excretion (ADME) in healthy subjects (ClinicalTrials.gov: NCT04992442), the absolute oral bioavailability of soticlestat was found to be 12.6%. Urinary excretion of soticlestat metabolites was the major route for elimination, with approximately 95% of the dose excreted in urine within 48 h. Urinary

excretion of the parent drug was low (< 1% of the dose), indicating that the total clearance of soticlestat is almost exclusively metabolic. The glucuronide metabolite of soticlestat contributed to 86% of the dose excreted in urine, suggesting that soticlestat is predominantly cleared by direct glucuronidation (Takeda, data on file).

Soticlestat at doses of 100, 200 and 300 mg b.i.d. showed a dose-dependent increase in systemic exposure (AUC) in a phase 1b/2a trial in adults with DEEs, with peak plasma soticlestat concentrations of 269.6, 639.8 and 975.3 ng/mL, respectively.[87] Mean oral clearance (CL/F) of soticlestat at doses of 100, 200 and 300 mg b.i.d. was 259.4, 195.8 and 190 L/h, respectively. A 55-day OLE of the same study showed an overall mean percentage change from baseline in plasma 24HC of -81.0%.

6.5 Drug interactions

An *in vitro* induction study using human hepatocytes indicated that soticlestat, over a concentration range of 3 to 100 µmol/L, resulted in little to no change (<2.0-fold change and <20% of the positive control) in CYP1A2, CYP2B6, and CYP3A4 messenger RNA levels; therefore, soticlestat is not an inducer of CYP1A2, CYP2B6 or CYP3A4. In human liver microsomes, soticlestat directly inhibited CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5 (using testosterone 6β-hydroxylation and midazolam 1′-hydroxylation as markers for the activity of these enzymes) with half-maximal inhibitory concentration (IC_{50}) values of 28, 30, 18, 73, and 30 µmol/L, respectively. At the highest concentration of 100 µmol/L tested, soticlestat directly inhibited CYP2D6 by 40%; thus, the IC_{50} value is assumed to be greater than 100 µmol/L. There was little or no evidence of direct inhibition of CYP1A2 or CYP2B6 by soticlestat (IC_{50} >100 µmol/L).

The potential inhibitory effects of lamotrigine, levetiracetam, valproic acid, and carbamazepine on the glucuronide conjugation of [^{14}C]-soticlestat were examined in human liver microsomes. The percentage to the control activity of elimination ratio of soticlestat and that of formation ratio of the glucuronide metabolite were evaluated at up to 100 µmol/L for lamotrigine and carbamazepine and at up to 1000 µmol/L for levetiracetam and valproic acid. The results indicate that these ASMs have little potency in inhibiting the glucuronide conjugation of soticlestat in human liver microsomes.

Two studies are currently ongoing to evaluate the drug interaction potential of soticlestat in healthy adults: a) a study with a UGT1A9 inhibitor (mefenamic acid) and a strong CYP3A4

inhibitor (itraconazole) (ClinicalTrials.gov: NCT05064449), and b) a study with a strong CYP3A4 inducer (rifampin) (ClinicalTrials.gov: NCT05098041). Direct glucuronidation via UGT1A9 and UGT2B4 is the predominant clearance pathway of soticlestat, while oxidative metabolism via CYP3A4 is a minor clearance pathway, based on a recently completed human ADME study (ClinicalTrials.gov: NCT04992442).

6.6 Efficacy data

Soticlestat efficacy was evaluated in ELEKTRA, a multicenter phase 2, randomized, double-blind, placebo-controlled study of soticlestat up to 300 mg b.i.d. (weight-adjusted in pediatric patients <60 kg) with either Dravet syndrome (n = 51) or Lennox-Gastaut syndrome (n = 88) (ClinicalTrials.gov: NCT03650452). ELEKTRA achieved its primary endpoint, with the soticlestat-treated combined patient population demonstrating a placebo-adjusted median reduction in seizure frequency of 30.5% during the 12-week maintenance period (p = 0.0007, n = 120). Over the 20-week full treatment period, patients with Dravet syndrome demonstrated a placebo-adjusted median convulsive seizure frequency reduction of 46.0% (p = 0.0007; Figure 4A) from baseline, and those with Lennox-Gastaut syndrome demonstrated a placebo-adjusted median drop seizure frequency reduction of 14.8% (p = 0.1279; Figure 4B).[89]

6.7 Adverse effects

In ELEKTRA, most TEAEs were mild or moderate. No deaths were reported. The incidence of TEAEs in ELEKTRA was similar between the soticlestat and placebo groups, at 80.3% and 74.3% of patients, respectively. Serious TEAEs were observed in 15.5% of patients in the soticlestat group and 18.6% of patients in the placebo group. TEAEs observed in this study with at least 5% difference from placebo were lethargy (soticlestat, 7.0%; placebo, 0%) and constipation (soticlestat, 5.6%; placebo, 0%).

6.8 Planned studies

Based on the efficacy and safety demonstrated in patients with Dravet syndrome and Lennox-Gastaut syndrome in ELEKTRA, participants are currently being recruited for phase 3 clinical trials. SKYLINE is a randomized, double-blind, placebo-controlled study to evaluate the

efficacy, safety and tolerability of soticlestat in pediatric and young adult patients (aged 2–21 years) with Dravet syndrome (ClinicalTrials.gov: NCT04940624). SKYWAY is a randomized, double-blind, placebo-controlled study to assess the efficacy, safety and tolerability of soticlestat in pediatric and adult patients (aged 2–35 years) with Lennox-Gastaut syndrome (ClinicalTrials.gov: NCT04938427). Patients from both of these studies will also have the option to roll over into the OLE study ENDYMION 2, which will assess the long-term safety and tolerability of soticlestat (ClinicalTrials.gov: NCT05163314).

7 STK-001

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7.1 Introduction and rationale for development

Dravet syndrome is a severe and progressive genetic epilepsy characterized by frequent, prolonged, and refractory seizures, typically beginning within the first year of life. Available therapies do not adequately control seizures in about 90% of patients with Dravet syndrome, and they do not address other comorbidities of the disease, including intellectual disability, ataxia/motor abnormalities, behavioral problems, speech impairment, sleep disturbances, and a high risk for SUDEP. Complications of Dravet syndrome often contribute to poor quality of life for patients and their caregivers.[90] In approximately 85% of cases, Dravet syndrome is caused by spontaneous, heterozygous loss-of-function mutations in the *SCN1A* gene, which encodes for the voltage-gated sodium channel type 1 α subunit ($Na_v1.1$) protein.[91,92] Upregulating $Na_v1.1$ protein may restore fully functioning neurons and thereby prevent seizures and reduce non-seizure related comorbidities in patients with Dravet syndrome.

7.2 Pharmacology

7.2.1 Activity profile in animal models of seizures and epilepsy

A single dose of STK-001 administered by the intracerebroventricular route (i.c.v.) at postnatal day (PND) 2 or PND 14 in the Dravet syndrome mouse model resulted in increased

productive mRNA transcript and increased $Na_V1.1$ protein in the brain, as well as significantly reduced incidence of SUDEP.[93]

7.2.2 Mechanism of action

STK-001 was developed using TANGO (Targeted Augmentation of Nuclear Gene Output) technology, which uses antisense oligonucleotides (ASOs) to specifically increase productive mRNA levels, leading to optimal protein expression.[94] Pre-mRNA normally undergoes splicing to remove introns and join exons together to form mature mRNA templates for protein production. Pre-mRNA can also be spliced to generate non-productive transcripts (for example by incorporating exons that introduce a premature stop codon or lead to a frame shift). Such is the case with *SCN1A*, where the inclusion of an alternative exon leads to a nonproductive transcript that is degraded by nonsense-mediated mRNA decay which results in reduced protein expression. STK-001 blocks the incorporation of this alternative exon to increase productive *SCN1A* mRNA expression. TANGO specifically increases expression of $Na_V1.1$ mRNA only in tissues with endogenous gene expression. In the case of an autosomal dominant haploinsufficiencies such as Dravet syndrome, TANGO can upregulate productive mRNA from the wild-type (WT) allele and operates in a mutation-independent manner.

A series of *in vitro* studies demonstrated that STK-001, an ASO, is a potent and selective modulator of productive *SCN1A* mRNA levels.[93] In addition, i.c.v. administration of STK-001 to WT mice resulted in a dose-dependent reduction of non-productive mRNA transcripts, an increase in productive mRNA transcripts, and upregulation of $Na_V1.1$ protein levels in brain. Similar results have been obtained with intrathecal administration of STK-001 in adult rats and non-human primates. The specificity of STK-001 for the *SCN1A* transcript was also assessed via a bioinformatic analysis for human *SCN1A* transcripts and an evaluation of brain samples of neonate mice, and it was demonstrated that STK-001 is highly selective for binding, with low likelihood of off-target effects.

7.3 Toxicology

STK-001 was well-tolerated in single and multiple-dose toxicology studies in non-human primates.

7.4 Pharmacokinetic profile

The first clinical study of STK-001 (MONARCH, STK-001-DS-101, ClinicalTrials.gov: NCT04442295) is an open-label study to investigate the safety and tolerability as well as the pharmacokinetics in plasma and the cerebrospinal fluid (CSF) drug exposure following single and multiple ascending doses (SAD and MAD) of intrathecally-administered STK-001 in children and adolescents aged 2-18 years with Dravet syndrome. To date, a total of 22 patients, grouped by age (2-12 and 13-18 years), were administered STK-001 either on Day 1 as SAD (10, 20, or 30 mg) or on Day 1, Week 4 and Week 8 as MAD (20 mg). Data cutoff was 19 October 2021, after all patients in 30 mg SAD completed visit 5 (day 85) and those in the 20 mg MAD completed visit 7 (week 12). All patients received ≥1 dose of STK-001.

Plasma STK-001 AUC_{last} was similar for the 20 mg SAD cohort and the first dose for the 20 mg MAD cohort. STK-001 CSF levels were detected to last collection available, day 169 for 10 and 20 mg SAD cohort, and day 85 for 30 mg SAD cohort. Overall, mean CSF concentration at day 85 increased with increasing dose from 10 mg to 30 mg. Mean CSF levels after the second MAD dose were higher compared to levels after the first dose, indicating accumulation of STK-001 in CNS tissues with repeated monthly dosing. Observed plasma and CSF levels in patients were in good agreement with animal model predictions. Based on pre-clinical modelling experiments, projected plasma, CSF, and brain levels were strongly correlated across time and dose following single intrathecal STK-001 doses. Thus, CSF and/or plasma levels in MONARCH can be used to estimate STK-001 levels in patients' brains.

ASOs are metabolized in humans by endonucleases and exonucleases but not by liver microsomes and CYP isozymes.[95] Therefore, ASOs are less likely to be involved in potential pharmacokinetic interactions with other co-administered drugs metabolized by CYP enzymes.

7.5 Drug interactions

Studies evaluating potential pharmacokinetic drug interactions with STK-001 have not been conducted.

7.6 Efficacy data

In the ongoing MONARCH study, 70.6% (12/17) of patients in SAD cohorts (10, 20, and 30 mg) and MAD cohort (20 mg) of STK-001 experienced a reduction from baseline in convulsive seizure frequency measured from Day 29 to Day 84 after receiving their first dose of STK-001, including all (7/7) patients in the younger 2-12 years age group. Across all cohorts, median convulsive seizure frequency reductions of 17% to 37% from baseline from Day 29 to Day 84 were observed.

7.7 Adverse effects

In MONARCH, as of data cutoff (19 October 2021), the most common TEAEs were headache, vomiting, seizure, irritability, back pain, fall, and pyrexia. Four patients had study drug-related TEAEs, 2 in the 10 mg SAD, 1 in the 20 mg SAD, none in 30 mg SAD and 1 in the 20 mg MAD cohorts. Five patients had serious TEAEs, none of which was considered related to the study drug. No patients withdrew or died due to TEAEs. No new clinically significant weakness was reported on physical examination. No increase in seizures was identified in a 1-hour EEG recorded about 24 h post-dose, and there were no clinically significant changes in laboratory safety tests which were considered to be related to the study drug.

7.8 Planned studies

MONARCH is ongoing, with plans to enroll approximately 90 patients across 20 sites in the USA. SWALLOWTAIL (ClinicalTrials.gov: NCT04740476), the OLE study, is designed to evaluate the long-term safety and tolerability of repeated doses of STK-001. Enrollment and dosing in SWALLOWTAIL are underway. Additionally, the phase 1/2a ADMIRAL study is also ongoing in the UK. Similar to MONARCH, ADMIRAL is an open-label study of patients with Dravet syndrome aged 2 to <18 years. The primary objectives of the study are to assess the safety and tolerability of multiple doses of STK-001 (up to 70 mg), as well as to characterize the plasma pharmacokinetics and CSF drug exposure. Secondary objectives are to assess the effects of multiple doses of STK-001 on percent change from baseline in convulsive seizure frequency and on overall clinical status and quality of life. Stoke plans to enroll up to 60 patients in the study across multiple sites in the UK.

8 XEN1101

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8.1 Introduction and rationale for development

XEN1101 is a novel, potent, selective KCNQ2/3 (Kv7.2/7.3) potassium channel opener being developed for the treatment of focal-onset seizures and major depressive disorder. The first generation Kv7 channel modulator retigabine/ezogabine was shown to be clinically effective in the treatment of focal epilepsy but was removed from the market for commercial reasons. XEN1101 was developed to be more potent and target-selective than ezogabine without the capacity to form pigmented dimers, potentially providing an improved benefit-risk profile.

Preliminary results with XEN1101were presented in the EILAT XIV progress report[52] and updated in the EILAT XV progress report.[96] The study design of a phase 2 trial was described in the EILAT XV progress report[96], with preliminary results presented here.

8.2 Pharmacology

8.2.1 Activity in experimental models of seizures and epilepsy

In *in vivo* assays, XEN1101 was protective against both electrically- and chemically-induced seizures in rodents, thereby suggesting the potential for broad spectrum use in epilepsy. The activity profile of XEN1101 in experimental models of seizures and epilepsy has been reported in greater detail in previous EILAT XV progress reports.[52,96]

8.2.2 Mechanism of action

The voltage gated K_V7 family comprises 5 subunit channels, $K_V7.1$ to $K_V7.5$. XEN1101 is a highly selective opener of subtypes $K_V7.2$ to $K_V7.5$, which influence neuronal excitability. The $K_V7.1$ subtype, involved in cardiac action potential repolarization, is not activated by XEN1101. In *in vitro* assays, XEN1101 was approximately 4-fold selective for Kv7.2/7.3 (half-maximal effective concentration, EC_{50}: 27 nM) relative to $K_V7.4$ and $K_V7.5$ subtype

channels (EC_{50}: 94 nM and 113 nM, respectively). XEN1101 also exhibits >100-fold selectivity against other non-Kv ion channels and receptors.

By enhancing the open state of $K_V7.2/7.3$ channels, XEN1101 favors a hyperpolarized resting membrane potential and thus reduces rapid action potential spiking. This mechanism has been shown clinically to be effective for treatment of focal seizures in adults with epilepsy with the $K_V7.2/7.3$ channel opener ezogabine.[97] The ability of XEN1101 to suppress cortical and corticospinal excitability in adult humans was previously demonstrated.[98]

8.3 Toxicology

Initiation of clinical development of XEN1101 was supported by a comprehensive nonclinical development program as reported earlier.[52,96] During toxicology assessment with daily oral administration of XEN1101 to cynomolgus monkeys for up to 39 weeks, transient clinical signs of tremors and decreased activity were observed in males given up to 4 mg/kg/day. Based on these results and earlier preclinical studies, the NOAEL was considered to be 4 mg/kg/day, associated with a C_{max} of 218 ng/mL and $AUC_{0\text{-}24h}$ of 1950 ng x h/mL. XEN1101 also exhibited no genotoxicity in Ames, chromosomal aberration, and rat micronucleus assays.

8.4 Pharmacokinetic and metabolic profile

The pharmacokinetic profile of XEN1101 has been described in previous EILAT progress reports[52,96] and found to be suitable for once daily dosing without titration. In early safety, tolerability and pharmacokinetic studies, oral XEN1101 was found to be safe and well tolerated at single doses up to 30 mg and multiple doses up to 25 mg once daily for 10 days. Taking XEN1101 after a high-fat meal enhanced the extent of absorption relative to the fasted state. The absorption rate was relatively slow with a median time to peak plasma concentration (t_{max}) of 4-6 h in the fed state. Treatment with XEN1101 25 mg once daily resulted in a C_{max} of 97 ng/mL and an AUC_{inf} of 19,400 ng x h/mL after 10 consecutive days of dosing. XEN1101 displayed a long terminal elimination half-life (approximately 4-10 days), but apparent steady-state plasma levels were achieved in approximately 1 week after dosing.

In vitro phenotyping assays suggest that CYP3A4 is a major enzyme involved in the metabolism of XEN1101. Other CYP isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6) play a very limited or no role in the metabolism of XEN1101. Both rat and human mass-balance studies conducted with [^{14}C]-XEN1101 indicate that hepatic excretion was the main route of elimination following p.o. dosing. Following 10 days of XEN1101 dosing at 25 mg once daily, the amount of XEN1101 excreted in the urine over a 24-h collection period was about 0.01% of the total administered dose.

In a phase 2a placebo-controlled, double-blind crossover study, single 20 mg doses of XEN1101 induced plasma concentration dependent elevations in resting motor threshold and decreased amplitudes of potentials evoked by transcranial magnetic stimulation (TMS), indicative of reductions in corticospinal and cortical excitability, respectively.[98] Correlations between pharmacodynamic and pharmacokinetic data from this study were used to help set the dose range to be studied in the Phase 2b study.

8.5 Drug interactions

In *in vitro* studies, XEN1101 elicited no inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 at concentrations above anticipated efficacious levels, suggesting low potential for drug-drug interactions through inhibition of these CYP isoenzymes.

While XEN1101 shows high metabolic stability in cryopreserved human hepatocytes, its metabolism *in vitro* was inhibited by the selective CYP3A4 inhibitor ketoconazole, suggesting a role of CYP3A4 in metabolic clearance. Only a single preliminary clinical drug-drug interaction cohort from an ongoing clinical pharmacology study has been conducted to date. In this study, a single dose of itraconazole (400 mg oral solution) was given 1.5 h prior to an 11th daily dose of XEN1101 (20 mg oral capsule once daily) in normal volunteers. Preliminary analysis suggested a modest effect of itraconazole, a strong inhibitor of CYP3A4, on overall XEN1101 exposure, with an approximately 10% increase in C_{max} and a <30% increase in $AUC_{0\text{-}24}$. Population pharmacokinetics analysis of the Phase 2b study described below predicted an increase of about 32% in these parameters at steady state in the presence of CYP3A4 inhibitors. Conversely, the model predicted that concomitant use of a single CYP3A4 inducer decreases XEN1101 C_{max} by 12% and $AUC_{0\text{-}24}$ by 21.6%. Concomitant use of two or more CYP inducers was predicted to decrease C_{max} by 27% and $AUC_{0\text{-}24}$ by 39%.

8.6 Efficacy data

X-TOLE was a Phase 2b randomized, double-blind, placebo-controlled, multicenter, study designed to evaluate the efficacy, safety, and tolerability of XEN1101 compared to placebo when administered once daily as adjunctive treatment in adults diagnosed with focal epilepsy.[99] As described previously[96], patients enrolled in this study had ≥4 countable focal seizures per month (recorded using an eDiary). Baseline focal seizure frequency was established over an 8-week period prior to the double-blind period. At baseline, patients were receiving stable treatment with 1-3 ASMs. Treatment with implanted neurostimulators and/or cannabinoids was also permitted, as was use of benzodiazepines as rescue medications for seizure clustering. Over half of the patients were taking a CYP3A4 inducing ASM during the study.

A total of 325 patients with a median of 13.5 focal seizures per month at baseline were treated after being randomized to one of four treatment groups in a 2:1:1:2 ratio (25 mg, 20 mg, 10 mg, and placebo), stratified by use of background CYP inducing ASMs. After completion of the 8-week double-blind period, eligible patients could elect to enroll in an OLE with a continued dosage of XEN1101 20 mg once daily for up to 3 years to evaluate long-term safety, tolerability, and efficacy.

The primary endpoint of the study was the median percent change in monthly (28 days) focal seizure frequency from baseline over the double-blind period versus placebo as well as the frequency of TEAEs. Secondary endpoints included the proportion of patients achieving ≥50% reduction in focal seizure frequency (50% responder rate) and percent change from baseline focal seizure frequency for each week of the double-blind period. Additionally, the physician-rated Clinical Global Impression of Change (CGI-C) and patient-reported Patient Global Impression of Change (PGI-C) scores were collected. Safety evaluations included TEAE monitoring, clinical laboratory tests, vital signs, ECGs, neurologic and physical examinations, and Columbia-Suicide Severity Rating Scale assessment.

The study met all the primary and key secondary efficacy endpoints, with XEN1101 demonstrating statistically significant, dose-dependent reductions from baseline in monthly focal seizure frequency compared to placebo. A statistically significant dose-response trend was observed in monthly focal seizure frequency compared to placebo. The median percent reductions from baseline in focal seizure frequency were 33.2% ($p = 0.035$, n=46), 46.4% ($p < 0.001$, n=51), and 52.8% ($p < 0.001$, n=112) in the 10 mg, 20 mg, and 25 mg groups, respectively, compared to placebo (18.2%, n=114) (Figure 5).

A pre-specified weekly assessment of seizure frequency was also conducted followed by a *post hoc* statistical pair-wise comparison between placebo and each treatment, yielding similar results. At Week 1, XEN1101 demonstrated a dose-dependent reduction of 39.1% ($p < 0.05$), 41.5% ($p = 0.06$) and 55.4% ($p < 0.001$) in the 10 mg, 20 mg, and 25 mg groups, respectively, from baseline in median focal seizure compared to placebo (20.2%). Consistent with lack of need for titration, there was a marked reduction in median focal seizure at week 1 in all doses compared with placebo. The 50% responder rates were 28.3% at 10 mg ($p = 0.037$), 43.1% at 20 mg ($p < 0.001$) and 54.5% at 25 mg ($p < 0.001$) compared to 14.9% for placebo. These marked reductions in seizure frequency were associated with statistically significant improvements in overall health status as assessed both by physician and patient reporting. The proportion of patients considered "much improved" or "very much improved" in the 25 mg XEN1101 group was 46.4% ($p < 0.001$) on the CGI-C scale compared to 22.8% in the placebo group, and 42.9% ($p < 0.001$) on the PGI-C scale compared to 21.9% in the placebo group.

X-TOLE included a "difficult-to-treat" patient population given that the median seizure frequency was 13.5/month at baseline, 50.8% of study subjects were taking 3 concomitant ASMs, and the median number of ASMs taken prior to study entry was 6. Additional *post hoc* analyses were performed in the 25 mg treatment group to assess the role of disease severity on median percent change in seizure frequency. Compared with baseline, subjects with ≤8.5 seizures/month at baseline experienced a 70.6% reduction compared to 50.8% for those with >8.5 seizures/month. Median monthly focal seizure reduction was 58% in subjects who failed ≤6 ASMs at baseline and 43% in subjects who failed >6 ASMs. Median monthly focal seizure reduction was 60.9% for subjects with 1-2 concomitant ASMs and 50.8% for subjects with 3 concomitant ASMs.

The number of subjects who did not complete the double-blind treatment period for any reason was 5 (4.4%) in the placebo group and 1 (2.1%), 8 (15.7%), and 26 (22.6%) in the 10 mg, 20 mg and 25 mg groups, respectively. After completion of the double-blind period, 96.5% of study completers entered the OLE study, which is ongoing. *Post hoc* analyses suggest that efficacy may be more robust in patients with less severe disease, which mirrors the likely use of XEN1101 if approved.

8.7 Adverse effect profile

XEN1101 was generally well-tolerated. The incidence of TEAEs was higher in the active treatment groups, with 67.4%, 68.6%, and 85.1% of patients in the 10 mg, 20 mg, and 25 mg XEN1101 groups, respectively, experiencing at least one TEAE, compared to 62.3% of patients in the placebo group. The majority of TEAEs were reported as mild or moderate in intensity. Across all XEN1101 dose groups (n=211), the most common TEAEs were dizziness (n = 52, 24.6%), somnolence (n = 33, 15.6%), fatigue (n = 23, 10.9%), and headache (n = 21, 10.0%). The breakdown of subjects with dizziness across dose groups including placebo was as follows: 8 (7.0%) in the placebo group, 3 (6.5%) in the 10 mg group, 13 (25.5%) in the 20 mg group, and 36 (31.6%) in the 25 mg group. There were no safety signals of concern from clinical laboratory evaluations, vital signs, or ECGs. No TEAEs of pigmentary abnormalities were reported during the double-blind period of the study, nor to date in the preliminary analysis of the ongoing OLE study. The incidence of serious TEAEs was similar in all 4 arms of the study with 3 (2.6%) subjects in placebo, 2 (4.3%) in the 10 mg, 2 (3.9%) in the 20 mg, and 3 (2.6%) in the 25 mg group. No deaths occurred during the study. These results were consistent with those reported for other ASMs used in patients with focal seizures.

8.8 Planned studies

The ongoing OLE of the Phase 2b study will reach completion in October 2024. Planning is ongoing for an End of Phase 2 meeting with the FDA to discuss phase 3 study design and continuing development. Enrollment is currently ongoing in two phase 2 randomized, placebo controlled trials of XEN1101 for the treatment of major depressive disorder.

9 CONCLUSIONS

The seven compounds in more advanced clinical development presented in this article are being developed through different development strategies. Bumetanide, originally a diuretic, and the weight-loss medication lorcaserin are examples of repurposed compounds. Bumetanide derivatives illustrate approaches to improve efficacy and safety profiles by structural modification of a prototype drug. Other compounds are designed to exert their effects by novel mechanisms, such as acting on specific $GABA_A$ receptor subtypes (darigabat) or serotonin receptor subtypes (lorcaserin), inhibition of cholesterol 24-hydroxylase (soticlestat), and augmentation of gene output (STK-001). A remarkable proportion of these compounds aim for narrrow indications such as treatment of seizures associated with Dravet

syndrome, Lennox-Gastaut syndrome, or other rare DEEs. This appears to reflect a shift in ASM development towards orphan indications and the targeting of less common, but often more severe epilepsy syndromes.

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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.

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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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Figure Legends

Figure 1. Schematic representation of the arrangement of subunits in the $GABA_A$ receptor. The $GABA_A$ receptor is a heteropentameric arrangement, with the most common arrangement of subunits consisting of two α, two β, and one γ. The GABA binding site occurs at the interface of the α and β subunits. When the γ subunit is γ2 and the α subunit is either α1, α2, α3, or α5 (but not α4 or α6), a benzodiazepine recognition site is formed at the interface of these subunits. When activated, $GABA_A$ receptors generally permit the flow of chloride ions along their concentration gradient which is predominantly from outside to inside the neuron, thereby resulting in a hyperpolarisation of the membrane potential that reduces excitability and the probability of the neuron firing further action potentials.

Figure 2. Schematic illustration of the double-blind, randomized, placebo-controlled, parallel-group, phase 2 clinical trial of darigabat in patients with drug-resistant focal epilepsy (CVL-SZ-001, ClinicalTrials.gov: NCT04244175). The trial compares darigabat at 25 mg b.i.d. and 7.5 mg b.i.d. with placebo and comprises of a 2-week titration period followed by an 8-week maintenance period and a 3-week taper period. Patients who complete the 8-week maintenance phase are eligible to enter the 57-week open label extension trial (ClinicalTrials.gov: NCT04686786). Abbreviations: R = randomisation; RO = receptor occupancy.

Figure 3. Treatment effects of soticlestat in *Scn1a*$^{+/-}$ mice. (A) Kaplan–Meier plot comparing 40-day survival between sex and treatment groups (0.02% soticlestat, n = 30/sex; vehicle control, n = 50/sex. $^{**}p < 0.0011$; $^{***}p < 0.0001$, log rank Mantel–Cox). (B) The average percentage of generalized tonic-clonic seizure (GTCS) events that progressed to hindlimb extension (HLE) differed between treatment groups. Symbols represent each individual mouse (mice with no GTCS are not included). The horizontal line represents the median, and error bars represent 95% confidence interval (0.02% soticlestat, n = 4; vehicle control, n = 54. $^{*}p < 0.003$, Mann–Whitney).

Figure 4. Median change and placebo-adjusted change from baseline in: (A) convulsive seizure frequency in patients with Dravet syndrome (DS), and (B) drop seizure frequency in patients with Lennox-Gastaut syndrome (LGS). [a]Rank-transformed ANCOVA adjusting for baseline seizure frequency and protocol amendment cohort. [b]Hodges–Lehmann estimation of the median treatment difference (percentage change from baseline in soticlestat vs percentage change from baseline in placebo). ANCOVA, analysis of covariance

Figure 5. Median percent change in seizure frequency from baseline after treatment with XEN1101 or placebo.