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Progress Report on New Antiepileptic Drugs: A Summary of the Fifteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XV). I. Drugs in Preclinical and Early Clinical Development

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Summary

Since 1992, the Eilat Conferences have provided a forum for all stakeholders in the epilepsy community to appraise the latest data on new antiepileptic drugs and emergency seizure treatments, including in recent years, updates on progress with the development of novel monitoring and therapeutic devices. Because of the Covid-19 pandemic, the Fifteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XV) was held as a fully virtual conference on July 27-30, 2020 for the sessions on drugs and on August 3, 2020 for the sessions on devices, and was attended during the five days by over 500 participants from 63 countries. This Progress Report summarizes

key preclinical and initial (Phase I) clinical data on eight investigational treatments which are currently in early development, including 2-deoxy-D-glucose, GAO-3-02, JNJ-40411813, NBI-921352, NTX-001, *sec*-butylpropylacetamide (SPD), XEN1101, and XEN496. This report provides an overview of current scenarios in the area of treatment discovery and development. The information presented illustrates a variety of innovative strategies, including exploration of compounds with novel mechanisms of action, transplantation of interneurons into epileptogenic brain regions, and the targeting of rare, previously neglected syndromes.

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Key Points

- The Fifteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XV) was held as a virtual conference on July 27-30 and August 3, 2020.
- This article summarizes currently available data on eight novel treatments in early development.
- Key preclinical and clinical (Phase I) findings are discussed for 2-deoxy-D-glucose, GAO-3-02, JNJ-40411813, NBI-921352, NTX-001, *sec*-butylpropylacetamide (SPD), XEN1101, and XEN496
- The information presented illustrates a variety of innovative research strategies, including exploration of compounds with novel mechanisms of action and transplantation of interneurons into epileptogenic brain regions.

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1. INTRODUCTION

Despite the introduction of over 20 second-generation antiepileptic drugs (AEDs) during the past three decades, including 10 new AEDs between 2008-2020, more than 30% of people with epilepsy do not attain seizure control with currently available medications, and for some epilepsy syndromes seizure outcome is even worse.¹ Hence, there is still an urgent need for newer, more effective treatments for epilepsy. Efforts to develop treatments with improved tolerability and safety are also warranted.

Since 1992, the Eilat Conferences have provided a forum for stakeholders involved in clinical care, basic and clinical research and regulatory agencies to meet on a biennial basis and discuss the latest advances in the discovery and development of new epilepsy treatments. Since 2016, sessions dedicated to discussion of novel therapeutic devices as well as devices for seizure monitoring were added to the Conferences' program. Because of the disruption caused by the Covid-19 pandemic, the 2020 Fifteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XV) was held virtually on July 27-30, 2020 for the sessions on drugs, and on August 3, 2020 for the sessions on devices. A total of 534 participants from 63 countries attended the Conference, with about 75% of attendees being healthcare professionals / academic researchers and 25% being professionals working in the pharmaceutical industry. The virtual format did not prevent lively interactions among participants, and presenters were able to address in real time the many questions raised by the audience. Nevertheless, the face-to-face discussions, the social interaction and the networking opportunities made possible by the physical presence of participants were surely missed. Accordingly, the Organizing Committee is confident that the next Conference scheduled for 2022 will revert to the traditional format and unique boutique atmosphere which has characterized the EILAT Conferences since their inception.

The core of EILAT XV was the presentation of data on compounds that are currently under development. Members of the organizing committee used all information available to them to identify eligible compounds and presenters. For drugs developed by the pharmaceutical industry, presenters and authors of the summary reports were selected in consultation with the companies responsible for development. The primary authors of this article conducted a thorough review of the summaries, edited sections as appropriate, and interacted extensively with the authors of the individual summaries to ensure that the material included is presented clearly and as informative as possible. The subsequent sections provide summaries of key data on eight treatments in preclinical or early (phase I) clinical development, including 2-deoxy-D-glucose, GAO-3-02, JNJ-40411813, NBI-921352, NTX-001, SPD, XEN1101, and XEN496 (also known as retigabine or ezogabine, but

now being developed in a multi-particulate “sprinkle” oral formulation specifically for the treatment of *KCNQ2* encephalopathy). Data on compounds in more advanced clinical development in epilepsy are presented in an accompanying article.²

The list of investigational treatments discussed in the EILAT XV Progress Reports should not be regarded as exhaustive. Other molecules currently in development discussed in the EILAT XIV Progress Reports^{3,4} include adenosine and adenosine kinase inhibitors, cannabidivarin, FV082, FV137, huperzine A (BIS-001), JJ-5511118 and analogs, ketone-enhanced antiseizure compounds, middle chain fatty acids, OV329, oxynytone, padsevoni and TAK-935 (OV935). The EILAT XV Conference also hosted presentations on antisense oligonucleotide therapy for Lafora disease, bumetanide derivatives, and omaveloxolone (RTA-408), but presenters or sponsors did not wish to have a summary of these compounds included in the Progress Report. Additional investigational drugs for seizures /epilepsy are listed in the proceedings of the 15th Antiepileptic Drug and Device Trials Conference (AEDD), which took place in Miami, Florida on May 22–24, 2019.⁵ The AEDD Conference is also a biennial event dedicated to drug and device development in epilepsy, with a format similar to the EILAT Conference but generally with greater emphasis on methodological issues. The AEDD proceedings, however, do not provide detailed information on compounds presented at the meeting.⁵

As is the case for all currently available drugs for the treatment of epilepsy, the majority of compounds under development act on seizures rather than on the underlying epilepsy. Thus, “antiseizure medications (ASMs)” would be a more appropriate term than ‘antiepileptic drugs (AEDs)’ to designate this class of therapeutic agents.⁶ In spite of this, in the present report and in the accompanying article we decided to retain the term ‘AEDs’ because such term is traditionally incorporated in the name of the Eilat Conferences.

2. 2-DEOXY-D-GLUCOSE

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Molecular structure of 2-deoxy-D-glucose and glucose here

2.1 Introduction and rationale for development

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2-deoxy-D-glucose is a glucose analogue differing from glucose only by removal of an oxygen atom at the 2 position, which prevents the isomerization of glucose-6-phosphate to fructose-6-phosphate, and thereby reversibly inhibits glycolysis. The actions of 2-deoxy-D-glucose in models of seizures and epilepsy and its ability to reduce metabolic flux in the glycolytic pathway were discovered during investigation of mechanisms of carbohydrate restriction underlying the efficacy of the ketogenic diet.^{7,8} These effects have been described previously in EILAT XIII and XIV Progress Reports,^{9, 4} with new observations and updates reported here.

2.2 Pharmacology

2-deoxy-D-glucose shows protective activity in preclinical models spanning acute seizures, chronic epilepsy, and traumatic brain injury.

2.2.1 Actions in acute experimental seizure models

Multiple studies wherein 2-deoxy-D-glucose was administered per os (p.o.) or parenterally at doses of 37.5-250 mg/kg have demonstrated anticonvulsant effects *in vivo* in rodent acute seizure models including the mouse 6 Hz model and Frings audiogenic seizure-susceptible mouse model of reflex seizures. 2-deoxy-D-glucose dose-dependently delays the onset and reduces the duration and severity of status epilepticus (SE) induced by pilocarpine in mice.¹⁰ In rats, 2-deoxy-D-glucose delays the onset, and reduces the duration and severity of SE induced by both pilocarpine and kainic acid.¹¹ Ictal epileptic regions with high metabolic and energy demand during SE have focal increased blood flow. This enables temporally regulated delivery of O₂ and glucose with millisecond control and micron precision at the level of capillaries by neurovascular coupling. As a result, 2-deoxy-D-glucose is delivered preferentially into neural circuits underlying SE. This property offers a novel therapeutic opportunity for focal and generalized seizure control compared to currently available drugs.

2.2.2 Actions in chronic experimental epilepsy models

2-Deoxy-D-glucose also shows antiepileptogenic effects in the chronic kindling model of experimental epilepsy. At doses of 37.5-250 mg/kg administered by gavage or intraperitoneally (i.p.) 30 min before conventional twice-daily kindling stimulation of the perforant path or olfactory bulb in rats, 2-deoxy-D-glucose produced a twofold slowing of progression of repeated seizures to the milestone of evoked bilateral tonic-clonic (Class V) seizures.⁸ These effects were also observed

when the minimal effective dose of 37.5 mg/kg was administered as long as 10-15 min after each evoked seizure as a consequence of enhanced postictal delivery of 2-deoxy-D-glucose by increased postictal blood flow mediated by neurovascular coupling. The efficacy of 2-deoxy-D-glucose when administered 10-15 min after seizure-induction implies that 2-deoxy-D-glucose may provide novel therapeutic opportunities for treatment of acute repetitive seizures as well as SE. Further evidence for the effects of glycolytic inhibition against seizure-induced epileptogenesis includes reduction of kindling progression in a rapid-kindling protocol (12 kindling stimulations per day at 30-min intervals for four consecutive days) by administration of fructose 1,6 biphosphate, which diminishes glycolysis by diverting glucose into the pentose phosphate pathway.¹²

2.2.3 Actions in a chronic model of posttraumatic epilepsy induced by experimental controlled cortical impact

As described in EILAT XIII and XIV Progress Reports,^{4,9} 2-deoxy-D-glucose exerts protective effects against secondary neurological damage and associated behavioral effects in the controlled cortical impact model of traumatic brain injury in both Sprague-Dawley rats and Perforant Path Kindling Susceptible (PPKS) rats, a rodent strain with enhanced susceptibility to kindling progression.¹³ In PPKS rats, treatment with 2-deoxy-D-glucose at a dose of 250 mg/kg/day by oral gavage for 2 weeks after controlled cortical impact dose-dependently reduced the incidence of post-traumatic seizures by ~60%, which was not observed with doses of 50 mg/kg/day. Dosing with 250 mg/kg/day for 3 days and 1 day also reduced the incidence of focal and generalized spike-wave seizures by ~50% and ~33% respectively, demonstrating that treatment periods as short as 1-14 days can be effective in protecting against the development of epilepsy in this model.¹⁴

2.2.4 Mechanisms of acute and chronic actions

Results of experiments on the mechanisms of action of 2-deoxy-D-glucose have been described in the Eilat XIV Progress Report.⁴ More recent studies have confirmed that the activity-dependent actions of 2-deoxy-D-glucose in CA3 neurons in elevated extracellular $[K^+]_o$ medium take place presynaptically as indicated by: 1) reduction in the frequency but not amplitude of miniature excitatory postsynaptic currents (mEPSCs), 2) reduction in the frequency but not amplitude of excitatory postsynaptic burst currents, and 3) lesser but significant reduction of frequency of pharmacologically isolated inhibitory postsynaptic currents compared to excitatory currents without changes in amplitude.¹⁵

2.3 Toxicology

In preclinical toxicology studies including four studies in rats and two studies in beagle dogs, 2-deoxy-D-glucose was found to induce dose-dependent cardiac myocyte vacuolation in rats. The most recent toxicological studies have demonstrated that doses of 125 mg/kg/day by gavage in rats (surface area-adjusted human equivalent dose ~ 75 mg/kg) produce reversible cardiac myocyte vacuolation after 14 days of treatment. Vacuolation was not observed at 8 days of treatment. The vacuolation induced by this dose after 14 days of treatment, as well as after 14 days and longer treatment durations with highest administered doses of 375 mg/kg/day in rats, demonstrated complete reversibility as consistent with autophagy induced by metabolic stress. In a small human study of 2-deoxy-D-glucose as adjuvant therapy for prostate and advanced malignancies, grade 3 asymptomatic QTc prolongation was observed at doses of 60/mg/kg/day but not at doses of 45 mg/kg/day,¹⁶ a finding which will likely guide potential dosing in patients with epilepsy. Prolonged and repeated episodes of hypoglycemia in patients with diabetes and repeated daily episodes of symptomatic glycolytic inhibition by 2-deoxy-D-glucose in rats may have proconvulsant effects. Still, the doses and durations of administration of 2-deoxy-D-glucose defined in preclinical efficacy and toxicology studies support safety and efficacy of limited short-term, repetitive dosing of 2-deoxy-D-glucose for acute conditions such as SE, acute repetitive seizures, and immediately after brain injury to potentially prevent long-term adverse consequences.

2.4 Pharmacokinetics

The pharmacokinetics of 2-deoxy-D-glucose are summarized in the EILAT XIV Progress Report.⁴ A non good-laboratory-practice (GLP) range-finding study in Beagle dogs dosed with 2-deoxy-D-glucose orally and intravenously (i.v.) demonstrated an absolute oral bioavailability in the range of 59.6-87.4%.

2.5 Planned studies

The novel acute and chronic mechanisms of 2-deoxy-D-glucose, including focal enhanced delivery to brain regions with high metabolic and energetic demands by in vivo neurovascular coupling, are unique compared to currently marketed AEDs. Following initial preclinical toxicological observations of dose-dependent cardiac myocyte vacuolation with features of reversible autophagy, additional toxicological studies have demonstrated full reversibility and safety of limited duration of

treatment (8 days) even at the highest examined doses. With this preclinical safety profile and demonstration of safety and tolerability at daily doses of 45 mg/kg/day p.o. for 3 weeks in clinical cancer trials,¹⁶ initial development plans for 2-deoxy-D-glucose have shifted from chronic treatment of drug resistant epilepsy to limited repetitive dosing for SE, acute repetitive seizures, and focal brain delivery in combination with brain stimulation device therapies. Focal delivery of 2-deoxy-D-glucose to ictal brain regions with high metabolic energy demands is anticipated to be advantageous and without risk of cardiac toxicity. As also demonstrated in preliminary studies in rodent models of post-traumatic epilepsy and post-traumatic stress disorder, acute repetitive dosing of 2-deoxy-D-glucose after both major and mild traumatic brain injury may prevent delayed consequences such as post-traumatic epilepsy and post-traumatic stress disorder. Immediate development plans include Phase II clinical trials to assess safety, tolerability, and pharmacokinetics of acute oral and i.v. formulations in patients with epilepsy.

3. GAO-3-02

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3.1 Introduction and rationale for development

GAO-3-02 is a drug candidate for epilepsy, currently developed by GAOMA Therapeutics. GAO-3-02 was initially selected through a phenotypic screening approach based on its ability to resolve neuroinflammation, improve cognition and limit seizures. By using this approach, the objective was to identify an active ingredient displaying a new mechanism of action in comparison with standard antiseizure drugs.

GAO-3-02 is a derivative of an endogenous molecule, the N-docosahexaenoyl ethanolamine (synaptamide). The objective in generating GAO-3-02 was to obtain a molecule preserving synaptamide's pharmacological properties,¹⁷ while optimizing its biological fluid solubility and its stability against enzymatic degradation. Indeed, in addition to the already known anti-inflammatory property of synaptamide, we demonstrated its beneficial effects on cognition. To date, GAOMA

Therapeutics has completed various preclinical proof-of-concept studies, demonstrating the efficacy of GAO-3-02 on both seizures and cognition.

3.2 Pharmacology

3.2.1 Activity profile in the amygdala kindling model

The anticonvulsant effect of i.p. administered GAO-3-02 was tested in fully amygdala-kindled male Sprague-Dawley rats ($n = 13$), a model of difficult-to-treat focal-onset seizures.¹⁸ Briefly, rats were first injected with single doses of GAO-3-02 at 5, 10 and 50 mg/kg on days 1, 6 and 9, respectively. During weeks 3, 4 and 5, these same rats received a single daily dose of 5, 10 and 20 mg/kg for 4 consecutive days, respectively (see Table 1). In the case of single GAO-3-02 doses, rats were stimulated 45 min after GAO-3-02 administration. In the case of repeated GAO-3-02 doses, rats were stimulated 24 h after the 4th administration only.

Single dose testing. At Day 1 (D1), after a dose of 5 mg/kg, seizure severity decreased by $23 \pm 9\%$ ($P = 0.025$). When rats received a higher dose of GAO-3-02 (10 mg/kg) at D6, the decrease in seizure severity was not significantly different from that observed immediately after the 5 mg/kg dose. However, a delayed further decrease in seizure severity was observed at D7, reaching $-46 \pm 12\%$ compared to D0 ($P = 0.0027$). Increasing the GAO-3-02 dose to 50 mg/kg at D9 enhanced the decrease in seizure severity at D10, reaching $-62 \pm 10\%$ compared to D0 ($P < 0.0001$).

Evaluation of the effect of GAO-3-02 revealed 3 groups of rats: those starting to respond at 5 mg/kg (7/13), at 10 mg/kg (2/13) and at 50 mg/kg (4/13). When focusing on rats responding at the 5 mg/kg dose, the reduction in seizure severity reached $-43 \pm 13\%$ at D1 compared to D0 ($P = 0.0148$). Intriguingly, the greatest reduction was observed 2 days after the 10 mg/kg dose ($-69 \pm 13\%$ compared to D0 ($P = 0.0019$)). In all rat subgroups, the maximum effect on seizure severity was delayed 24 to 48 h after GAO-3-02 administration.

Repeated dose testing. Using the same population of rats, we next examined the effect of repeated doses of GAO-3-02 (5, 10 and 20 mg/kg, each administered daily for 4 consecutive days). At the daily dose of 5 mg/kg, the average seizure severity was kept at a level close to that previously obtained with the single dose of 50 mg/kg ($-54 \pm 14\%$ compared to D0, $P = 0.0028$). The proportion of seizure-free rats increased with repeated dosing in comparison with the single dose administration: 31% of the rats were seizure-free 24 and 48 h after a single dose of GAO-3-02 at 10 mg/kg compared to 54% after repeated doses of 5 mg/kg. When focusing on rats responding at the 5

mg/kg dose (7/13 rats), the reduction in seizure severity was greatest after repeated doses of 5 mg/kg ($-86 \pm 14\%$ compared to D0, $P < 0.001$), with 6/7 rats (86%) being seizure-free at that time.

Treatment withdrawal. We examined whether absence of seizures or decrease in seizure severity was maintained at 7, 15, 42 and 56 days after the last administration of GAO-3-02. At these time points, the number of rats that still presented with stage 0 seizures was 6/13, 6/13, 4/13 and 4/13, respectively.

Overall, GAO-3-02 exerts a more potent and longer-lasting effect on seizure severity compared to levetiracetam and brivaracetam.¹⁸

3.2.2 Activity profile in other models

GAO-3-02 is ineffective in reducing generalized tonic-clonic seizures induced by subcutaneously (s.c.) administered pentylentetrazole (PTZ) in Swiss mice (22-25g) at doses up to 20 mg/kg i.p., following either single or repeated dosing (4 consecutive days).¹⁸ The lack of activity in the PTZ model is not surprising in view of GAO-3-02 putative mechanism of action (see below).

We previously showed that spatial learning in the Morris Water Maze is impaired following pilocarpine-induced SE in weanling male Sprague-Dawley rats.¹⁹ We investigated whether GAO-3-02 treatment in the early stages post-SE protected spatial learning after onset of epilepsy. To this end, 45 rats were included in the study. Rats subjected to SE ($n = 30$) were treated with vehicle ($n = 15/30$) or GAO-3-02 (2 mg/kg i.p., $n = 15/30$) one hour after pilocarpine-induced SE onset and once daily for one week (total of seven injections). Healthy control rats ($n = 15$) received vehicle only. Spatial learning assessment in the Morris Water Maze started 6 weeks after pilocarpine-induced SE (equivalent to 5 weeks after the last administration of treatment, and about 2 weeks after the onset of epilepsy). The experiment had a testing period of four consecutive days and confirmed that spatial learning is impaired in rats subjected to pilocarpine-induced SE. Indeed, while healthy control rats showed learning over the 2-4 training days with significantly lower latencies to reach the platform compared with day 1, performance of rats subjected to SE was significantly impaired at days 1-4 ($P < 0.001$). Moreover, only 85% of rats subjected to SE were able to locate the hidden platform in the water maze, despite 12 attempts over four days of testing, whereas, all of the healthy control rats successfully found the platform from the second testing day. Treatment with GAO-3-02 significantly reduced the learning impairment caused by SE. Indeed, the latencies to find the platform in GAO-3-02-treated rats were shorter than those observed in rats subjected to SE and treated with vehicle, on testing day 3 (43.8 ± 5.7 sec versus 58.0 ± 6.5 sec, $P < 0.05$) and testing day 4 (39.0 ± 5.0 sec versus 55.0 ± 7.5 sec, $P < 0.05$). In addition, all GAO-3-02-treated rats were able

to find the platform at testing day 4. These results reveal the sustained effect of GAO-3-02 on the reduction of spatial learning impairment caused by SE.

3.2.3 Mechanism of action

A comprehensive understanding of GAO-3-02 mechanism of action is part of current work at GAOMA. GAO-3-02 has a structure close to synaptamide, which was recently described as a specific ligand of the adhesion G-protein-coupled receptor 110 (GPR110).^{17,20} Because of their structural similarity, GAO-3-02 and synaptamide are expected to share similar mechanisms of action. However, GAO-3-02 is more water soluble than synaptamide and is expected to show a higher stability against enzymatic degradation. It is also expected to display a substantial blood brain barrier passage such as synaptamide.²¹

Previous reports have shown that GPR110 activation by synaptamide leads to an increase of the cyclic adenosine monophosphate (cAMP)/protein-kinase A (PKA) signaling pathway and to attenuation of the expression of pro-inflammatory markers such as interleukin-1 β (IL-1 β), IL-6, tumor necrosis factor α (TNF α) and nitric oxide synthetase 2 (NOS2).²² In line with the anti-inflammatory effect mediated by synaptamide/GPR110 signaling, we demonstrated that GAO-3-02 concentration-dependently resolved the inflammatory response induced by IL-1 β in Immortalized Human Microglial cells (-85% at 150 nM, $P < 0.0001$). Because fever can precipitate seizures, especially in children, we also tested the effects of GAO-3-02 in rat pups treated with lipopolysaccharide (LPS), a bacterial endotoxin commonly used as a fever-inducing pro-inflammatory stimulus. When administered immediately after LPS at a dose of 2 mg/kg i.p. GAO-3-02 significantly reduced the inflammatory response by -89% ($P = 0.0037$).

GPR110 activation by synaptamide has been shown to promote hippocampal development, neurite outgrowth and synaptogenesis,¹⁷ key mechanisms of brain plasticity associated with improved cognitive functions. Based on this premise, we found that GAO-3-02 increases substantially the magnitude of CA1 hippocampal long-term potentiation (LTP), a key cellular and molecular mechanism underlying learning and memory,²³ in a pilocarpine-induced SE model of temporal lobe epilepsy. Indeed, while CA1 pyramidal neurons in slices prepared from healthy rats, perfused with saline, exhibited robust LTP (Fig. 1A; 162.3 ± 5.8 % $t = 45-50$ min, $p < 0.001$) and neurons in slices prepared from rats subjected to pilocarpine-induced SE, perfused with saline, failed to express LTP (Fig. 1A; 109.6 ± 6.1 %; $t = 45-50$ min, $P = 0.13$), the magnitude of LTP was significantly enhanced when slices, prepared from pilocarpine-induced SE rats, were perfused with GAO-3-02 at 100 nM (Fig. 1A; 132.2 ± 5.01 %; $t = 45-50$ min, $P < 0.001$) or 400 nM (Fig. 1A; 159.9 ± 10.7 %, $t = 45-50$

min, $P < 0.001$). We also demonstrated a significant LTP induction following administration of GAO-3-02 i.p. (2, 5 and 10 mg/kg), or p.o. (30 and 100 mg/kg) in rats subjected to pilocarpine-induced SE (Fig.1B-D).

Taken together, results obtained to date suggest that GAO-3-02 has an antiseizure effect and a beneficial effect on cognition, likely through reducing neuroinflammation and fine-tuning synaptic plasticity.

Figure 1 here

3.3 Toxicology

A formal toxicological study with GAO-3-02 has not yet been conducted. However, no deleterious signs (including weight modification, grooming, coat quality, lack of porphyrin) were observed in mice and rats exposed to high doses (up to 100 mg/kg p.o.) for up to 3 weeks. We observed that rats subjected to pilocarpine-induced SE at the age of 21 days significantly lose weight over the next days. Daily administration of GAO-3-02 (10 mg/kg i.p., 30 or 100 mg/kg p.o.) allowed the animals to recover their normal weight from the third day of treatment, suggesting beneficial effects and a good tolerability of the molecule.

3.4 Pharmacokinetics and metabolic profile

GAO-3-02 is at a preclinical development stage and no human data are available yet.

Due to its amphiphilic and lipidic nature, GAO-3-02 is expected to partly interact with lipoproteins and some plasma proteins such as serum albumin. The designed chemical structure of GAO-3-02 protects it against enzymatic attack on different sites of the molecule. Formation of specific oxygenated derivatives on its lipid moiety is expected *in vivo*.

3.5 Drug interactions

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

3.6 Planned studies

Planned studies include more comprehensive studies to evaluate GAO-3-02's mechanism of action. We will examine whether GAO-3-02 binds to GPR110 and/or other targets and how receptor(s)

activation can influence the resolution of inflammation, improvement of cognition and reduction in seizure frequency and/or severity in different animal models of epilepsy. In parallel, pharmacokinetic studies will also be conducted in appropriate animal models. Comprehensive IND-enabling studies are then planned over 2021 and 2022.

4. JNJ-40411813

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Molecular structure of JNJ-40411813 here

4.1 Introduction and rationale for development

The metabotropic glutamate receptor type 2 (mGlu2), which is expressed predominantly presynaptically, is involved in limiting excessive glutamate release through a feedback inhibition mechanism.²⁴ As summarized in the EILAT XIV Progress Report,⁴ inhibition of glutamatergic transmission by mGlu2 agonists and positive allosteric modulators (PAMs) is associated with seizure protecting properties in some experimental models.

In previous investigations with two mGlu2 PAMs (the Phase II clinical candidate JNJ-40411813/ADX71149 and the preclinical tool compound JNJ-46356479), evidence was provided for a potential synergistic antiseizure activity between these compounds and levetiracetam, which is known to modulate glutamate release by acting at the synaptic vesicle protein SV2A.^{4,25,26} In collaboration with UCB, we now describe new data to strengthen the pharmacodynamic interaction between those two targets. Firstly, we confirm the synergistic antiseizure activity in the 6 Hz model in an independent laboratory. Secondly, we highlight the beneficial effects of the combination of mGlu2 PAM and levetiracetam in additional preclinical epilepsy models. Finally, we investigate whether the pharmacodynamic interaction is (more) specific to levetiracetam compared to other AEDs.

4.2. Pharmacology

4.2.1 Activity profile in experimental models of seizures and epilepsy

The results of previous studies on the activity of JNJ-40411813 in seizure models have been summarized in the EILAT XIV Progress Report.⁴ In summary, JNJ-40411813 was inactive in the maximal electroshock seizure (MES) model, the s.c. PTZ model and the mouse corneal kindling model at doses up to 100 mg/kg s.c. However, it did show dose-dependent antiseizure activity after s.c. administration in the mouse 6 Hz model, with median effective dose (ED₅₀) values of 12.2 mg/kg at 32 mA stimulation and 21.0 mg/kg at 44 mA stimulation. Another mGlu2 PAM, JNJ-46356479, was also found to be active in that model, with modest activity in the mouse corneal kindling model.

In collaboration with UCB, JNJ-40411813 administered by the i.p. route has been tested in the audiogenic seizure-susceptible mouse model and found to be inactive against the three stages of convulsions, up to the highest tested dose of 100 mg/kg. In the same model, JNJ-46356479 was also not able to protect against wild running and clonic convulsions but suppressed tonic convulsions with a calculated ED₅₀ value of 19.6 mg/kg after i.p. administration.

Importantly, published data suggested a potential pharmacodynamic interaction between mGlu2 PAMs and levetiracetam.^{25,26} Administering an inactive dose of mGlu2 PAM caused a significant left-ward shift of the dose-response of levetiracetam in the 44 mA 6 Hz model. To strengthen this finding, we collaborated with UCB to perform additional experiments. Confirming the data in the same preclinical model in independent laboratories carries potential risks: small differences in experimental set-up and/or use of different mouse strains can influence the results, exemplified by calculated ED₅₀ values for levetiracetam (ED₅₀ = 345 mg/kg i.p. for levetiracetam, as reported in Metcalf et al 2017, versus 43 mg/kg i.p. in the current studies performed at UCB).²⁷ JNJ-40411813 showed moderate protective effects against focal seizures in this model, with a statistically significant effect only at the dose of 150 mg/kg i.p. JNJ-46356479 afforded a dose-dependent antiseizure effect, with an ED₅₀ of 10.1 mg/kg after i.p. administration.

Interaction studies, combining levetiracetam (administered i.p. 60 min before stimulation) and an inactive dose of JNJ-40411813 (10 mg/kg, administered i.p. 15 min before stimulation), or JNJ-46356479 (3 mg/kg, administered i.p. 15 min prior to stimulation), confirmed a potentiation of the antiseizure effect of levetiracetam (pretreatment times represent the time to peak pharmacodynamic effects of levetiracetam, JNJ-40411813 and JNJ-46356479, respectively). The ED₅₀ value for levetiracetam alone was 43 mg/kg. The ED₅₀ decreased to 4.9 and 1.3 mg/kg when JNJ-40411813 or JNJ-46356479 was co-administered (ED₅₀ shift of 9 and 33, respectively).

Furthermore, interaction studies combining diazepam (administered i.p. 30 min before stimulation) or carbamazepine (administered i.p. 15 min before stimulation) and an inactive dose of JNJ-40411813, or JNJ-46356479, were performed for comparison. Potentiation of the protective effect induced by diazepam or carbamazepine was also observed, but to a lesser extent than the one observed with levetiracetam. The ED₅₀ value for diazepam alone was 0.57 mg/kg and decreased to 0.33 and 0.25 when JNJ-40411813 or JNJ-46356479 was respectively co-administered. The ED₅₀ value calculated for carbamazepine alone was 25.4 mg/kg, decreasing to 8.3 and 7.5 mg/kg when JNJ-40411813 or JNJ-46356479 was respectively co-administered.

Another set of experiments was conducted in fully amygdala-kindled rats. In this model, JNJ-46356479 (30 mg/kg p.o.) slightly decreased the mean seizure severity score while JNJ-40411813 (30 mg/kg p.o.) was without any effect in this model. Both mGlu2 PAMs slightly reduced the behavioral seizure duration and did not affect significantly the EEG after-discharge duration at the tested dose. In co-administration studies, JNJ-40411813 at doses of at 10 and 30 mg/kg p.o. significantly enhanced the protective effects of levetiracetam (170 mg/kg i.p.) against seizure severity score and behavioral seizure duration (Figure 2).

Figure 2 here

JNJ-46356479 was able to significantly potentiate the effects of levetiracetam only at the maximal tested dose of 30 mg/kg. p.o. Overall, the results reported above strongly suggest an increased potency and efficacy of levetiracetam in combination with mGlu2 PAMs and a mechanistic link between the presynaptic targets SV2A and mGlu2.

4.2.2 Mechanism of action

JNJ-40411813 binds to an allosteric modulator site independent of the agonist binding site on the mGlu2 receptor. The *in vitro* and *in vivo* data supporting this conclusion has been described in the EILAT XIV Progress Report.⁴

4.3 Toxicology

The toxicological profile of JNJ-40411813 has been described elsewhere.⁴ In short, the preclinical package has been used to support clinical studies with up to 3 months of dosing at a dose of 150 mg twice daily (b.i.d.).

4.4 Pharmacokinetics and drug interactions

The pharmacokinetics and drug interaction potential of JNJ-40411813 in humans have been described in the EILAT XIV Progress Report.⁴ In repeated-dose studies with doses up to 225 mg b.i.d., the half-life of JNJ-40411813 was found to be approximately 24 h after 7 days of dosing.

4.5 Efficacy and tolerability

In Phase I clinical studies completed to date, 286 healthy subjects have received JNJ-40411813 at doses ranging from 5 to 1,000 mg (single-dose studies) or 50 to 225 mg b.i.d. (multiple-dose studies). In these studies, JNJ-40411813 was found to be generally well tolerated.

JNJ-40411813 has been studied in an exploratory add-on study in 92 patients with stable schizophrenia (placebo, 50 mg and 150 mg b.i.d., 4 weeks double-blind treatment period, followed by maximum 10 weeks open label). The results indicated a possible benefit in patients with prominent negative symptoms (Janssen, data on file). Based on results of an exploratory study with doses up to 150 mg b.i.d. in 121 patients with anxious depression (2 double blind periods of 4 weeks each), no further development is envisaged in this indication.²⁸ A post-hoc analysis of 5 patients with co-morbid panic disorder included in the latter study showed complete remission of panic symptoms within 2-4 weeks of treatment with JNJ-40411813.²⁹ JNJ-40411813 appeared to be safe and well tolerated in these studies. Dizziness was the most noteworthy treatment-emergent adverse event (TEAE) at the highest dose, and dose titration seemed to reduce its incidence.

4.6 Planned studies

The clinical development plan is described in the EILAT XIV Progress Report.⁴

5. NBI-921352 (XEN901)

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5.1 Introduction and rationale for development

NBI-921352 (formerly XEN901), a heterocyclic sulfonamide, is a novel, small molecule, selective inhibitor of Nav1.6 sodium channels currently being developed for the treatment of epilepsy. Preclinical data has been previously published in the EILAT XIV Progress Report.⁴

Current available AEDs that block voltage-gated sodium channels (Nav) are non-selective for Nav channel isoforms. Nav1.1 is the primary isoform in inhibitory neurons and inhibition of Nav1.1 is therefore predicted to be proconvulsant.^{30,31} In contrast, Nav1.6 and Nav1.2 are expressed in excitatory neurons, and inhibition of either or both should have antiseizure activity.³² It is expected that a drug that selectively targets Nav1.6 will retain, and potentially improve, antiseizure efficacy, while displaying an improved safety profile relative to non-specific Nav inhibitors.

Gain-of-function mutations in the *SCN8A* gene that encodes the Nav1.6 channel result in a severe type of childhood epileptic encephalopathy (EIEE13 or SCN8A developmental and epileptic encephalopathy [SCN8A-DEE]),³³ and a selective inhibitor of Nav1.6 could address the underlying etiology in this condition.

5.2 Pharmacology

5.2.1 Activity in experimental models of seizures and epilepsy

NBI-921352 protects against electrically induced seizures as tested in the MES test in mice and rats and in a modified 6 Hz assay in transgenic mice expressing a gain-of-function *SCN8A* mutation.⁴

5.2.2 Mechanism of action

NBI-921352 inhibits human Nav1.6 current with a half-maximal inhibitory concentration (IC₅₀) of 51 nM and exhibits, at minimum, 130-fold selectivity against other human Nav subtypes (Nav1.1-Nav1.7).⁴ Radioligand binding assays showed that NBI-921352 is selective against more than 50 targets including receptors, enzymes and transporters.

5.3 Toxicology

NBI-921352 has been studied in a comprehensive toxicology package including non-GLP single- and repeated-dose studies and 14-week pivotal GLP toxicology studies in rats and dogs along with

safety pharmacology studies and a GLP battery of genotoxicity studies.⁴ The in-life phases of the chronic GLP toxicology studies (i.e., 6-month rat and 9-month dog studies) are completed and data analysis is ongoing. NBI-921352 exhibited no genetic toxicity in GLP Ames, chromosomal aberration, and rat micronucleus assays. There were no organ system findings or unexpected safety pharmacology effects in the central nervous system (CNS), respiratory or cardiovascular systems that would preclude further clinical development of NBI-921352.

5.4 Pharmacokinetics and metabolic profile

To date, two Phase I studies have been completed with NBI-921352 in healthy subjects. The first-in-human study was a single center, randomized, double-blind, placebo-controlled, pharmacokinetic and safety study in 95 male and female subjects aged 18-55 years. Subjects received single ascending oral doses of an active pharmaceutical ingredient-in-capsule (API-in-capsule) formulation of 5-80 mg NBI-921352 or placebo (3:1 randomization), or multiple ascending doses of NBI-921352 in an API-in-capsule formulation at 15 mg b.i.d., 23 mg b.i.d., 50 mg once daily, or 75 mg once daily or placebo (b.i.d. or once daily) for 7 days. An immediate release (IR) tablet was also administered at a single 45 mg dose and repeat doses of 45 mg b.i.d. administered for 7 days.

Following single oral administration of 5, 10, 15, 30, 45 and 80 mg in-capsule form, NBI-921352 was rapidly absorbed with median time to peak plasma concentration (t_{max}) occurring between 1 and 1.5 h after dosing across the dose range. Peak plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) values increased approximately dose-proportionally. The terminal elimination half-life ranged from 7.5 to 10.6 h across all dose levels (5 to 80 mg). The tablet formulation displayed similar pharmacokinetics to the API-in-capsule formulation. Following multiple oral administration of NBI-921352 at 15 and 23 mg b.i.d., 50 and 75 mg once daily as an API-in-capsule formulation, or 45 mg b.i.d. as a tablet for 7 days, exposure generally increased with total daily dose. Steady state was reached within 2-4 days. Peak to trough fluctuation values were higher with once daily administration than with b.i.d. administration, indicating that b.i.d. dosing provides more stable exposure to NBI-921352.

A second Phase I study with NBI-921352 has been recently completed. This was an open-label, 2 cohort study to assess drug-drug interactions (DDI cohort), food effect on the pediatric formulation, and bioequivalence of adult vs pediatric formulations of NBI-921352 (FE/BE cohort). In the DDI cohort, 18 male and female subjects aged 18-55 years received single oral doses of 100 mg NBI-921352 as an immediate-release tablet (adult) formulation on Day 1 and on Day 12. Phenytoin, a strong inducer of cytochrome 3A4 (CYP3A4) and moderate inducer of CYP1A2 and CYP2C19,

was administered at a dose of 100 mg three times daily (t.i.d.) from Day 3 to Day 12, when a single (morning) dose was given 1 hour prior to NBI-921352. Blood samples were obtained up to 48 h after each NBI-921352 dose. The FE/BE part of the study followed a 3-period crossover design. Twenty-four male and female subjects aged 18-55 years were randomized to receive all three treatments (50 mg NBI-921352 pediatric (granule) formulation in fed state, 50 mg NBI-921352 pediatric (granule) formulation in fasted state and 50 mg NBI-921352 adult (tablet) formulation in fasted state) in one of 3 different treatment sequences. Each NBI-921352 dose was separated by a minimum washout period of 72 h. Blood samples were obtained up to 48 h after each dose.

For the DDI cohort, 17 subjects completed the study. Following 100 mg NBI-921352 administration, mean C_{max} and AUC_{0-inf} of 3300 ng/mL and 19820 ng*h/mL, respectively, were observed on Day 1. Similar exposures (mean C_{max} of 3720 ng/mL and AUC_{0-inf} 18050 ng*h/mL) were observed on Day 12 after 10 days of phenytoin dosing. NBI-921352 was rapidly absorbed with a median t_{max} of 1 h for both doses.

For the FE/BE cohort, all 24 subjects completed the study. Mean C_{max} levels of 2240 ng/mL, 2020 ng/mL and 1250 ng/mL were observed for the adult formulation-fasted, pediatric formulation-fasted and pediatric formulation-fed groups respectively. Similar AUC exposures were observed across formulations and in the fed or fasted states, with mean AUC_{0-inf} values of 12,380 ng*h/mL, 11,300 ng*h/mL and 11,770 ng*h/mL observed for the adult formulation-fasted, pediatric formulation-fasted and pediatric formulation-fed groups respectively (Figure 3). Maximum plasma concentrations were delayed by 2 h under fed conditions after intake of a single 50 mg dose of the pediatric formulation. Following the administration of NBI-921352 with a high-fat meal compared to fasted conditions, C_{max} decreased by 40% while AUC remained unchanged (Figure 3). The pediatric and adult formulations were bioequivalent. Less than 0.1% of the administered dose was excreted in the urine as unchanged drug.

Figure 3 here

In *in vitro* studies with test systems expressing human recombinant CYP enzymes, NBI-921352 was primarily metabolized by CYP3A4, CYP2D6, and CYP2C9. Elucidation of the full metabolite profile of NBI-921352 in humans is currently ongoing.

5.5 Drug interactions

In the DDI study described in the previous section, phenytoin trough levels reached steady state by Day 7 (fifth phenytoin dosing day). In the presence of phenytoin, the NBI-921352 C_{max} increased

by 21.5%, AUC_{0-inf} decreased by 7.3% and no changes were observed in t_{max} . These results indicate that the enzyme inducing agent phenytoin has no significant effect on overall NBI-921352 exposure.

Figure 4 here

5.6 Efficacy data

No efficacy studies in epilepsy patients have been completed to date. In the first-in-human study, transcranial magnetic stimulation (TMS) measurements and EEG were assessed in eight healthy subjects in the multiple ascending dose cohorts assigned to receive 50 and 75 mg once daily with C_{max} plasma levels > 1000 ng/ml and compared to three placebo-treated subjects. TMS measures were recorded at baseline and on Days 5 and 6. In these participants, repeat dose NBI-921352 administration showed a trend towards increased resting motor threshold (2.0% with NBI-921352 vs. 0.67% with placebo) and active motor threshold (2.25% with NBI-921352 vs. 0% NBI-921352 with placebo) and decreased amplitude of TMS evoked potential at 180 ms. The observed changes in TMS parameters suggest that NBI-921352 reduced corticospinal and cortical excitability at the doses used in this pilot study.

5.7 Adverse effects

NBI-921352 was well tolerated in both Phase I studies. In the first-in-human study, no serious or severe TEAEs or TEAEs leading to withdrawal were reported. In the single ascending dose capsule cohort, 83.3% of subjects administered NBI-921352 reported at least one TEAE in comparison to 20.0% of subjects who received placebo. In the repeat-dose capsule cohort, 52.2% of subjects administered NBI-921352 reported at least one TEAE in comparison to 57.1% of those who received placebo. The most common TEAEs were medical device site reaction (13.0%), headache (8.7%) and nausea (8.7%). In both the single ascending dose and the repeat dose capsule cohort, TEAEs in subjects administered NBI-921352 were mostly mild in intensity, and no subject experienced severe TEAEs. There were no clinically significant observations or trends in vital signs or electrocardiograms (ECGs) in any of the study cohorts.

No serious TEAEs occurred in the DDI, food effect and bioequivalence study and no subjects were withdrawn due to TEAEs. In the DDI cohort, 83.3% of subjects reported at least one TEAE, the most common TEAEs being headache (11.0%), dizziness (8.0%), and nausea (7.0%). In the FE/BE cohort, 29.2% of subjects reported at least one TEAE, and the most common TEAEs were dizziness (25.0%) and headache (20.8%). In the DDI, food effect and bioequivalence study, the majority of

TEAEs were rated as mild in intensity, and no subject experienced severe TEAEs. There were no clinically significant NBI-921352 effects on vital signs or ECG values in either cohort of this study, except for increased heart rate in one subject from the DDI cohort, accompanying an episode of nausea and vomiting.

5.8 Planned studies

Phase II studies with NBI-921352 for SCN8A developmental and epileptic encephalopathy and adult focal epilepsy are being planned.

6. NTX-001 (interneuron transplantation)

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6.1 Introduction and rationale for development

A promising strategy to overcome intractable epilepsy is the transplantation of inhibitory interneurons to restore balance to the hyperexcitable epileptic neural network. It is hypothesized that interneuron loss plays a major role in seizure generation and propagation since tissue resected from patients with epilepsy and brain tissue from animal models of epilepsy display a loss and/or a dysfunction of interneurons.³⁴ Selective ablation of interneurons in rodents has been shown to induce seizures.³⁵ Conversely, chemo- and optogenetic studies to selectively activate interneurons in epileptic rodent models have demonstrated seizure suppression.³⁶ In addition to having antiseizure properties, augmentation of inhibitory interneuron activity could slow the progression of epilepsy and hippocampal pathology.³⁷ Therefore, γ -aminobutyric acid (GABA)ergic interneurons are an important target for the development of antiseizure drugs, and direct transplantation of interneuron cells to restore local inhibitory tone to epileptic networks represents a novel therapeutic strategy. Most of the hippocampal and cortical interneurons originate from the medial ganglionic eminence (MGE) during embryonic development. Neurona Therapeutics is developing a human medial ganglionic eminence-type GABAergic interneuron therapeutic (NTX-001) derived from human embryonic stem cells.

6.2 Pharmacology

6.2.1 Activity profile in experimental models of seizures and epilepsy

Several proof-of-concept studies have evaluated the impact of transplanted primary rodent GABAergic interneuron precursor cells from the MGE on seizure suppression in preclinical models of epilepsy. For example, Baraban and colleagues³⁸ transplanted mouse MGE cells into a potassium channel mutant mouse (Kv1.1), which displays spontaneous seizures, and reported a 90% reduction in seizure frequency at 1 month post-transplant. Similarly, the same group found that mouse MGE cell transplants were able to reduce spontaneous seizures by 80-90% in the pilocarpine mouse model of chronic epilepsy from 2-7 months post-transplant.^{39,40} Other groups have also reported some degree of seizure reduction after cell transplantation: Henderson et al.⁴¹ described a significant decrease in seizure frequency between 1 and 2 months post-transplant in the pilocarpine mouse model, but not at earlier or later time-points. Similarly, transplantation of rat MGE cells reduced spontaneous seizures in the systemic kainate rat model by 43% at 3 months post-transplant.⁴² Other groups have studied human interneuron transplantation with non-clinical-grade cells and have shown similar antiseizure effects of 72-90% seizure reduction in the systemic kainate rat or pilocarpine mouse model of epilepsy.^{43,44}

The engraftment, functional synaptic integration, and seizure suppression reported in these rodent and human MGE cell transplantation studies support further investigation of inhibitory interneuron cell therapy for drug-resistant focal epilepsies. Neurona Therapeutics is developing a clinical-grade human MGE-type GABAergic interneuron therapeutic for potential first-in-human clinical trials. We have demonstrated significant reduction in electrographic seizure frequency of 70-80% in human interneuron-transplanted mice compared to a vehicle-injected control group in the focal kainate mouse model of pharmacoresistant mesial temporal lobe epilepsy.⁴⁵ Furthermore, the cumulative duration of seizures was reduced significantly over time post-transplant (Fig. 5). These antiseizure effects were long-lasting and measured at 4 - 9.5 months post-transplant (MPT). Overall, two thirds of cell-transplanted mice (16/24) became seizure-free at 6-7 MPT, whereas none of the vehicle-injected animals (0/17) achieved seizure freedom.

Figure 5 here

6.2.2 Mechanisms of action

Antiseizure effects observed after transplantation of NTX-001 are comparable to studies in the same animal model with several approved AEDs that increase GABAergic inhibition. For example, 3 mg/kg diazepam i.p. reduced the number of electrographic seizures by 73% in the hour after injection compared to vehicle-injected mice.⁴⁶ However, an interneuron cell therapy can potentially

increase GABAergic inhibition with a more targeted administration than systemic GABA enhancing drugs, and possibly with fewer side effects.

Preclinically, NTX-001 cells are administered by stereotactic intracranial injection. The human interneurons disperse a few millimeters from sites of injection and persist long-term (Fig. 6).⁴⁵ The grafted human cells express markers of an MGE-type cortical/hippocampal interneuron lineage, including LIM/homeobox protein 6 (LHX6), musculoaponeurotic fibrosarcoma factor (MAF), MAF B transcription factor (MAFB), and glutamic acid decarboxylase (GAD) 1/2. Electrophysiological recordings in organotypic slices from mice at up to 16 months post-transplant have shown that the grafted human interneurons are functionally integrated, fire action potential trains with interneuron-type firing patterns, and express cortical/hippocampal interneuron subtype markers. The NTX-001 cells produce and secrete GABA in the cell culture supernatant, as measured by HPLC or ELISA. GABA release is correlated with cell seeding density and duration of cell culture. These data are consistent with a proposed mechanism of action that the interneuron cell therapy can increase local synaptic inhibition of neural circuits.

Figure 6 here

6.3 Toxicology

Potential adverse effects of increased GABAergic tone could be sedation, motor impairment, and the development of tolerance, which could result in a recurrence of seizures. A modified Irwin Screen has been performed on mice repeatedly after transplantation up to 9 months post-transplant and suggests that transplantation of NTX-001 is well-tolerated and does not cause obvious signs of sedation or motor impairment. Other behavioral testing paradigms such as Open Field test and Learning and Memory tests have not revealed increased anxiety or cognitive deficits compared to untreated epileptic mice.

Another possible concern would be the potential for proliferative expansion of transplanted cells. All animals are examined histologically and we never observed graft-derived tumors, ectopic or teratomatous tissue between 1 to 12 months post-transplant, which is as long as has been studied. Since we are using a xenograft approach in preclinical testing, human cells have been transplanted into immunodeficient rodents, which have not displayed any signs of graft rejection. Preliminary cell dose escalation has been performed. Increased doses of up to ~10-fold more cells than the efficacious dose to fully suppress electrographic seizures injected per hippocampus have not resulted in distortion of tissues, off-target distribution of grafted cells into adjacent brain regions, or any significant differences in the fate of grafted cells. In addition, the higher doses have been comparably efficacious and have not resulted in behavioral abnormalities

6.4 Efficacy and adverse effects

There have been no studies to date to assess NTX-001 in patients with epilepsy.

6.5 Planned studies

Dose escalation and preliminary safety studies are ongoing. Investigational New Drug (IND)-enabling GLP safety, toxicology, and biodistribution studies in rodents will be performed to support an IND application and Phase I/IIa clinical study of NTX-001 for drug-resistant mesial temporal lobe epilepsy.

7. *sec*-Butylpropylacetamide

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Molecular structure of valnoctamide and sec-butylpropylacetamide here

7.1 Introduction

sec-Butylpropylacetamide (SPD) is a one-carbon homologue of valnoctamide, a chiral constitutional isomer of valproic acid's corresponding amide (valpromide). Unlike valpromide, in rats and mice SPD acts as a drug on its own with no biotransformation to its corresponding acid. SPD and its individual stereoisomers possess a unique and broad-spectrum antiseizure profile superior to that of valproic acid, with ED₅₀ values 3-15 times more potent than those of valproic acid.⁴⁷ SPD possesses two chiral centers in its molecule (denoted above with the asterisk *) and exhibits stereoselective pharmacokinetics and pharmacodynamics (anticonvulsant activity) in rats.^{3,48-51}

Since the EILAT XIV Progress Report³ new preclinical data with SPD have been acquired.^{52,53}

7.2 Update on pharmacokinetics and pharmacodynamics

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SPD has proven to be active in six different rat models of benzodiazepine-resistant SE induced by pilocarpine, the rodenticide tetramethylenedisulfotetramine (TETS) and the nerve agents soman, paraoxon, VX, and sarin.^{47,54,55} In the benzodiazepine-resistant pilocarpine- and soman-induced SE models, SPD and its individual stereoisomers terminated seizures even when given 30 and 60 min after seizure onset.⁴⁹⁻⁵¹

The issue of drug chirality is cardinal in the design, discovery and development of new drug candidates due to the role of stereospecificity and molecular recognition in mediating pharmacological activity. Thus, most new chiral drugs are currently being developed as a single individual stereoisomer rather than racemic mixtures, because stereoisomers are often readily distinguished by biological systems.⁵⁶ Consequently, we investigated thoroughly the potential of the CNS-active SPD individual stereoisomer (2S,3S)-SPD by studying for the first time its pharmacokinetics in pigs as well as the correlation between its pharmacokinetics and pharmacodynamics (PK-PD) utilizing the MES seizure threshold (MEST) test in rats.⁵² The MEST model has been suggested as an addition or as an alternative to the conventional MES test.⁵⁷ Since (2S,3S)-SPD was found previously to be active in the MES model with better potency than valproic acid,⁴⁸ it was considered that the MEST test could be valuable in further defining PK-PD correlations. In the MES test (2S,3S)-SPD and its antipode (2R,3R)-SPD were the most potent out of the four SPD individual stereoisomers.^{48,50} Although in some models of antiseizure activity (2S,3S)-SPD was less potent, we elected to proceed with (2S,3S)-SPD due to its lower teratogenic potential.⁴⁸

In addition to being developed as a second-generation drug to valproic acid for chronic oral dosing, an intramuscular (i.m.) formulation of (2S,3S)-SPD is currently being developed as a potential treatment for acute repetitive seizures.⁵² Because the treatment of acute repetitive seizures requires a rapid onset of activity, the MEST test allowed comparative evaluation of (2S,3S)-SPD i.m. injection (in comparison to diazepam) on seizure susceptibility at 4, 7, 10 and 20 min after dosing in rats.⁵²

The pharmacokinetics of two parenteral formulations (F_A and F_B) of (2S,3S)-SPD were studied following i.m. injection (20, 40 mg/kg and 60 mg/kg) to Sprague Dawley rats, and its main pharmacokinetic parameters were calculated. Plasma and brain samples from four rats were collected 4, 7, 10, 20 min after dosing. The same two formulations (F_A and F_B) were administered i.m. to female pigs (two-month old 20 kg swine) at a dose of 12 mg/kg ($n = 2$ for each formulation). Plasma concentrations of (2S,3S)-SPD were quantified by a liquid chromatographic tandem mass spectrometry method assay.⁵²

Following i.m. administration of the two formulations of (2S,3S)-SPD 40 mg/kg to rats, C_{\max} values of 9.4 mg/L (F_A) and 7.0 mg/L (F_B) were obtained at 1 h (F_A) and 1.3 h (F_B) after dosing. (2S,3S)-SPD plasma exposure (AUC) was similar for both formulations, half-life ranged from 0.9-2.3 h for both i.m formulations and plasma concentrations at 10 min after dosing were 3.5 mg/L (F_A) and 2.3 mg/L (F_B).⁵² In the rat MEST study, (2S,3S)-SPD (formulation F_A) exhibited a similar pharmacodynamic profile as diazepam, was effective at 4 min after i.m. injection, and reached its peak effect at 20 min after dosing. No sedation was apparent at visual inspection in (2S,3S)-SPD-treated rats. A good correlation was found between (2S,3S)-SPD plasma and brain levels and its activity in the MEST test.⁵²

Following i.m. administration (12 mg/kg) of formulations F_A and F_B to pigs, C_{\max} values of 0.9 mg/L were obtained at 0.75 (F_A) and 0.42 h (F_B) after dosing. Plasma (2S,3S)-SPD AUC values were similar after both formulations, half-life ranged from 6.1-9.7 h and plasma concentrations at 10 min after dosing were 0.26 mg/L (F_A) and 0.46 mg/L (F_B).

PK-PD analysis of the activity of (2S,3S)-SPD in the rat-MEST model (60 mA) in relation to its plasma and brain concentration showed that the effect of (2S,3S)-SPD can already be detected at plasma concentrations of about 5 mg/L and brain concentrations of about 4 mg/L, with correlation coefficients (r^2) of 0.646 (plasma) and 0.653 (brain).

To date, the pharmacokinetic and activity profile of SPD has been investigated solely in mice and rats, except for one study in guinea pigs.⁵¹ Domestic pigs are closely related to humans in terms of anatomy, genetics and physiology, and represent an excellent animal model to study various human diseases.⁵⁸ Consequently, pigs are increasingly being used as an alternative to dogs or monkeys as the choice of non-rodent species in preclinical toxicological and pharmacokinetic testing of pharmaceuticals.

Comparison of (2S,3S)-SPD main pharmacokinetic parameters (normalized to body weight) between pigs and rats shows that its clearance is similar in these two species, ranging between 1-2 L/h/kg. (2S,3S)-SPD half-life in pigs is longer due to its higher volume of distribution in this species, which is 4-7 times larger than in rats. Due to the larger volume of distribution, higher doses than 12 mg/kg of (2S,3S)-SPD are needed in pigs in order to reach the plasma concentrations found to be effective in the MEST test in rats (4-5 mg/L).

Further studies are ongoing to develop new parenteral formulations of (2S,3S)-SPD that will enable better solubility at higher concentrations than those obtained with formulations F_A and F_B . In fact, allometric calculations based on pharmacokinetic data in rats and pigs suggest that parenteral

formulations containing higher doses of (2S,3S)-SPD will be needed to achieve in humans plasma concentrations comparable to those which are effective in the MEST test in rats.

8. XEN1101

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8.1 Introduction and rationale for development

XEN1101 is a novel positive allosteric modulator of neuronal Kv7.2-7.5 (KCNQ2-5) potassium channels, currently in a Phase IIb clinical trial as a treatment for focal epilepsy. As detailed in the EILAT XIV Progress Report, this second generation Kv7 channel opener is more potent and target-selective than the first generation Kv7 channel modulator, retigabine.⁴ Chemically, XEN1101 is a Biopharmaceutics Classification System (BCS) Class 2 small molecule. Unlike retigabine, XEN1101 does not possess the requisite structural features for the formation of chromophoric phenazinium-type dimers, which have been implicated in the pigmentary abnormalities observed with long-term retigabine exposure. Furthermore, XEN1101 is structurally distinct from any currently marketed AEDs.

8.2 Pharmacology

8.2.1 Activity in experimental models of seizures and epilepsy

XEN1101 is effective in several models of electrically and chemically induced seizures in rodents.⁴ Its potency *in vivo* was recently reassessed in comparison to retigabine in the MES test, using a 60 Hz bipolar stimulus with CF-1 mice. Under the conditions of this assay, XEN1101 reduced the fraction of mice seizing with a half-maximal effective concentration (EC₅₀), based on plasma concentrations at time of testing, of 220 nM, which was 16-fold more potent than retigabine (EC₅₀ = 3500 nM).

8.2.2 Mechanism of action

XEN1101 is a selective Kv7 channel opener. In experiments conducted in Chinese Hamster Ovary (CHO) cell lines expressing heteromeric $K_{v7.2/7.3}$ and $K_{v7.3/7.5}$ potassium channels and homomeric $K_{v7.4}$ channels, XEN1101 was approximately 4-fold more selective for $K_{v7.2/7.3}$ over $K_{v7.3/7.5}$ and $K_{v7.4}$ channels.⁴

In recent patch clamp studies conducted with HEK cells expressing $K_{v7.2/7.3}$ channels, XEN1101 ($EC_{50} = 42$ nM) was found to be ~22 times more potent than retigabine ($EC_{50} = 920$ nM) for causing an approximate -40 mV leftward shift in the half maximal voltage of activation for the channel, while producing a similar increase in maximal conductance ΔG_{max} of ~38% versus vehicle.

8.3 Toxicology

Recently completed 6-month rat and 9-month cynomolgus monkey toxicology studies were supportive of long-term clinical studies. In terms of CNS tolerability, monkeys are more sensitive than rats, as early signs of dose-limiting CNS signs (e.g., sedation, ataxia) were observed at lower plasma concentrations in monkeys.

In 26-week rat studies, the no-observed-adverse-effect level (NOAEL) was considered to be 8 mg/kg/day for males and 10 mg/kg/day for females. In the 39-week monkey study, daily oral administration of XEN1101 at dose levels of 1, 2.5, or 4 mg/kg/day was well tolerated and did not result in test article-related mortality and AEs. Under the conditions of the study, the NOAEL was considered to be 4 mg/kg/day.

In embryo-fetal developmental toxicity studies, the NOAEL of XEN1101 in rats and rabbits for embryo-fetal developmental toxicity was considered to be 6 mg/kg/day and 10 mg/kg/day, respectively.

8.4 Pharmacokinetics

Preliminary results of a single ascending dose (5, 10, 15, 20, 25 and 30 mg) and multiple ascending dose (15 and 25 mg once-daily for up to 10 days) pharmacokinetic and tolerability study of XEN1101 in healthy subjects were disclosed in the EILAT XIV Progress Report⁴ and are updated here. The effect of consumption of a high fat meal on the pharmacokinetics of single doses of XEN1101 (20 mg) was assessed using a crossover design. Food enhanced XEN1101 absorption, which was more pronounced in subjects with lower XEN1101 plasma concentrations in the fasted

state. XEN1101 displayed a prolonged absorption and elimination phase. The absorption rate was relatively slow (median t_{\max} of 4-6 h) in the fed state. Administration of 25 mg doses once daily resulted in a C_{\max} of 97 ng/mL after 10 consecutive days of dosing. XEN1101 displayed a long terminal elimination half-life (approximately 4-10 days), potentially reflecting slow release from a tissue compartment, but near steady-state trough plasma levels (no statistical difference between trough levels on successive days) were achieved in approximately 1 week after dosing, with full steady state expected to be achieved in approximately 3 weeks. XEN1101 had one major metabolite detectable in plasma at concentrations of approximately 20% AUC of the parent compound, while the parent drug was minimally excreted in urine. This primary hepatic oxidative metabolite appeared to be inactive on KCNQ channels in preliminary assays.

8.5 Drug interactions

No clinical drug-drug interaction studies with XEN1101 have been completed to date. *In vitro* enzyme and transporter studies suggest a low potential for causing drug-drug interactions.⁴ While showing good stability in human hepatocyte preparations, reflected in its long half-life *in vivo*, *in vitro* CYP450 reaction phenotyping showed that XEN1101 is susceptible to CYP3A4-mediated metabolism.

8.6 Effect on cortical excitability

In a cross-over design TMS placebo-controlled study in adult healthy subjects, a 20 mg single dose of XEN1101 increased resting motor threshold of maximum stimulator output by 4.4% at a plasma level of 44 ng/mL, 6 h after dosing (Figure 7), and decreased significantly TMS evoked potential amplitudes,⁵⁹ indicating reduced corticospinal and cortical excitability.

Figure 7 here

8.7 Adverse effects

XEN1101 was well tolerated in healthy subjects after single doses up to 30 mg in a fasted state and after single and multiple doses of 25 mg once daily in a fed state, with the majority of reported AEs being mild. The most common treatment-related AEs affected the CNS (e.g., somnolence, dizziness, headache, blurring of vision, memory and speech disorders), and all were reversible.

8.8 Planned and ongoing studies

No efficacy studies of XEN1101 in patients with epilepsy have been completed yet. The objectives of the first ongoing Phase II clinical study are to evaluate the efficacy, safety, tolerability and pharmacokinetics of XEN1101 in adults with refractory focal seizures. Dose selection was guided by the results of the TMS study,⁵⁹ as well as XEN1101's pharmacokinetic and side effect profile in Phase I studies.

This multicenter double-blind parallel-group trial (Figure 8) is being conducted at ~90 sites in Europe and North America. Adults (n = ~300) with focal epilepsy are randomized to 8 weeks of treatment with XEN1101 (25 mg, 20 mg or 10 mg once daily) or to placebo (randomization ratio 2:1:1:2). Patients are stratified in each treatment arm by background CYP inducing AEDs. Eligibility criteria include ≥ 4 countable focal seizures per month during an 8 week baseline period. Plasma samples for pharmacokinetics will be collected during the double-blind phase. After completion of the double-blind phase subjects may be eligible to enroll in a 12-month open-label extension study with treatment of 20 mg XEN1101 once daily. Safety evaluations include AE monitoring, laboratory tests, vital signs, ECGs, neurologic and physical examinations and Columbia-Suicide Severity Rating Scale assessment.

Figure 8 here

The primary endpoint will be the median percent change from baseline in monthly focal seizure rate with XEN1101 compared to placebo in the 8-week double blind phase. Secondary endpoints will include evaluation of responder rates, as well as changes in seizure frequency with time and quality of life assessments. The primary efficacy endpoint may include multiple hypothesis tests, with study-wide type I error controlled through a closed-testing procedure.

9. XEN496 (retigabine)

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Molecular structure of XEN496 (retigabine) here

9.1 Introduction and rationale for development

XEN496 is a new oral formulation of N-[2-amino-4-(4-fluorobenzylamino)-phenyl] carbamic acid ethyl ester (INN: retigabine, USAN: ezogabine) being developed by Xenon Pharmaceuticals Inc. as a precision medicine for the treatment of seizures associated with *KCNQ2* developmental and epileptic encephalopathy. *KCNQ2* encephalopathy is a rare, severe neurodevelopmental disorder characterized by multiple, daily, refractory seizures presenting within the first weeks of life and profound neurocognitive impairment. The electroencephalogram (EEG) at onset of the disease shows a burst suppression pattern later evolving into multifocal epileptiform activity.⁶⁰ No drug has been specifically studied in and approved for the treatment of seizures associated with *KCNQ2* encephalopathy. Sodium channel blockers have been reported to decrease seizure frequency in patients with *KCNQ2* encephalopathy with varying degrees of success, with 37.8% of individuals achieving seizure freedom, while in others there is limited efficacy.⁶¹

The *KCNQ* (Kv7) family consists of 5 related genes (*KCNQ1-5* [encoding Kv7.1-7.5]) that are highly expressed in the CNS, peripheral nervous system, heart, and smooth muscle organs such as the bladder. The *KCNQ2* gene codes for the Kv7.2 voltage-gated potassium channel subunit, which can form functional heterotetramers with Kv7.3 to form active Kv7.2/7.3 potassium channels. Certain loss-of-function missense mutations in the *KCNQ2* gene lead to over-excitability of neurons causing *KCNQ2* encephalopathy.

Retigabine, a Kv7 potassium channel modulator, was previously approved for adjunctive treatment of focal seizures, including focal to bilateral tonic-clonic seizures, in adult patients, but was withdrawn from the global market in 2017 by GlaxoSmithKline for commercial reasons. Retigabine potentiates Kv7-mediated potassium current, and thus targets the underlying etiology of *KCNQ2* encephalopathy. Predicated upon this mechanistic rationale, physicians have used retigabine off-label in infants and young children with *KCNQ2* encephalopathy with positive responses related to seizure control, as well as improvements in behavior and development, suggesting that retigabine could be highly efficacious in this population.^{60,62}

XEN496 is a multi-particulate “sprinkle” (granule) pediatric oral formulation of retigabine which allows for flexible weight-based dosing in this population. Its dissolution profile is consistent with an immediate-release product, and it is expected to be compatible with baby bottles and pediatric

nasogastric feeding tubes due to negligible non-specific binding to these materials, and small particle size. Packaged in single-use sachets or sprinkle capsules of varying fill weights, XEN496 is intended to be administered to infants and to young children in breast milk or formula, juice or soft foods.

9.2 Pharmacology

9.2.1 Activity profile in experimental models of seizures and epilepsy

Retigabine has been studied in two strains of knock-in mice bearing known loss of function mutations in *Kcnaq2*, the mouse orthologue of *KCNQ2*, to evaluate its effects on kainic acid-induced seizures in comparison to phenobarbital.⁶³ In both strains of mutant mice, retigabine significantly attenuated kainic acid-induced seizures, not only in terms of frequency of seizures, but also in terms of suppression of the EEG spike bursts, and more markedly than phenobarbital. The key role that these *KCNQ* channels play in maintaining neuronal excitability is further highlighted by the fact that relatively moderate (~25%) reductions in Kv7 channel activity due to impaired expression or function in either subunit, can lead to a loss of control over neuronal excitability in the brain resulting in a seizure predisposition.⁶⁴ This finding is in concordance with preclinical data in which transgenic *KCNQ2*^{+/-} mice, deficient in one copy of the *KCNQ2* gene, show increased sensitivity to the chemoconvulsive agent PTZ.⁶⁵ Consequently, strategies to increase activity of Kv7.2/7.3 represent a novel targeted means to restore the control of neuronal excitability in patients with *KCNQ2* encephalopathy more specifically.

9.2.2 Mechanisms of action

In vitro studies indicate that retigabine enhances transmembrane potassium currents mediated by the *KCNQ2* (Kv7.2 to 7.5) family of ion channels. Retigabine activates the Kv7.2/7.3 channels, generating the M current which stabilizes the resting membrane potential, inhibits burst firing and controls neuronal excitability.⁶⁶ In *KCNQ2* encephalopathy, a significant loss of function of the Kv7.2/7.3 heterotetramer channel function causes an impaired M current. *In vitro* studies suggest that retigabine may also exert its therapeutic effects through augmentation of GABA-mediated currents.⁶⁶

9.3 Toxicology

Retigabine has been studied in a comprehensive toxicology package to support the original approval, including GLP compliant repeat-dose studies in mice, rats, and dogs, and non-GLP studies in monkeys. These studies include a battery of genotoxicity and safety pharmacology studies, reproductive and developmental studies, carcinogenicity studies in mice and rats and juvenile toxicity studies in rats. Dose-limiting toxicity of retigabine appeared to be CNS related. Systemic exposures achieved at the NOAEL in the toxicity studies were lower than plasma retigabine levels achieved clinically at therapeutic doses, reflecting the greater sensitivity of the animal models to the toxic effects of retigabine.⁶⁷ Urinary bladder toxicity was also seen in mice, rats, and dogs.⁶⁸ Retigabine did not impair fertility, reproductive performance or embryonic development and was not carcinogenic in rats, but increased the frequency of lung neoplasms in a neonatal mouse carcinogenicity study.⁶⁶ Retigabine was negative in the *in vitro* Ames assay, the *in vitro* CHO *Hprt* gene mutation assay, and the *in vivo* mouse micronucleus assay. It was positive in the *in vitro* chromosomal aberration assay in human lymphocytes. The major circulating metabolite of retigabine, N-acetyl-retigabine, was negative in the *in vitro* Ames assay, but positive in the *in vitro* chromosomal aberration assay in CHO cells.⁶⁶ In juvenile animal studies, increased sensitivity to retigabine indicated by acute neurotoxicity and urinary bladder toxicity was observed in young juvenile rats compared with adults.⁶⁸

9.4 Pharmacokinetics and metabolic profile

To date, there have been no pharmacokinetic studies of XEN496 in healthy subjects or patients.

Results of studies conducted during development of the product marketed by GlaxoSmithKline showed that the pharmacokinetic profile of retigabine is approximately linear in daily doses between 600 mg and 1,200 mg in adults with epilepsy, with no unexpected accumulation following repeated administration. After both single and multiple oral doses, retigabine is rapidly absorbed with median t_{max} values generally between 0.5 and 2 h.⁶⁹ Absolute oral bioavailability relative to an intravenous dose is approximately 60%.¹² High-fat food does not affect the extent to which retigabine is absorbed based on plasma AUC values, but it increases C_{max} by approximately 38% and delays t_{max} by 0.75 h.⁶⁹

Retigabine is eliminated partly by renal excretion, with 36% of the dose being recovered unchanged in urine, and partly by N-acetylation and subsequent N-glucuronidation.⁶⁹ The parent drug and N-acetyl-retigabine have similar elimination half-lives of 7 to 11 h.

9.5 Drug interactions

In vitro studies have shown that retigabine does not inhibit enzyme activity for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5. In addition, retigabine and N-acetyl-retigabine do not induce CYP1A2 or CYP3A4/5 activity. Therefore, retigabine is not expected to alter the pharmacokinetics of substrates of the major CYP isoenzymes through inhibition or induction.⁶⁶

Carbamazepine has been shown to reduce the retigabine exposure at doses between 600 and 2400 mg/day (31% decrease in AUC, 23% decrease in C_{max}).⁶⁶ A reduction in retigabine exposure has also been observed with phenytoin at doses between 120 and 600 mg/day (34% decrease in AUC, 18% decrease in C_{max}). US prescribing information recommended considering an increase in dosage of retigabine when co-administered with carbamazepine or phenytoin. Retigabine has been shown to have a small effect on lamotrigine exposure (18% decrease in AUC), but no modifications to lamotrigine dose are recommended when given concomitantly with retigabine.⁶⁶ No drug interactions have been observed between retigabine and topiramate, valproate or phenobarbital.⁶⁶

A population pharmacokinetic analysis using pooled data from Phase III trials showed no significant impact of retigabine on the pharmacokinetics of zonisamide, valproic acid, clonazepam, gabapentin, levetiracetam, oxcarbazepine, phenobarbital, pregabalin, topiramate, clobazam and lamotrigine.⁶⁶ Likewise, no significant impact of these AEDs on the pharmacokinetics of retigabine was found.

9.6 Efficacy data

At this time, XEN496 has not been evaluated in patients. Retigabine has been extensively studied prior to its initial approval as adjunctive treatment of focal seizures in patients aged 18 years and older.⁷⁰

9.7 Adverse effects

At the time of writing, there have been no completed clinical trials with XEN496.

The most common TEAEs ($\geq 4\%$ and twice placebo rate) reported in adult patients that received retigabine during the controlled clinical studies for the product originally approved for the treatment of focal seizures were dizziness (23%), somnolence (22%), fatigue (15%), confusional state (9%), vertigo (8%), tremor (8%), abnormal coordination (7%), diplopia (7%), disturbance in attention

(6%), memory impairment (6%), asthenia (5%), blurred vision (5%), gait disturbance (4%), aphasia (4%), dysarthria (4%), and balance disorder (4%). AEs were mostly dose related and of mild to moderate intensity.⁶⁶ Dose-related weight gain has also been observed in clinical trials with retigabine. A QT study with the originally approved product demonstrated a mean 7.7-msec QT prolongation within 3 h of dosing in healthy adult subjects that received 1200 mg/day, and thus prescribing information recommends monitoring of the QT interval when the product is used with medicines known to increase QT or patients with known QT prolongation or with comorbidities that elevate the risk of QT prolongation.⁶⁶

Retinal pigmentary abnormalities, similar to those observed in retinal pigment dystrophies, have been observed in clinical trials with retigabine, generally in patients who had taken the drug for long periods of time.⁶⁶ Approximately one-third of patients who had eye examinations performed after 4 years of treatment had retinal pigmentary abnormalities.⁶⁶ Vision loss due to pigmentary abnormalities has not been reported in patients treated with retigabine. Blue discoloration of the skin, lips, nails and additional tissues has been observed in approximately 10% of patients in long-term clinical trials with retigabine.⁶⁶ In patients with unresolved events at discontinuation and ≥ 1 post-treatment follow-up, dermatological discoloration resolved completely in 9-66% of patients across studies.⁷¹

In placebo-controlled trials, urinary hesitation (2.2%), urinary retention (0.9%) and dysuria (2.3%) have been observed in patients treated with retigabine (compared with < 1% in patients receiving placebo).⁶⁶ Urinary retention has also been reported in pediatric patients with *KCNQ2* encephalopathy treated with retigabine off-label, but in all cases it was reversible with dose modification and with no long term consequences.^{60,62} TEAEs observed during off-label use of retigabine in *KCNQ2* encephalopathy included urinary retention (38-46%), somnolence (9%) and chromaturia (9%).^{60,62}

9.8 Ongoing and planned studies

Xenon Pharmaceuticals is currently conducting an open label, randomized, single dose, two-way crossover study to evaluate the effect of food on the pharmacokinetics of XEN496 and its major metabolite. After completion of the ongoing pharmacokinetic study, we plan to initiate a Phase III pivotal study, which we believe to be the first controlled trial in children with a monogenic epileptic and developmental encephalopathy testing a molecule that targets the specific disease mechanism. Furthermore, it is anticipated that XEN496 will be tested in infants with *KCNQ2* encephalopathy in an open label extension study beyond the maintenance phase of the Phase III trial, enabling an

opportunity to assess changes in development and other features of the complex phenotype of this disease.

CONCLUSIONS

The information presented in this article demonstrates that intense efforts are ongoing to discover and develop novel epilepsy treatments with potentially improved efficacy and safety. The variety of pursued development strategies range from addressing metabolic targets (2-deoxy-D-glucose) to mimicking the actions of endogenous ligands (GAO-3-02), exploiting synergism with established medications (JNJ-40411813), improving selectivity of action at ion channels involved in control of neuronal excitability (NBI-921352 and XEN1101), modifying the structure of existing agents to avoid undesired off-target effects (SPD), and integration of functional inhibitory networks in epileptogenic brain regions through grafting of human interneurons (NTX-001). Of note, some of these treatments aim at addressing less commonly targeted indications, such as SE and acute repetitive seizures (2-deoxy-D-glucose), and some developmental and epileptic encephalopathies (NBI-921352 and XEN496). While results of experiments conducted with these treatments in animal models of seizures and epilepsy are promising, well designed clinical studies will be needed to evaluate their potential value in the treatment of patients with epilepsy. Results of those studies are eagerly awaited.

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DISCLOSURES ON CONFLICT OF INTEREST

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2-deoxy-D-glucose: Thomas Sutula is an inventor in multiple patents issued to the Wisconsin Alumni Research Foundation.

GAO-3-02: The authors of the GAO-3-02 summary are co-founders of GAOMA Therapeutics.

JNJ-40411813: Marc Ceusters and Hilde Lavreysen are full-time employees of Janssen Research and Development. Rafal M. Kaminski was a full-time employee of UCB Pharma at the time when these experiments were performed, while Karine Leclercq and Chiara Rospo continue their full-time employment with UCB Pharma.

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XEN496: All authors are employees of Xenon Pharmaceuticals Inc.

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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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GAO-3-02 injection mode and dose (mg/kg; i.p.)	Single-dose testing										Repeated dose testing			Treatment withdrawal				
	1x 5	1x 10					1x 50				4x 5	4x 10	4x 20	Days after last 20 mg/kg treatment				
Time scale	D0	D1	D2	D5	D6	D7	D8	D9	D10	D11	W3	W4	W5	+7	+15	+42	+56	
Whole rat population. Number of rats (n/13) at:																		
Stage 5	13	8	6	9	8	5	5	5	2	3	6	5	5	5	3	6	6	
Stage 4	0	0	1	1	1	0	1	1	1	2	0	1	1	0	2	1	2	
Stage 3	0	2	3	2	2	3	2	1	2	2	0	1	1	1	1	2	1	
Stage 2	0	1	1	0	0	0	0	2	0	1	0	0	0	1	1	0	0	
Stage 1	0	2	1	1	1	1	1	1	5	3	0	0	0	0	0	0	0	
Stage 0	0	0	1	0	1	4	4	3	3	2	7	6	6	6	6	4	4	
Seizure-free rats	0%	0%	8%	0%	8%	31%	31%	23%	23%	15%	54%	46%	46%	46%	46%	46%	31%	31%
Mean Racine Seizure Score	5.00	3.85	3.54	4.31	3.92	2.69	2.77	2.85	1.92	2.62	2.31	2.46	2.46	2.31	2.15	3.08	3.15	
SEM	0.00	0.45	0.48	0.35	0.47	0.61	0.62	0.59	0.51	0.53	0.72	0.68	0.68	0.66	0.62	0.62	0.63	
Significance (compared to D0)		*	**	n.s.	*	**	**	**	***	***	**	**	**	**	***	**	*	
Rats responding to the dose of 5 mg/kg (7/13). Number of rats at:																		
Stage 5	7	2	0	3	3	1	0	1	0	1	1	1	1	2	2	2	2	
Stage 4	0	0	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	
Stage 3	0	2	3	2	2	2	2	1	2	1	0	1	1	1	1	1	1	
Stage 2	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	
Stage 1	0	2	1	1	0	1	1	1	3	2	0	0	0	0	0	0	0	
Stage 0	0	0	1	0	1	3	3	2	1	2	6	4	4	4	4	4	4	
Seizure-free rats	0%	0%	14%	0%	14%	43%	43%	29%	14%	29%	86%	57%	57%	57%	57%	57%	57%	
Mean Racine Seizure Score	5.00	2.86	2.29	3.71	3.57	1.71	1.57	2.14	1.86	2.00	0.71	1.71	1.71	1.86	1.86	1.86	1.86	
SEM	0.00	0.63	0.52	0.57	0.69	0.75	0.65	0.74	0.55	0.76	0.71	0.84	0.84	0.91	0.91	0.91	0.91	

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Significance (compared to D0)

* | ** | n.s. | n.s. | ** | ** | ** | ** | ** | *** | ** | ** | * | * | * | *

Table 1.

Sustained anti-seizure effect of GAO-3-02 in fully amygdala-kindled rats. All rats (n = 13) reached at least 5 consecutive stage 5 seizures by day 0 (D0) of the study. Rats were administered GAO-3-02 as a single 5, 10 and 50 mg/kg dose (i.p.) on D1, D6 and D9, respectively. On week 3 (W3), W4 and W5, rats received a daily dose over 4 consecutive days with 5, 10 or 20 mg/kg of GAO-3-02 (i.p.), respectively. Results are expressed as mean ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001; level of significance of the decrease in Racine Seizure Score compared to D0, *post hoc* Fisher's Least Significant Difference (LSD) test following one-way analysis of variance with repeated measures; n.s., not significant.

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LEGENDS OF FIGURES

Figure 1. GAO-3-02 rescued and prevented LTP deficit in hippocampal CA1 pyramidal neurons following pilocarpine-induced status epilepticus (SE). (A) Induction of LTP by Theta Burst Pairing protocol stimulation (TBP, indicated by arrow) in hippocampal slices from healthy rats (Ctrl) and from rats subjected to pilocarpine-induced SE.⁷² Slices were perfused either with saline (Ctrl or SE) or GAO-3-02 (SE GAO). (B-C) LTP induction in hippocampal slices from rats subjected to pilocarpine-induced SE and injected (i.p.) or administered (p.o.) with GAO-3-02 (SE GAO). GAO-3-02 was administered i.p. or p.o. 1 h after cessation of SE, then each day during 6 days then once every other day for 2 weeks. (D) Mean amplitude (\pm SEM) of excitatory post-synaptic potentials (EPSPs) measured during the last 5 min of recording in each condition. Summary data are presented as mean \pm SEM and numbers between brackets indicate the number of cells. #P < 0.05, ##P < 0.01, ###P < 0.001 Ctrl vs SE NaCl, *P < 0.05, **P < 0.01, ***P < 0.001 SE NaCl vs SE GAO.

Figure 2. Effect of levetiracetam (LEV, 170 mg/kg i.p. 60 min before stimulation) alone, and in combination with JNJ-40411813 (JNJ813, 3, 10 and 30 mg/kg p.o., 30 min before stimulation), on seizure duration recorded at supra-threshold stimulation in fully amygdala-kindled rats. Histograms represent mean seizure duration, with bars indicating SDs. VEH, vehicle. Doses in brackets expressed in mg/kg. * P < 0.05, ** P < 0.01, *** P < 0.001 paired t-test between pre- and post-drug responses; # P < 0.05, ## P < 0.01 unpaired t-test for post-drug treatment between levetiracetam alone and levetiracetam co-administered with JNJ-40411813.

Figure 3. Mean plasma concentrations of NBI-921352 after a 50 mg single dose given as IR tablets and granule (pediatric) formulation in a fasted state and 50 mg granule formulation in a fed state. IR, immediate release. PED, pediatric.

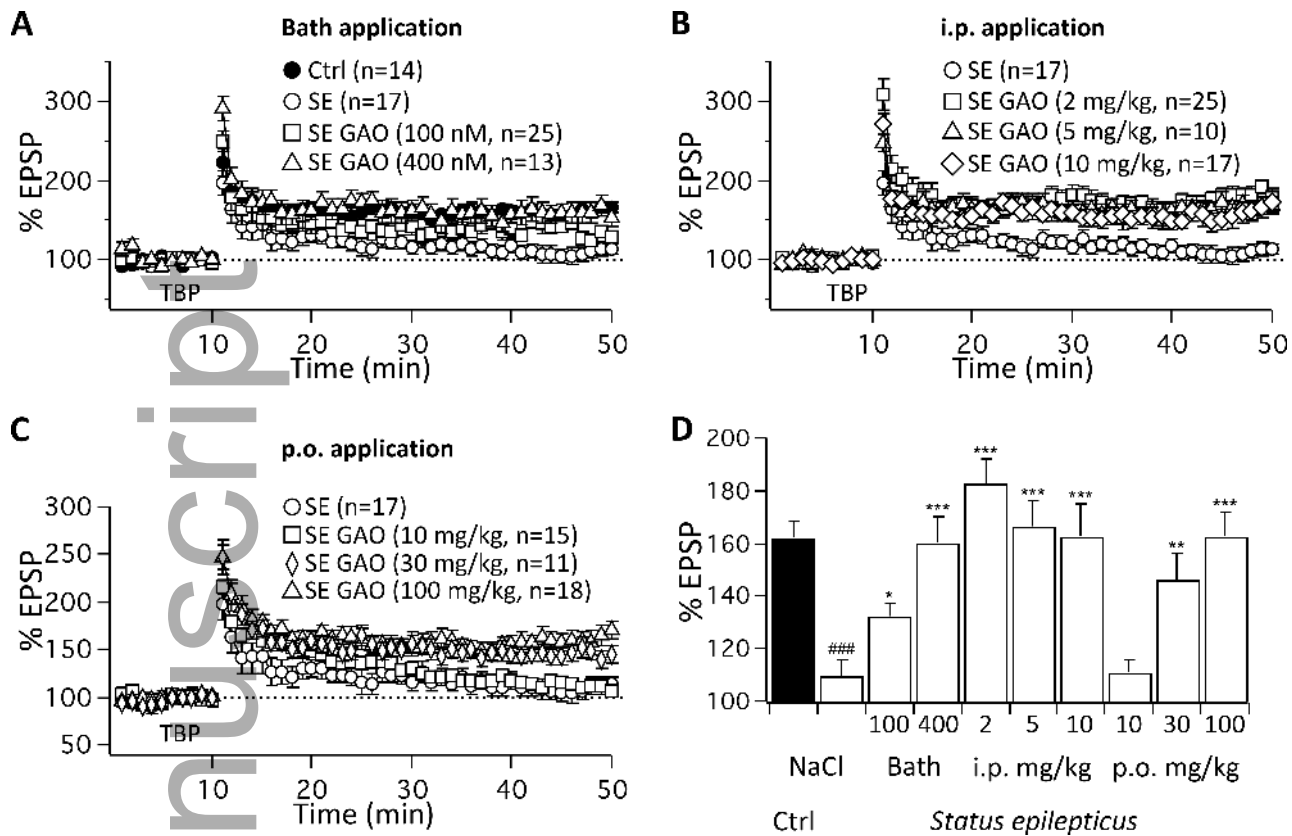
Figure 4. Mean plasma concentrations of NBI-921352 after a 100 mg single dose prior to phenytoin treatment (Day 1) and after 10-day treatment with phenytoin, 100 mg t.i.d. (Day 12).

Figure 5. Cumulative duration of electrographic seizures at different time-points post-transplant (4,5 and 6 months post-transplant; MPT) across 3 independent cell batches of NTX-001. The cumulative duration is normalized to the respective vehicle group at the same time-point. All data are mean \pm SEM. Statistics: Kruskal Wallis test, followed by Dunn's, *P < 0.05, **P < 0.01, ***P < 0.001.

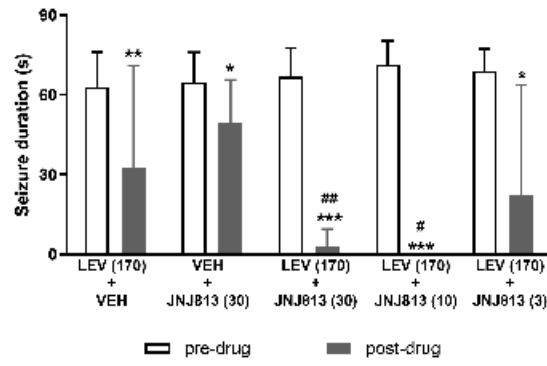
Figure 6. Human cells persist, disperse and express markers of interneurons up to 10 months post-transplant in the kainate treated hippocampus of a mouse. SST = Somatostatin, LHX6 = marker for MGE cells.

Figure 7. Effects of XEN1101 on resting motor threshold (RMT) assessed by transcranial magnetic stimulation (TMS)-electromyography (EMG). Data show change from baseline (% of maximum TMS stimulator output) on left axis, following a single 20 mg dose of XEN1101 (green circles) or placebo (blue squares) in a double-blind, randomized, crossover study in healthy subjects (n = 20). The right axis shows plasma levels of XEN1101 (red triangles) sampled at the same time points (2, 4, 6 h post-dose). Bars represent standard errors (SEM) *P < 0.05, **P < 0.01 (vs placebo).

Figure 8. Design of the Phase 2 trial of XEN1101 in patients with focal epilepsy. QD = once daily.

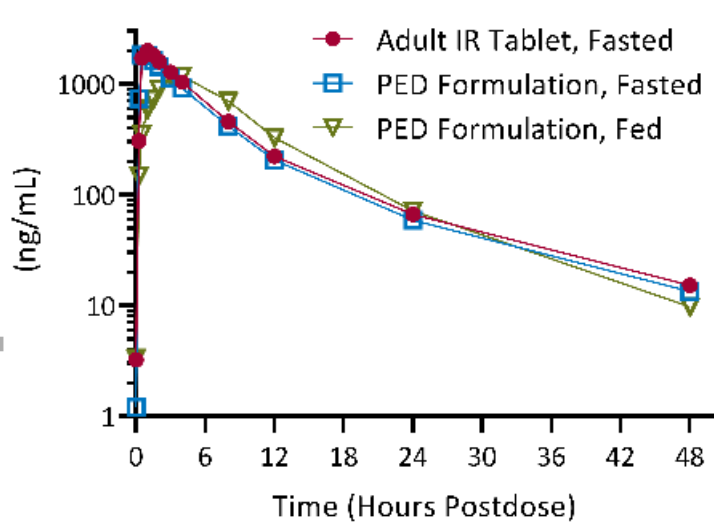


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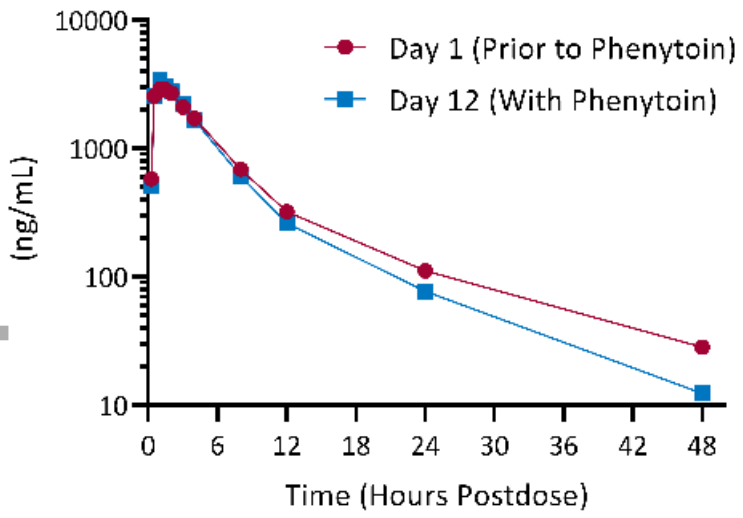
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Mean Plasma NBI-921352 Conc.



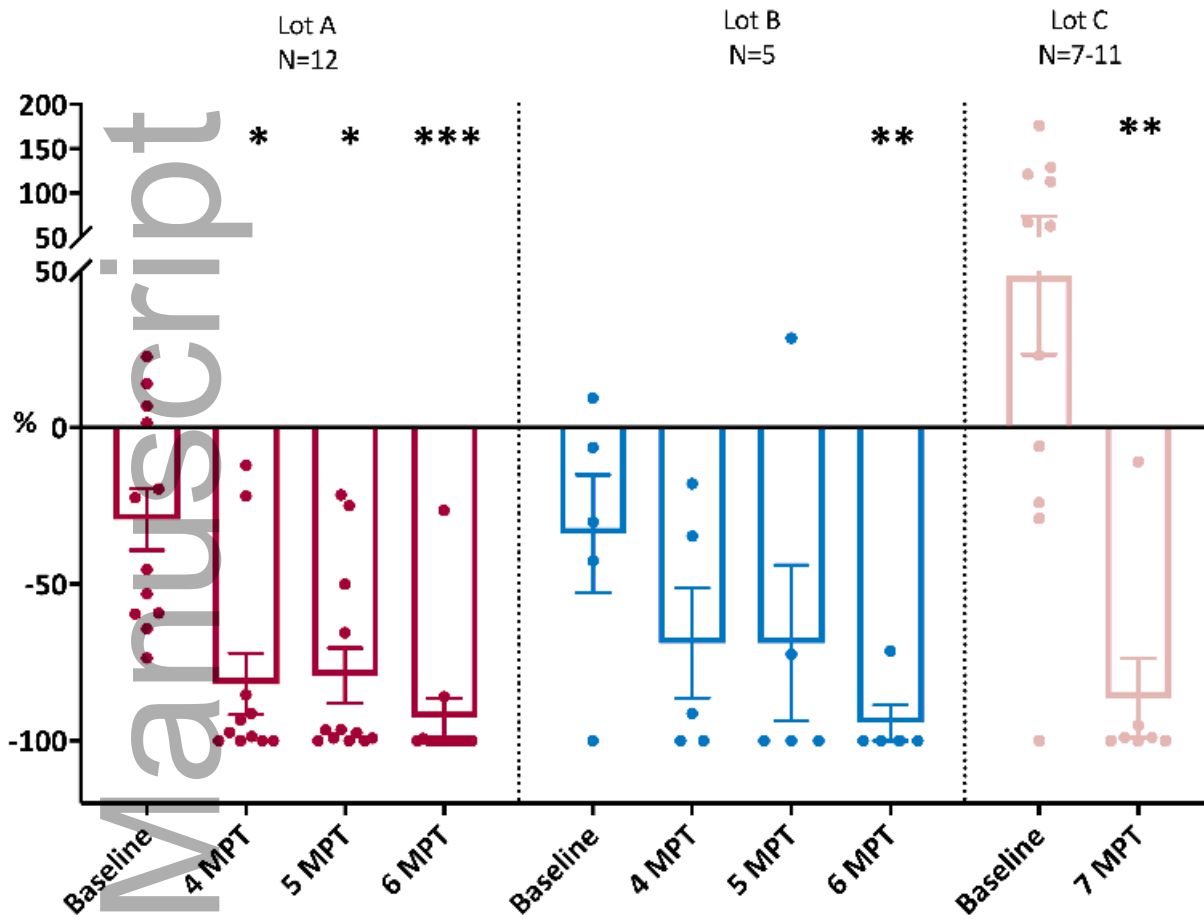
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Mean Plasma NB1-921352 Conc.

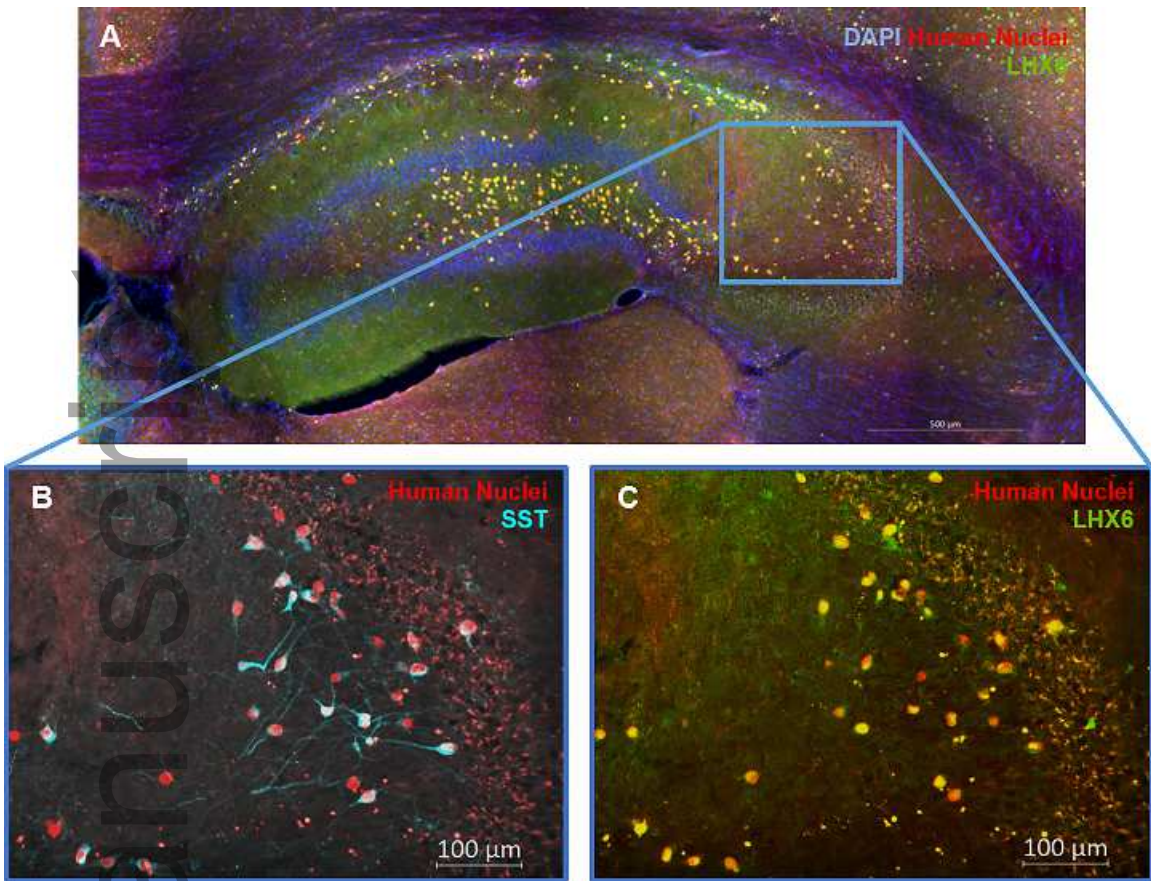


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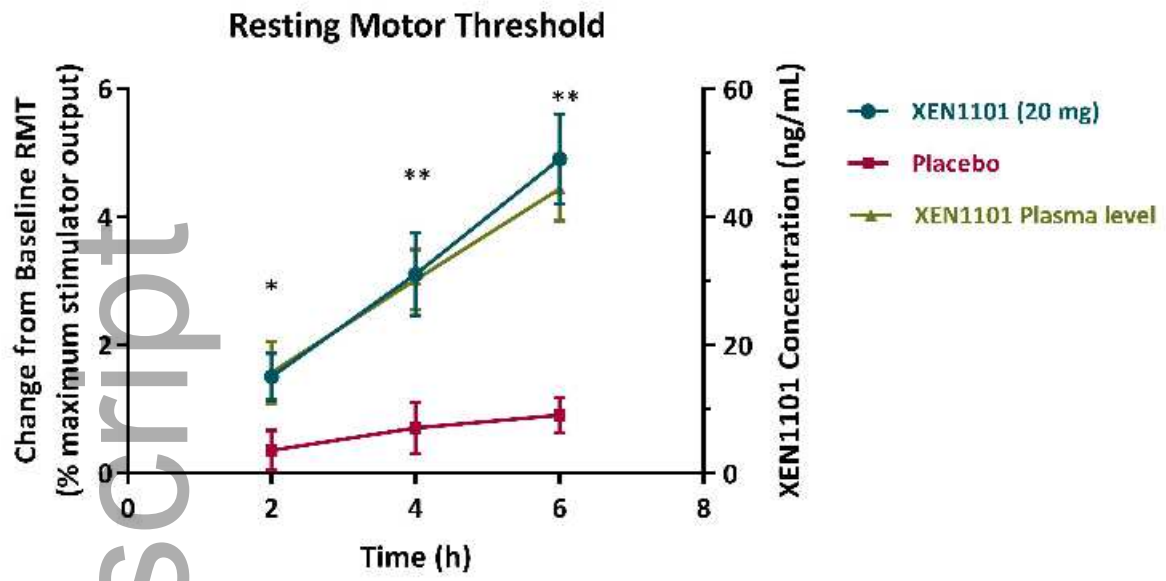
Cumulative Duration of Focal Seizures



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