Evaluation of OV329, a next-generation GABA-AT Inhibitor in a Series of Pharmaco-resistant Seizure Models Through the NINDS Epilepsy Therapy Screening Program

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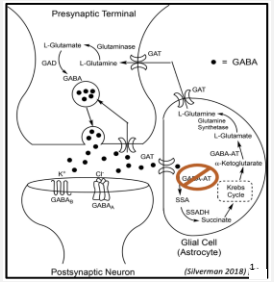
Poster #: 2.215

Background and Rationale



Reductions of GABA-mediated inhibitory signaling have been associated with seizures and epilepsy. One mechanism to increase GABAergic neurotransmission is the inhibition of GABA-amino transferase (GABA-AT), the primary catabolic enzyme of GABA. Administration of a highly potent and efficient GABA-AT inhibitor, OV329, is proposed to increase levels of GABA within the CNS through a mechanism of action similar to that of vigabatrin (VGB). OV329 is active in a series of clinically relevant and pharmacoresistant seizure models (Table, below). To further explore the potential efficacy of OV329 for treatment of drug resistant epilepsy, OV329 has been enrolled in the NIH/NINDS Epilepsy Therapy Screening Program (ETSP).

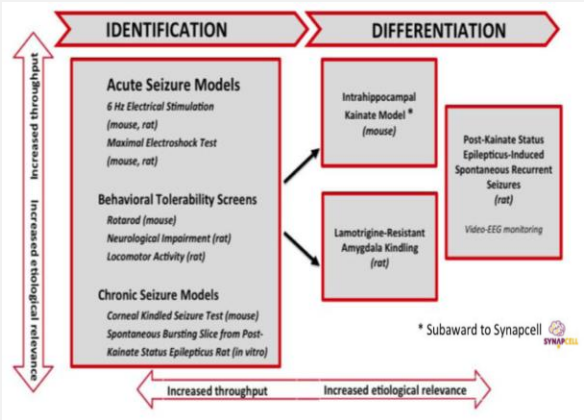
GABAergic synapse. Catabolism of GABA. GAD = glutamic acid decarboxylase; GAT = GABA transporter; GABA-AT = GABA aminotransferase. SSADH = succinic semialdehyde dehydrogenase.

OV329 Efficacy in Other Seizure Models

Model	Injury Model	OV329 Dose	Observations	Ref
NMDA-Induced model of Infantile Spasm (IS)	Acute	0.0025, 0.01, 0.1, and 1 mg/kg	Decreased seizure scores at ≥ 0.0025 mg/kg; ED_{50} estimated to be ~ 0.014 mg/kg	2, 3
Pentylenetetrazol (PTZ)-Induced Seizure	Acute	5, 20, and 40 mg/kg	Full protection at 40 mg/kg	4
Amygdala-kindled seizure model	Chronic	5, 20, 30, and 40 mg/kg	Active at 40 mg/kg	4

Methods

Epilepsy Therapy Screening Program



Results

Summary of MES Screening

Study (Site)	Oral doses (mg/kg)	Dose time (hours, pre-MES)	Protection	Untoward Effects (death post tonic extension)
Screen 1 (Utah), mouse	3, 10, 30	0.5, 2	None	0.5 h: 25-50% across doses 2 h: 25% 10 mg/kg only
Screen 2 (Utah), mouse	1, 3, 10	4, 6	None	6 h: 25% 1 mg/kg only
Screen 3 (Utah), rat	15	0.25, 0.5, 1, 2, 4	None	1 h: 25% 4h: 25%

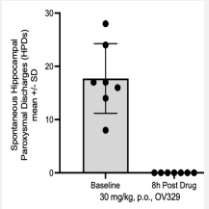
OV329 (p.o.) was assessed in the MES model[11] in three screening experiments using n = 4 animals/dose/timepoint and at least two timepoints.

Summary of 6 Hz Screening

Study (Site)	Oral doses (mg/kg)	Dose time (hours, pre-6Hz)	Protection	Untoward Effects
Screen 1 (Utah)	3, 10, 30	0.5, 2	1 of 4 mice, @ 2h (10 and 30 mg/kg)	None
Screen 2 (Utah)	1, 3, 10	4, 6	None	None
Screen 3 (Utah)	30, 60	0.25, 0.5, 1, 2, 4	None	4 of 4 mice sedated, 60 mg/kg @ 4 h
Replication (Washington)	30	0.25, 0.5, 1, 2, 4, 8, 24	None	3 of 4 mice adverse motor effects, 30 mg/kg @ 8 h

OV329 (p.o) was evaluated in the 6 Hz (44 mA) seizure model[12] using male mice (n = 4/dose/time point) for three screening studies and a replication study.

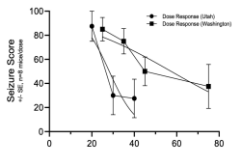
Intra-Hippocampal Kainate (IHK) Model of MTLE



OV329 (30 mg/kg, p.o.) was evaluated in the IHK-MTLE model[13]. A mean baseline count of 17.7 ± 6.6 HPDs was determined prior to drug administration. HPDs were abolished (0.0 % ± 0.0) in all animals when assessed at 8-hours. Two of the seven mice were reported to be comatose at this timepoint with the remainder of the subjects described as quiet. Efficacy of OV329 in this model has been noted at lower concentrations[3] and following repeat dosing (AES Poster #2.213)

Summary of CKM Screening

Study (Site)	Oral doses (mg/kg)	Dose time (hours, pre-corneal stim.)	Protection	Untoward Effects
Screen 1 (Utah)	1, 3, 10	2, 4	none	none
Screen 2 (Utah)	30, 60	2, 4, 8	30- 85% reduction in seizure scores; Greatest reductions at 8h	Only in 60 mg/kg group: ataxia, motor impairment, death (2 of 12 mice)
Dose Response (Utah)	20, 30, 40	8	ED_{50} = 27.9 mg/kg, p.o. (95%CL: 19 - 36.7 mg/kg)	none
Dose Response Replication (Washington)	25, 35, 45, 75	8	ED_{50} = 46.7 mg/kg, p.o. (95%CL: 25-75 mg/kg)	Only in 75 mg/kg group: motor impairment (5 of 8 mice)



Summary of CKM EC_{50} Calculations

Test		Time to Peak Effect (TPE)	ED_{50}[a]	95% Confidence Interval	p^b	z^c
Corneal Kindling	UU	8 hr	27.92	(19 - 36.7)	0.234	2.12
	UW	8 hr	46.70*	(25 - 75)		

[a] median effective dose (ED_{50}) UU = U of Utah; UW = U of Washington.
[b] proportion of the overlap
[c] test statistic z; reject the null hypothesis of the equality of the two means when z test is larger than 1.96 as long as p is less than 0.29

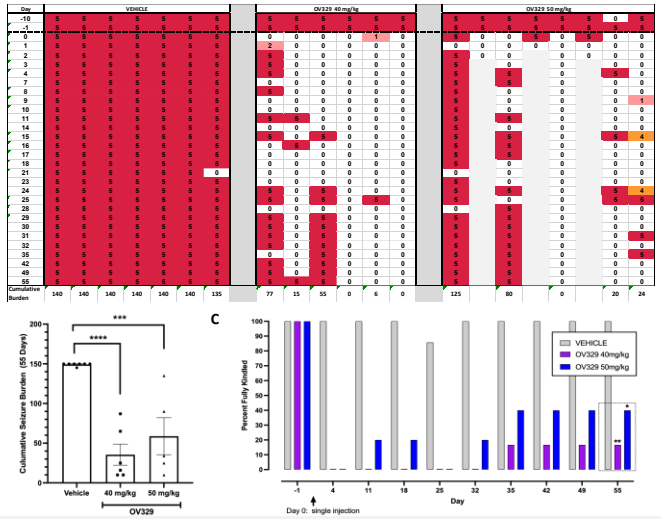
OV329 (p.o.)was assessed in the corneal kindling seizure test[14] in two screening experiments (n = 4 mice/dose/time) and two dose response experiments (n =8 mice/dose/time). Dose response studies were conducted in two different laboratories using an 8-hour pretreatment time.

CKM Longitudinal Data

A Groups of 8 fully kindled mice were treated with a single dose of either vehicle or OV329 (40 or 50 mg/kg, p.o). Seizures scores were recorded prior to drug administration (day -10, day -1), on the day of drug administration (day 0), daily (5 days/week) for 4 weeks following drug administration (days 1-32), and weekly for 4 subsequent weeks (days 35-55). Following treatment kindling status was verified in subsequent stimulation and, where five consecutive stage-5 seizures were observed, full kindled status was considered resumed. Greyed out regions indicate where animals died and thus seizure scores were no longer collected.

B Final cumulative seizure burden following treatment with either oral vehicle or OV329 (40 and 50 mg/kg). **P=0.0006, ****P<0.0001 (one-way ANOVA, Dunnett's post-hoc test).

C The percentage of fully kindled mice is shown for vehicle-treated (VEH; grey) and OV329 (40 mg/kg, purple; 50 mg/kg p.o, blue). Kindling status resumed immediately for VEH-treated animals (i.e. after 5 stimulations) whereas it was delayed or absent in mice treated with OV329 (16.7% and 40% recovered fully kindled status in 40 mg/kg and 50 mg/kg treatment groups, respectively). *P <0.05, **P<0.01, Fisher's exact test (vs. VEH) for the number of fully kindled mice on Day 55 post-dose.



Rotarod

- OV329 was assessed in this rotarod test in two screening experiments.[10]
- In the first screen, animals (8/group) were dosed (p.o.) with 3, 10, 30 mg/kg OV329 and tested at 0.5h or 2h after initial dose.
- In the second screen, animals were dosed with 1, 3, 10 mg/kg (p.o.) and examined at 4h or 6h post dosing.
- No motor impairment was seen following OV329 treatment in mice by the rotarod test in either screen.

Conclusions

Model	OV329 Efficacy
6 Hz Electrical Stim.	• No protection from seizure activity within tolerated dose range
Maximal Electroshock	• No protection from seizure activity within tolerated dose range
Corneal Kindling	• Significant protection from seizure activity with independent ED_{50} determinations 27.9 and 46.7 mg/kg, p.o. • Motor impairment/ataxia noted at doses of 60 mg/kg, p.o. and above • Long term protection from requirement of kindling after a single dose of 40 or 50 mg/kg, p.o.
IHK-MTLE	• Full protection from seizure activity at 30 mg/kg, p.o.; sedation noted (8h)
Rotarod	• No impairment noted up to 30 mg/kg, p.o., through 6h post-dost

Next Steps Ongoing and future studies will attempt to evaluate repeat dosing at lower dose levels in corneal kindled model. Efficacy in the Intrahippocampal Kainate MTLE models has been confirmed with doses as low as 3.0 mg/kg/day for up to 8 days of dosing (See AES Poster #2.213). Currently, OV329 is being evaluated in the Intra-Amygdala Kainate model of spontaneous recurrent seizures.

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References: **1.** Silverman, RB (2018) Chem Rev 118(7): 4037-4070. **2.** Davies P et al. AES 2021 Poster #1.270 **3.** Bialer, M. et al. Epilepsia. 2022;00:1-18 **4.** Feja M, et al. (2021) Epilepsia 62(12): 3091-3104. **5.** Shi XY, et al. (2015) Epilepsy Res 118: 29-33. **6.** Green AR, Murray TK (1989) J Pharm 41:879–80. **7.** Duveau V, et al. (2016) CNS Neurosci Ther 22:497-506. **8.** https://panache.ninds.nih.gov/Home/CurrentModels. **9.** Juncosa JI, et al. (2018) JACS, 140(6): p. 2151-2164. **10.** https://panache.ninds.nih.gov/TestDescription/TestRotarod. **11.** https://panache.ninds.nih.gov/TestDescription/TestMES. **12.** https://panache.ninds.nih.gov/TestDescription/Test6HZ. **13.** https://panache.ninds.nih.gov/TestDescription/TestIHKM. **14.** https://panache.ninds.nih.gov/TestDescription/TestCKST.

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