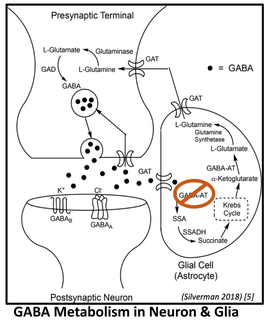
# Low, Repeat Dosing of OV329 Enhances GABA-AT Inhibition in Rodent Brain - Relationship between Pharmacokinetic and Pharmacodynamic Effects

Shing-Hong Lin[1],PhD; Andrea Vecchione[1]; Ray Colburn[2], PhD; Jay Mukherjee[1], PhD; Patrick D. Sarmiere[1], PhD

Poster #: 3.255

## Background and Rationale

GABA, the primary inhibitory neurotransmitter in the adult CNS, is catabolized by GABA-aminotransferase, GABA-AT. Therefore, inactivation of GABA-AT can elevate GABA level within CNS and potentially reduce neuronal hyperexcitability associated with seizures and epilepsies. OV329 is a highly potent, mechanism-based, GABA-AT inhibitor that holds the potential to be the best-in-class antiepileptic drug with a similar mechanism of action to vigabatrin (VGB) (1-4). Previously, we had shown that a low, repeat dose of OV329 (3mg/kg,QD,8-days) significantly reduced the number of focal seizures in a mouse model of mesial-temporal lobe epilepsy. The PK data in rodents suggests that OV329 peaks within 30 minutes and mostly gets eliminated within 4 hours with an approximate half-life of ~1.5 hours. Given the short exposure and delayed PD effect, it is critical to understand how OV329 inhibits GABA-AT activity in brain relative to its PK profile. Also, given OV329 is highly potent to GABA-AT, we examined its efficacy towards other ATs e.g., Aspartate/Alanine-AT (AST/ ALT), found in liver. The mechanistic insights gained here will potentially inform how to optimally use OV329 in clinic.

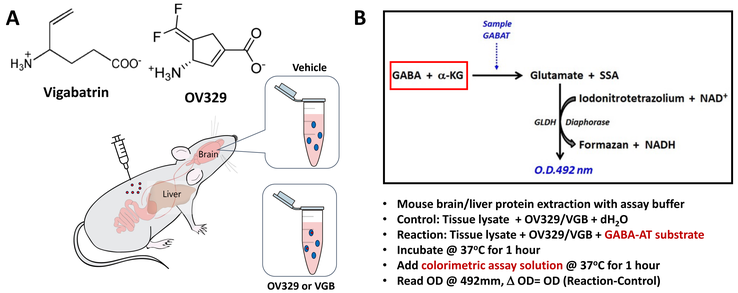



**GABA Metabolism in Neuron & Glia**

***OV329 is a mechanism-based inhibitor of GABA-AT and is markedly more potent than vigabatrin. We hypothesize that OV329 will be efficacious at lower concentrations than vigabatrin in drug-resistant epilepsies, offering a broader therapeutic window and use potential.***

## Methods

### Figure 1: Experimental Set Up and Principle of GABA-AT Activity Assay




- Mouse brain/liver protein extraction with assay buffer
- Control: Tissue lysate + OV329/VGB + $dH_2O$
- Reaction: Tissue lysate + OV329/VGB + GABA-AT substrate
- Incubate @ 37°C for 1 hour
- Add colorimetric assay solution @ 37°C for 1 hour
- Read OD @ 492mm, Δ OD= OD (Reaction-Control)

**A.** Structures of vigabatrin and OV329. Wildtype C57BL6 mice (8-12wks) were dosed with either IP or PO with OV329 or VGB. For target engagement study, mouse brain or liver tissue were collected at various time points and subjected to enzymatic analysis. For PK study and GABA detection, samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to detect OV329. For, GABA-AT assay and western blot, tissue homogenates were prepared, and protein concentration was measured by BCA assay. **B** The assay is based on the sequential GABAT-mediated transamination reaction and glutamate dehydrogenase (GLDH) reaction, which couples the reduction of Iodonitrotetrazolium to formazan allowing for sensitive detection of GABAT enzyme activity in cell or tissue extracts. GABA-AT converts GABA to glutamate and the assay measures the conversion of glutamate to formazan through a colorimetric reaction. To determine GABA-AT activity, ΔOD was measured between control (- substrate) and reaction (+ substrate) samples. Due to the high expression of GABA-AT in liver, its activity was monitored and served as control.

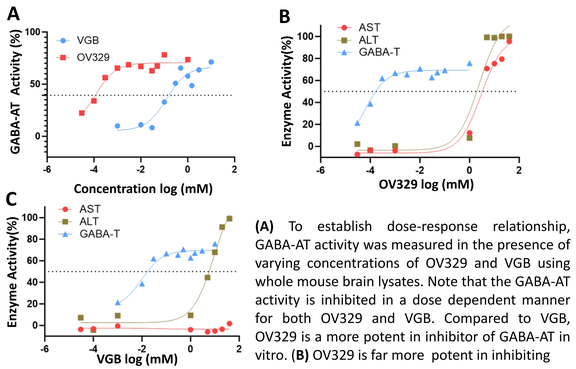
**References** **1.** Wild JM, et al. CNS Drugs. 2009; 23(11):965-82. **2.** Maguire MJ, et al., Epilepsia, 2010. 51(12): 2423-31. **3.** Juncosa JI, et al. J Am Chem Soc. 2018;140(6): 2151-64. **4.** Silverman R, Chem Rev 2018 Apr 11;118(7):4037-4070.

**Acknowledgements** This study was funded by Ovid Therapeutics Inc.

**Author affiliations** 1Ovid Therapeutics, 441 9th Avenue,14th floor New York, NY 10001; 2RW Colburn Consulting, 2 Kathryn Rd, Chalfont, PA 18914

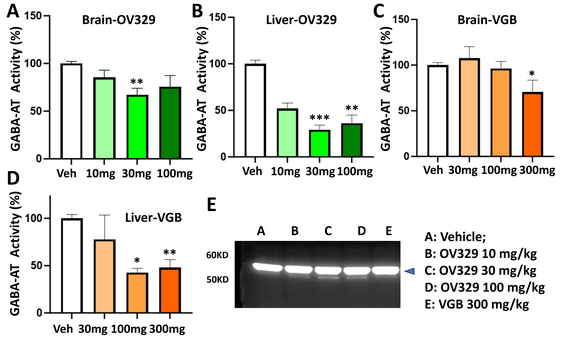
## Results

### Figure 2: OV329 is a Highly Potent Inhibitor of GABA-AT with Minimal Activity to AST and ALT



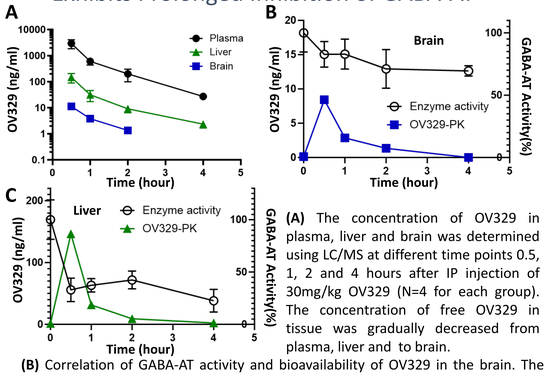

**(A)** To establish dose-response relationship, GABA-AT activity was measured in the presence of varying concentrations of OV329 and VGB using whole mouse brain lysates. Note that the GABA-AT activity is inhibited in a dose dependent manner for both OV329 and VGB. Compared to VGB, OV329 is a more potent in inhibitor of GABA-AT in vitro. **(B)** OV329 is far more potent in inhibiting GABA-AT ($IC_{50}$~57nM) in liver than both AST (IC50 ~2.94mM) and ALT is ($IC_{50}$ ~2.45mM). **(C)** VGB is almost 1000-fold more potent in inhibiting GABA-AT ($IC_{50}$ ~9.53μM) than ALT ($IC_{50}$ ~9.21 mM) and did not show any activity on AST. Mouse Liver or brain tissue homogenate (20 μg of total protein) was used in assay with varying concentrations of OV329 and VGB. All data points were duplicated. For each point, N=3.

### Figure 3: GABA-AT Activity is Significantly Reduced After Single Dose of OV329




Following 4 hrs of OV329 treatment, GABA-AT activity in brain **(A)** and liver (**B**) was compared to vehicle treated mice. Normalized enzyme activity (control = 100%) showing 30 mg/kg OV329 treatment significantly reduced GABA-AT activity in brain (P<0.01) and liver (P<0.01) whereas reduction of GABA-AT was not significant in both brain and liver for 10 mg/kg OV329. **(C,D)** Similar treatment with VGB showed GABA-AT inhibition in brain at 300mg/kg dose. Data: mean ± SEM, one-way ANOVA, non-parametric Kruskal-Wallis test with Dunn's comparison test, Vehicle: n=7 and OV329 n=6 per dose level. (*P<0.05, **P<0.01, ***P<0.001) **(E)** Western blot showing GABA-AT expression remain unchanged after 4 hrs of OV329 treatment.

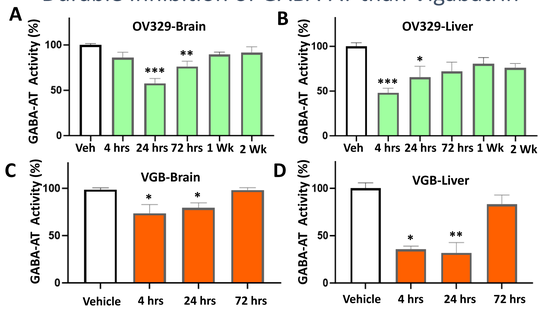
### Figure 4: OV329 is Rapidly Eliminated but Exhibits Prolonged Inhibition of GABA-AT




**(A)** The concentration of OV329 in plasma, liver and brain was determined using LC/MS at different time points 0.5, 1, 2 and 4 hours after IP injection of 30mg/kg OV329 (N=4 for each group). The concentration of free OV329 in tissue was gradually decreased from plasma, liver and to brain.

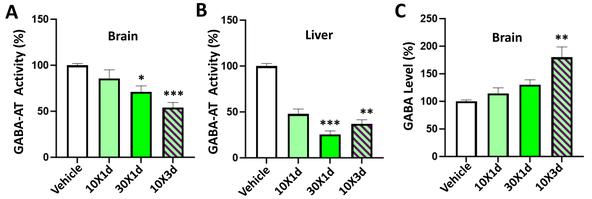
**(B)** Correlation of GABA-AT activity and bioavailability of OV329 in the brain. The peak availability of OV329 in the brain was at 30 minutes, but the peak inhibition of GABA-AT was at 4hours. **(C)** Correlation of GABA-AT activity and bioavailability of OV329 in the liver. The peak concentration of OV329 in liver was at 30 minutes, at which significant GABA-AT inhibition was observed.

### Figure 5: A Single Dose of OV329 has More Durable Inhibition of GABA-AT than Vigabatrin




GABA-AT activity in the brain and liver was measured at different time points after a single dose of 10 mg/kg OV329. **(A)** Normalized GABA-AT activity in brain was significantly reduced within 24 hours (P<0.001) and 72 hours (P<0.01) after initial dose of OV329. The effect was not significant at 4 hours (P=0.37), 1-week (P=0.41) and 2-weeks (P>0.99) when compared to vehicle. **(B)** In liver, significant inhibition of GABA-AT activity was observed at 4 hours (P<0.001) and 24 hours (P<0.05) but enzyme inhibition was not significant either after 72 hours (P=0.14), 1-week (P=0.73) or 2-weeks (P=0.17). N=7 for vehicle and N=5 for each OV329 group. **(C)** The effect of a single IP injection of 300 mg/kg VGB on brain GABA-AT was significant at 4 and 24 hrs (p<0.05) but back to control level within 72 hrs. **(D)** In liver, the effect of a single IP injection of 300 mg/kg VGB was significant at 4 (p<0.05) and 24 hrs (p<0.01) but no longer significant after 72 hrs. Data: mean ± SEM, one-way ANOVA, nonparametric Kruskal-Wallis test with Dunn's comparisons test. (*P<0.05, **P<0.01,***P<0.001)

### Figure 6: Repeat Dosing of OV329 Significantly Reduced GABA-AT Activity and Elevated Brain GABA Levels




GABA-AT activity in brain and liver was compared between single, acute dose and 3 daily doses of 10 mg/kg OV329. Normalized enzyme activity (Control=100%) showing repeat dosing of OV329 treatment significantly reduced GABA-AT activity in both brain **(A)** (p<0.01) and liver **(B)** (p<0.001). **(C)** Compared to vehicle, increased GABA level, determined by mass spectrometry, in total brain homogenate was observed after repeat dosing of OV329 (P<0.01); N=5-10, Data: mean ± SEM, nonparametric Kruskal-Wallis with Dunn's post hoc multiple comparisons test. (*P<0.05, **P<0.01, ***P<0.001)

### Figure 7: QD or BID Repeat Dosing of OV329 Significantly Reduced GABA-AT Activity in Brain

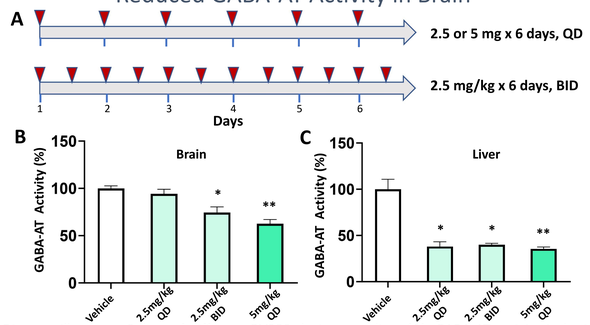



Diagram illustrates 6-days of different OV329 dosing paradigm **(A)**. GABA-AT activity in brain and liver was significantly reduced after 6 days of different OV329 dosing treatment (p<0.01). Normalized enzyme activity in brain showing significant reduction of GABA-AT activity after 5 mg/kg QD (P<0.01) and 2.5 mg/kg BID (P<0.05), but not with 2.5 mg/kg QD (P=0.99), compared to vehicle treated mice **(B)**. In contrast, in liver all treatment resulted in a significant inhibition of GABA-AT compared to vehicle (p<0.05 for 2.5xQD and BID and p<0.01 for 5xQD) **(C)**. Data; mean ± SEM, one-way ANOVA, non-parametric Kruskal-Wallis test with Dunn's multiple comparisons test, N=6 for all conditions.(*P<0.05, **P<0.01, ***P<0.001)

## Conclusions

- **Compared to VGB, OV329 is more potent in inactivating GABA-AT and the inhibitory effect on GABA-AT lasts longer *in vivo*.**
- **A significant level of GABA-AT inhibition with a concomitant change in GABA level in brain was achieved by daily repeat administration of OV329.**
- **The cumulative PD effect gained through low and repeat doses of OV329 might be a viable strategy to demonstrate target engagement & anti-seizure property of OV329.**