CNS-3D functional organoids predict clinical neurotoxicity outcomes for small molecules and anti-sense oligonucleotides

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Background and Purpose

Unanticipated central nervous system (CNS) neurotoxicity remains a major driver of late-stage clinical attrition, particularly for drugs that progress through traditional preclinical testing without revealing human-relevant liabilities. This challenge is further amplified for emerging modalities such as antisense oligonucleotides (ASOs), where CNS adverse events are difficult to predict based on sequence or target biology alone.

Human induced pluripotent stem cell (iPSC)-derived cortical organoids provide a physiologically-relevant system to model neuronal network activity and functional perturbations *in vitro*. In this study, we leverage a combination of 28bio CNS-3D Brain Organoids and AI-enabled predictive modeling to identify clinically observed seizure liability across both small molecules and ASOs.

Functional Measurements and Predictions on CNS-3D Organoids

CNS-3D Brain Organoids were differentiated following established methods[1,2,3] that provide stable, spontaneous electrophysiological network activity in high throughput screening compatible 384-well ultra-low attachment (ULA) plates. Functional responses were assessed using calcium imaging (FLIPR® Calcium 6 Kit) following 2 hours of exposure to various small molecule or ASO test articles. Activity recordings were analyzed using several open source and custom-built feature extractors, depending on therapeutic modality. Predictive models were constructed using gradient-boosted decision trees (XGBoost) for small molecules (**Figure 1**) and logistic regression for ASOs (**Figure 2**), enabling drug-level classification of seizure liability.

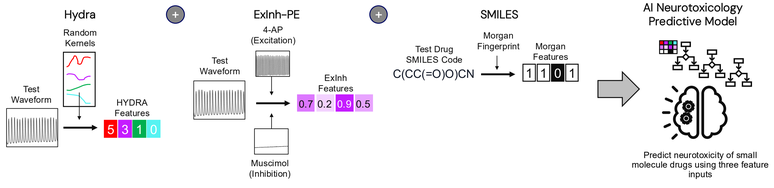

Figure 1 - AI neurotoxicology predictive model. Feature extraction captured spontaneous activity dynamics using (1) high-dimensional time-series representations – Hydra[4], (2) Excitation–inhibition phenotypic embeddings (ExInh-PE) built on excitatory (4-AP) and inhibitory (muscimol) phenotypic profiles and (3) chemical structure descriptors – Morgan fingerprints[5]. These features were used to train an AI neurotoxicology predictive model constructed using gradient-boosted decision trees (XGBoost) and validated via 5-fold cross-validation to avoid overfitting.

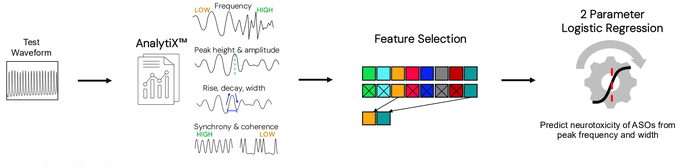

Figure 2 - Logistic regression architecture for ASO tolerability prediction. Feature extraction captured spontaneous activity dynamics using 28bio AnalytiX™ Software, a software package that quantifies the number, size, and shape of neural bursts from CNS-3D Brain Organoids in an automated fashion. Two features - peak frequency and peak width - were used to train a logistic regression model predicting ASO tolerability.

Small Molecule and ASO selection

Small molecule drugs were selected based on documented human clinical seizure outcomes. The panel comprises 30 seizure-inducing and 36 clinically safe drugs curated from clinical trial reports, FDA filings, and public drug databases (e.g., DailyMed, NeuroDerisk) (**Table 1**). Drugs were evaluated across pharmacokinetically guided exposure ranges (0.01 - 100x C_{max}) as previously described[3].

Twenty-four phosphorothioate gapmer ASOs with LNA-modified flanks were selected from a previously characterized panel[6]. The set included sequences with known *in vivo* neurobehavioral toxicity following intracerebroventricular (ICV) administration in mice. ASOs were selected to include clearly toxic, clearly safe, and borderline cases that were challenging to predict based on sequence alone (**Table 2**).

Table 1 - Clinically safe and seizure-associated small molecules.

Safe	5-Aminovaleric acid, Acetaminophen, Ambroxol, Aspirin, Beclamide, Bicifadine, BMS 204352, Caffeine, Cyclizine, Cyclothiazide, Diclofenac, Diethylstilbestrol, Dopamine, Droxidopa, Fipronil, Gabazine, Ibuprofen, Iprindole, Lamotrigine, Lazabemide, LY 450108, Mepazine, Naproxen, Nicotine, Nifedipine, Norepinephrine, NS 1643, Ondansetron, Orphenadrine, Ralfinamide, Rimegepant, Rislenemdaz, Sotuletinib, Tianeptine, Tropisetron, Xanomeline
Seizure	Amfepramone, Aminophylline, Aminopyridine, Bay K 8644, Bicuculline, Bicuculline methiodide, Bromocriptine, Bupropion, Carbamazepine, Clozapine, Ethosuximide, Famotidine, Flumazenil, Hydrastine, Indomethacin, Kainic Acid, Lindane, Maprotiline, Meperidine, Metrizamide, Mexiletine, Minaprine, NMDA, Penicillin, Quetiapine, Ropivacaine, Rufinamide, Strychnine, Tiagabine, Venlafaxine

Table 2 - ASO panel of well-tolerated and neurotoxic sequences. blue target = MAPT, orange target = STC1, grey = nontargeting; uppercase = LNA-modified; lowercase = DNA nucleotide

Clear Safe	CAACtgattccatcaCC, CCTgatcacaaacCCT, GCaacaatccaaCCAC, GAATattacaccATCC, GTAaccaactttCATC, CTTtatttccaaattcaCTT, AAAtctataataaccacCAC
Borderline Safe	CATCtctctagacatCT, AGCgtgatcttccATC, CGTgatcttccatCAC, GCGtgatcttcCAT, TATttatattccaCAGT
Clear Toxic	TTCaggtctggcaTGG, TTAgccctaaagtcccaGGT, TGGttagccctaaagtcCCA, TAGtcactctggTGA, CCAgcgtgatcttCCA, AAGatgaaatttgCTC, TCaatattattctTCTG, AACattcaagccTTTC
Borderline Toxic	CGTgatcttccATC, TACtagcccacccATC, TaTTTccaaattcacTTtTA, ATTTccaaattcactTTTAC

Predicting Human Seizure Liability for Small Molecules

The AI neurotoxicology predictive model distinguished 30 seizure-inducing drugs from 36 clinically safe drugs with a sensitivity of 83.3% and specificity of 88.9% at the selected operating point on the receiver operating characteristic (ROC) curve (**Figure 3A**). Predicted scores also distinguished seizure mechanism (**Figure 3B**) and tended to be higher for drugs that more frequently cause seizures clinically (**Figure 3C**).

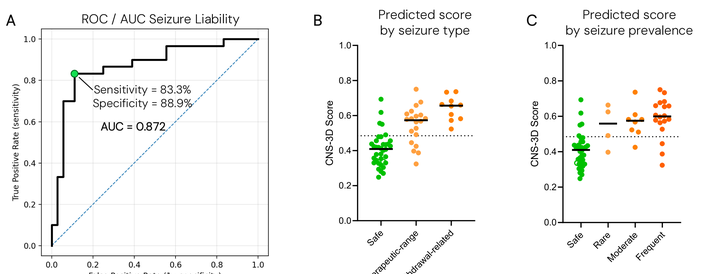

Figure 3 - Performance of small molecule human neurotoxicity predictions. (A) ROC curve for seizure liability prediction with AUC of 0.872; optimal threshold is highlighted in green and provides a sensitivity of 83.3% and specificity of 88.9%; blue dotted line indicates the performance expected under random classification. (B) Predicted scores for small molecule drugs grouped by seizure type. (C) Predicted scores for small molecule drugs grouped by seizure prevalence.

Predicting *In Vivo* Tolerability of ASOs

Current sequence-based predictors of ASO tolerability exhibit only 80% accuracy[6]. In contrast, CNS-3D Brain Organoids accurately predicted *in vivo* tolerability outcomes for ASOs, providing an area under the curve (AUC) of 0.931, with sensitivity of 91.7% and specificity of 91.7% (**Figure 4A**). A minimal feature set (peak frequency and peak width) was sufficient to distinguish toxic from well-tolerated ASOs, consistent with prior observations that network activity perturbations are key drivers of CNS liability. Predicted scores were also highly correlated with severity of *in vivo* tolerability (**Figure 4B**).

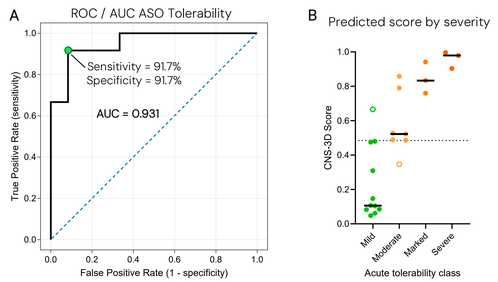

Figure 4 - Performance of ASO *in vivo* tolerability predictions. (A) ROC curve for *in vivo* tolerability prediction with AUC of 0.931; optimal threshold is highlighted in green and provides a sensitivity of 91.7% and specificity of 91.7%; blue dotted line indicates the performance expected under random classification. (B) Predicted scores for ASO panel grouped by acute tolerability class indicating strong relationship between CNS-3D scoring and severity of CNS effects. Open circles indicate false positive or false negative predictions.

Conclusions & Future Directions

This work demonstrates that human CNS-3D Brain Organoids, combined with AI-enabled predictive modeling, provide sensitive and specific prediction of seizure liability across therapeutic modalities. By leveraging functional network-level readouts, the technology captures clinically-relevant neurotoxicity signals that are not readily identified using traditional *in vitro* approaches, while avoiding false positive classifications that plague animal models. Key advances include:

- 83% sensitivity & 89% specificity in predicting seizure liability of small molecule drugs
- 92% sensitivity & 92% specificity in predicting CNS adverse effects caused by ASOs
- Balanced performance across both therapeutic modalities supports identification of seizure liabilities without sacrificing promising drug candidates.

Future efforts will focus on expanding predictive scope across CNS adverse event types (e.g. sedation, neuropsychiatric effects). Collectively, this work strongly supports the integration of human-relevant functional models into preclinical safety workflows.

References

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