Poster #290

Identification of a potential therapeutic compound for Rett syndrome using a highly homogenous human iPSC-derived cortical organoid screening platform

Nicholas Coungeris[1], Victoria Alstat[1], Cassiano Carromeu[2], Andrew LaCroix[1]
[1]AxoSim, Inc. Maple Grove, MN: [2]StemoniX, Maple Grove, MN

AxoSim Human Data, Faster.

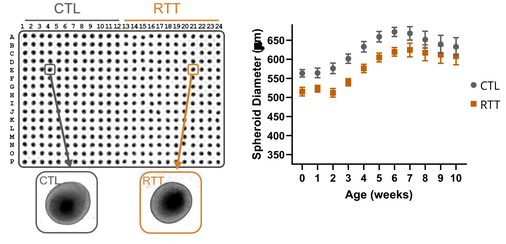
microBrain™ Functional Organoids

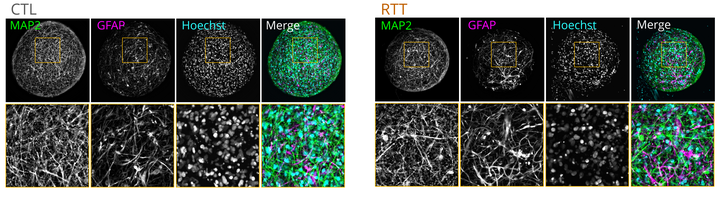
Overview

Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in *MECP2*. Despite decades of research and identification of several promising therapeutic candidates in 2D cultures and animal models of RTT, there are currently no disease-modifying treatments available for RTT patients. AxoSim leverages human induced pluripotent stem cells (iPSCs) to generate a homogenous functional screenable cortical organoid platform that can be used to efficiently screen large compound libraries in a reproducible manner. Using RTT patient-derived lines, these cortical organoids can be analyzed for their highly reproducible, physiologically relevant functional phenotype. AxoSim utilized the RTT microBrain™ platform to reveal the novel potential therapeutics, acetylcholinesterase (AChE) and histone deacetylase (HDAC) inhibitors, as the most promising targets based on an algorithm designed to quantify the shape, size and count of the functional activity. Further analysis revealed similar targets showed the same recovery fingerprint while having distinct recovery profiles from other biological targets. In summary, we show that AxoSim's microBrain™ model can be used to identify promising therapeutic compounds in a human-first fashion.

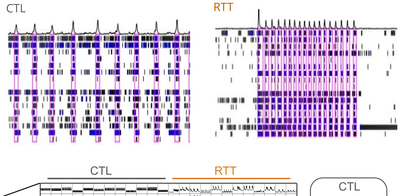
microBrain™ Functional Organoids

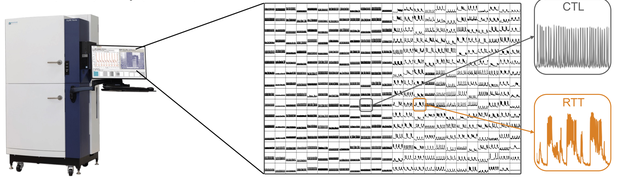
microBrain™ is a scalable cortical organoid platform that can be grown in high-throughput plate formats up to 384-wells. Here we show the similar growth trajectories of control and RTT patient-derived organoids throughout their differentiation (right). In addition, both cell lines show remarkably similar neuron to astrocyte ratios (below) making the platform ideal for reproducible data generation and comparison of other endpoints.





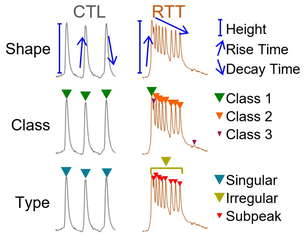
The hallmark of the microBrain™ platform is the spontaneous functional activity. However, we observe that, unlike the regularly synchronized bursting in the control organoids, the RTT organoids exhibit clustered bursts of hyperactivity. These functional differences are observed to be electrically-driven (MEA, top) and can be captured in high throughput using a calcium reporter dye and fluorometric imaging plate reader (FLIPR) assay (bottom).

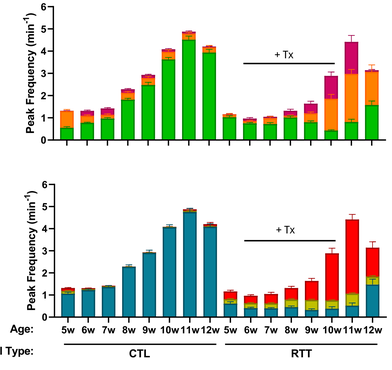




Functional Phenotypic Analysis

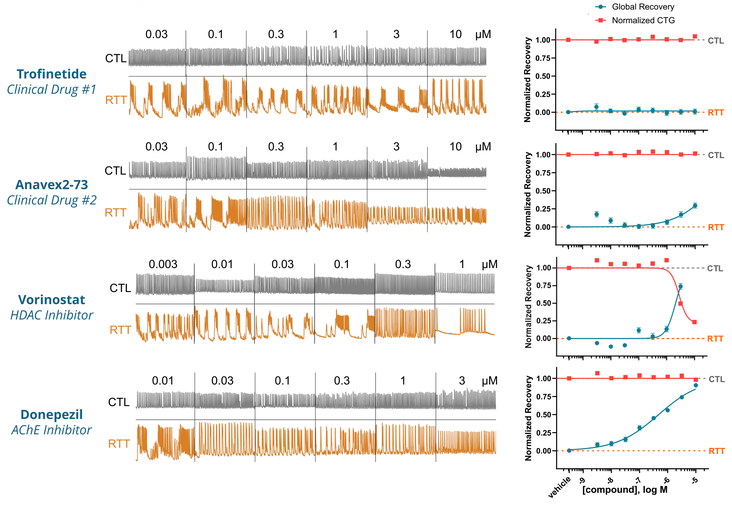
To analyze the unique phenotypic activity of the RTT microBrain™, over 50 features describing peak shape, class and type are extracted to fully characterize the functional activity. Overtime characterization shows the appearance of class 2 and 3 peaks in the RTT organoids (top) and irregular and subpeaks (bottom) that show maximal differences from control around 10-11 weeks.





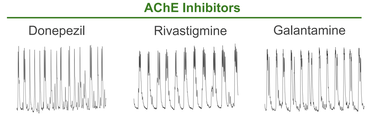
Functional Phenotypic Recovery

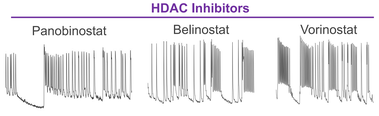
Applying the previously mentioned analysis to our SMART library screening data we can not only visualize the broad ranges of waveforms generated but also apply an in-depth characterization of the recovery responses. The global recovery metrics of two clinical candidates, Trofinetide and Anavex 2-73, show minimal to no recovery as high as 10µM. In contrast, molecules targeting two distinct pathways (AChE and HDAC) showed strong recovery. Recovery through HDAC inhibition (vorinostat) came at the cost of toxicity. Rescue through AChE inhibition was robust, pharmacologically gradual and did not result in any overt toxicity in the RTT patient-derived organoids.

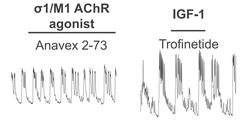


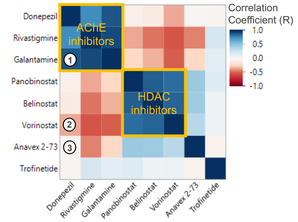
Target-Specific Rescue Profiles

We screened three AChE and HDAC inhibitors and compared a partial rescue dose to two clinical trial candidates. Correlation analysis show clear phenotypic fingerprints amongst donepezil, rivastigmine and galantamine while showing distinct features rescued by the HDAC inhibitors. Molecules targeting either AChE or HDAC also showed dramatically better rescue when compared to both clinical molecules (trofinetide, Anavex 2-73).

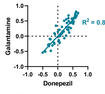




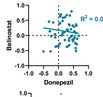




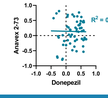
① Consistent pattern of feature recovery among AChE inhibitors



② AChE inhibitors are distinct from HDAC inhibitors



③ AChE inhibitors are distinct from Anavex 2-73



Future Directions

Here we showed how microBrain™ cortical organoids can be used to visualize, screen and analyze physiologically relevant functional phenotypes in neurodevelopmental disorders. Through a combination of model development, high-throughput screening, target validation, and correlation analysis, we identified AChE and HDAC inhibitors as potential therapeutics that could provide disease-modifying relief to RTT patients. Further investigation into these targets opens the potential for co-treatments and various re-purposing compounds in the clinical landscape.

While microBrain™ functional organoids contain the most highly represented cell types in the human brain (neurons and glia), the platform could be further enhanced by incorporating cell types not achievable through standard differentiation protocols (e.g. microglia). Preliminary investigations show successful incorporation of microglia into 2- and 5-week old organoids. The key finding in these optimization studies was the incorporation of IL-34 to support microglia survival. Further optimization and characterization of this proof-of-concept inflammatory organoid model could enable investigations into a diverse set of neurodegenerative diseases driven by neuroinflammation.