Clinically Related Rare Neurodevelopmental Disorders Display Distinct Functional Phenotypes in Human iPSC-derived Cortical Organoids

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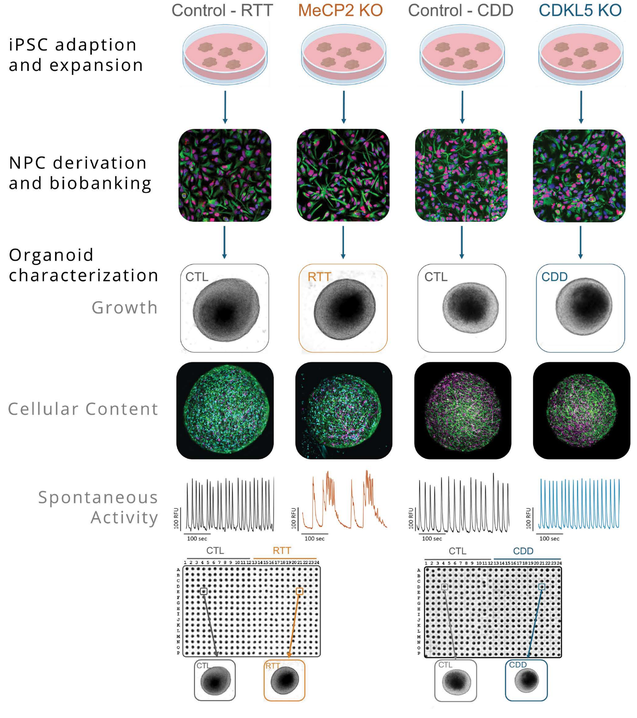
Overview

- Rett syndrome (RTT) and CDKL5 deficiency disorder (CDD) are rare X-linked neurodevelopmental disorders that share overlapping phenotypic features including cognitive defects, developmental delays, and seizures.
- microBrain™ is a scalable cortical organoid platform that can be grown in high-throughput plate formats up to 384-wells.
- RTT and CDD organoids were used to screen over 5000 compounds and identify promising biological targets and molecules that corrected aberrant function through unique mechanisms of action.

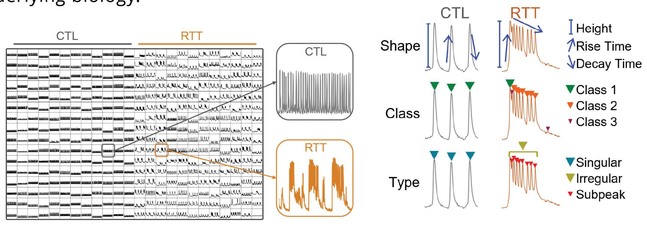
iPSC-derived Functional Organoids

- Patient-derived iPSCs with defined genetic mutations were used to derive neural progenitor cells
- Both healthy and KO organoids grow to similar sizes and show appropriate cellular composition but show striking differences in terms of their calcium bursting

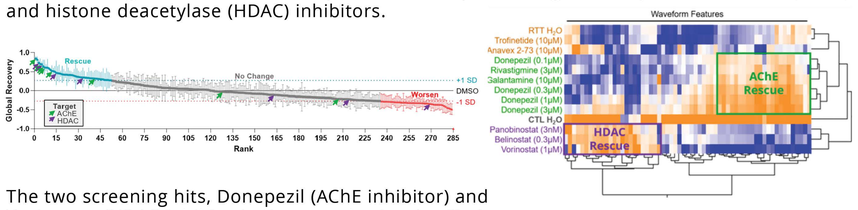


Drug Screening for Rett Syndrome

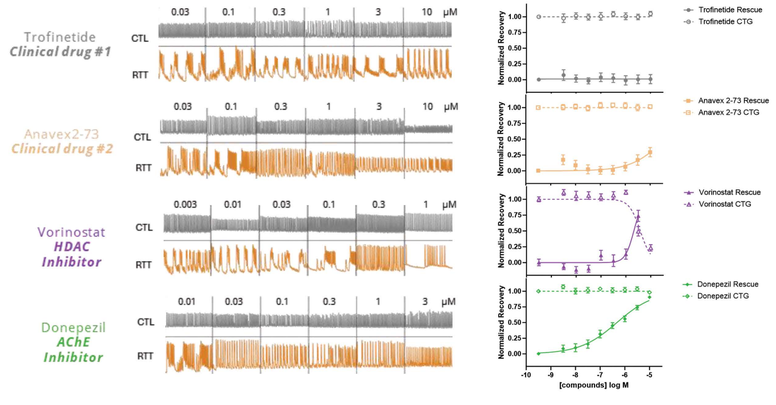
Rett organoids exhibit complex aberrant calcium oscillations (non-uniform peak heights and peak shape). AxoSim's AnalytiX software breaks down activity bursts by strength (class 1, 2, 3 peaks) and type (singular, irregular, and subpeaks) for a more comprehensive understanding of the underlying biology.



RTT patient organoids were used screen the SMART library, a set of 296 compounds curated by the International Rett Syndrome Foundation, over the course of 4 weeks to enable identification of disease-modifying therapeutics over short-acting symptomatic treatments. This screening revealed the novel potential therapeutic targets, acetylcholinesterase (AChE) and histone deacetylase (HDAC) inhibitors.

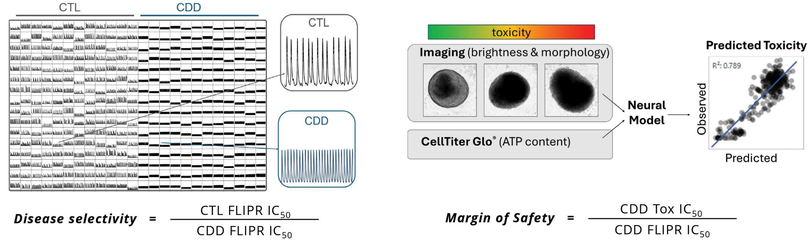


The two screening hits, Donepezil (AChE inhibitor) and Vorinostat (HDAC inhibitor), discovered using AxoSim's cortical organoid platform dramatically outperform existing clinical molecules available to patients with Rett Syndrome.



Drug Screening for CDD

CDD organoids exhibit a hyperexcitability phenotype (high calcium peak frequency). CDD organoids were treated in a chronic, 3-week dosing paradigm to identify disease-modifying therapeutics which restore peak frequency to that of control organoids.



Disease selectivity = $\frac{\text{CTL FLIPR IC}_{50}}{\text{CDD FLIPR IC}_{50}}$

Margin of Safety = $\frac{\text{CDD Tox IC}_{50}}{\text{CDD FLIPR IC}_{50}}$

Selective rescue = Disease selectivity ratio > 1

Non-specific = Disease selectivity ratio ≤1

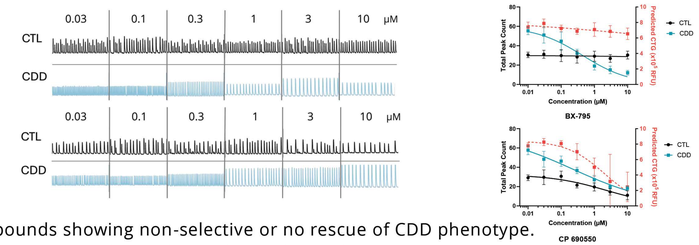
Safe compounds = Margin of safety ratio > 10

Toxic = Margin of safety ratio ≤ 10

Examples of compounds showing selective rescue of CDD phenotype.

VE-822
ATR Inhibitor
✓ Selective rescue
✓ Safe

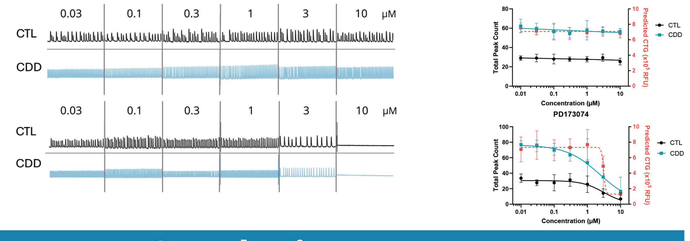
BX-795
PDKP1 Inhibitor
✓ Selective rescue
X Safe



Examples of compounds showing non-selective or no rescue of CDD phenotype.

CP 690550
JAK Inhibitor
X Selective rescue
✓ Safe

PD 173074
FGFR Inhibitor
X Selective rescue
X Safe



Conclusions

- AxoSim's cortical organoids recapitulate functional phenotypes characteristic of neurodevelopmental diseases
- RTT organoids were used to identify novel therapeutic targets AChE and HDAC inhibitors which outperform leading clinical candidates
- CDD organoids were used to identify 22 novel drug candidates spanning 15 biological targets in < 1 year through high-throughput functional screening