A nondestructive, image-based cytotoxicity assessment for combined safety and efficacy screening in human-iPSC derived organoids.

Poster #J468

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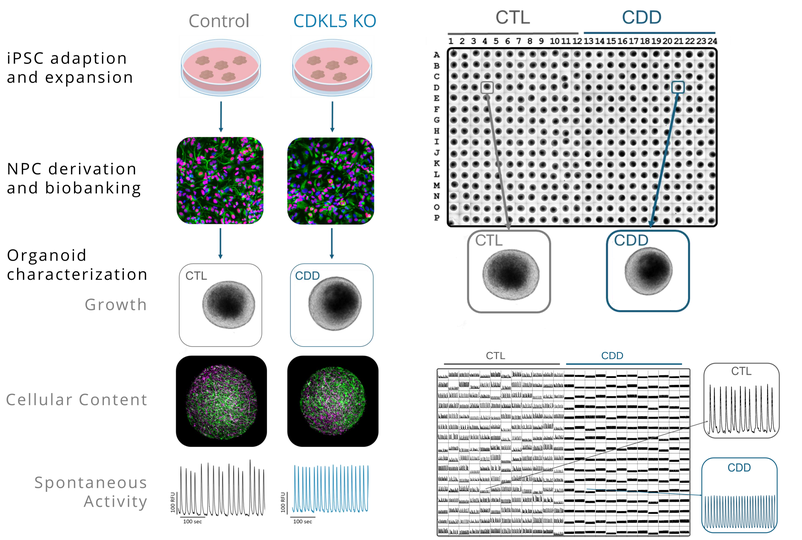
Background and Purpose

Drug development relies on multiple biochemical, cell-based, in vitro, and in vivo assays, each providing limited safety or efficacy data. We previously developed a human induced pluripotent stem cell (iPSC)-derived neural organoid system to model CDKL5-deficiency disorder (CDD). This model recapitulates key functional features of CDD, a rare X-linked neurodevelopmental disorder characterized by early onset seizures, developmental delay, and severe intellectual disability. This organoid model was previously used to identify promising drug candidates, though the safety profiles of the proposed drugs were unknown. Gold-standard ATP-based viability assays are destructive and can only be performed at the end of a drug treatment while longitudinal assays based on membrane rupture provide a non-destructive but costly and time-intensive means to monitor cell health. Here, we present a non-invasive, image-based viability assay used to monitor CDD patient-derived neural organoids, allowing for the combined safety and efficacy evaluation of potential therapeutics.

iPSC-derived Functional Organoids

microBrain™ is a scalable cortical organoid platform that can be grown in high-throughput plate formats up to 384-wells. When generating organoids using CDD patient cell lines, we observed striking differences from healthy parental control (CTL) in terms of their calcium bursting activity profiles. We verified the reproducibility of these distinct phenotypes across independent batches of organoid plates, showing consistency in the phenotypic fingerprint and strength across organoids, plates, and batches.

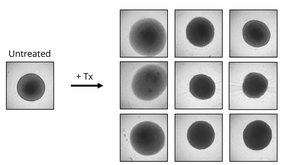


Scalable and Nondestructive Toxicity Endpoint

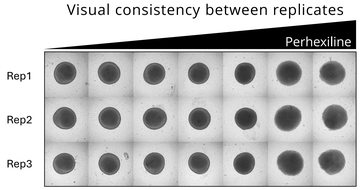
An imaging-based model to predict Cell Titer Glo (CTG) luminescence value was developed from brightfield images of CDD and CTL organoids treated with a broad range of neurotoxic agents in half-log, 7-point dose response for 4 days. A variety of morphological changes were observed following compound treatment (bottom left). Compounds caused highly reproducible morphological changes in organoids (bottom right).

Compound	Top Dose (uM)	Mechanism of Action
Valinomycin	300	Potassium ionophore
Berberine	300	Topoisomerase inhibitor & AMPK activator
Deltamethrin	100	Sodium channel activator
Hexachlorophene	100	Mitochondrial function disruptor
Simvastatin	100	HMG CoA reductase inhibitor
Clofazimine	30	ROS generator
Perhexiline	30	Fatty acid metabolism inhibitor
Ivermectin	30	Chloride channel activator
Staurosporine	10	Kinase inhibitor
Ixabepilone	3	Microtubule stabilizer
Rotenone	3	Mitochondrial complex I inhibitor
Vincristine	0.3	Microtubule disruption
Vinorelbine	0.3	Microtubule disruption
Bortezomib	0.1	Proteasome inhibitor

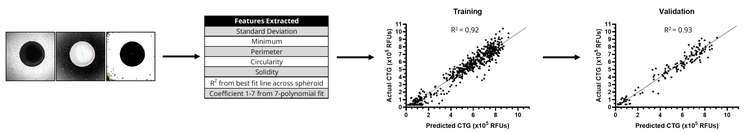
Diverse morphological changes



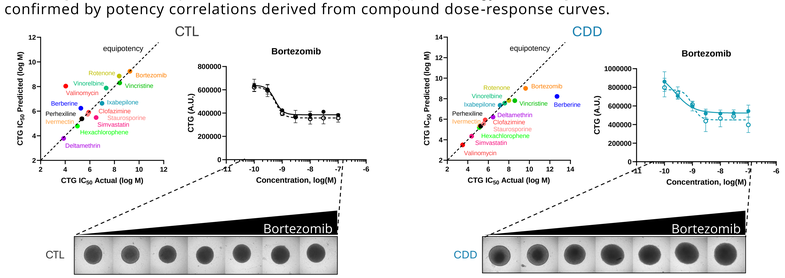
Visual consistency between replicates



ImageJ was used to filter brightfield images and extract features describing the intensities and textures in organoids. These morphological features were then used to train a neural network in JMP to predict luminescence value. The model was trained on 80% of the data with a 20% hold-out validation set.



The image-based toxicity model was able to accurately predict IC_{50} values regardless of cell line as confirmed by potency correlations derived from compound dose-response curves.



Safety and Efficacy Screening in CDD

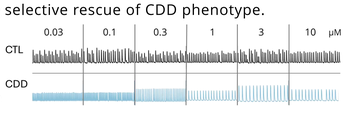
CDD and CTL organoids were screened in a chronic, 3-week dosing paradigm to identify disease-modifying therapeutics which restore peak frequency to that of control organoids. Hit compounds were identified based on the disease selectivity and toxicity score metrics shown below.

$$\textit{Disease selectivity} = \frac{\text{CTL FLIPR } IC_{50}}{\text{CDD FLIPR } IC_{50}} \qquad \textit{Toxicity Score} = \frac{\text{CDD Tox } IC_{50}}{\text{CDD FLIPR } IC_{50}}$$

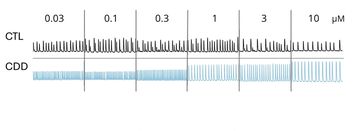
Selective rescue = Disease selectivity ratio > 1
Non-specific = Disease selectivity ratio ≤1
Safe compounds = Toxicity score > 10
Toxic = Toxicity score ≤ 10

Compounds showing selective rescue of CDD phenotype.

VE-822
ATR Inhibitor
✓ Selective rescue
✓ Safe

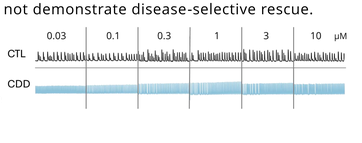


BX-795
PDKP1 Inhibitor
✓ Selective rescue
X Safe

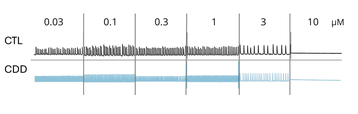


Compounds that did not demonstrate disease-selective rescue.

CP 690550
JAK Inhibitor
X Selective rescue
✓ Safe



PD 173074
FGFR Inhibitor
X Selective rescue
X Safe



Conclusions

- We developed an image-based toxicity assay for human iPSC-derived brain organoids, providing a nondestructive, cost-effective, and accurate alternative to gold standard viability assays.
- This novel toxicity endpoint is cell line agnostic – i.e. can be applied effectively to organoids derived from multiple donors.
- The non-destructive nature of this assay enabled simultaneous safety and efficacy testing in CDD patient-derived organoids, allowing for the identification of disease-selective neuromodulation and avoidance of drugs that cause neurotoxicity.