High throughput platform for ALS drug discovery by using human iPSC-derived motor neurons

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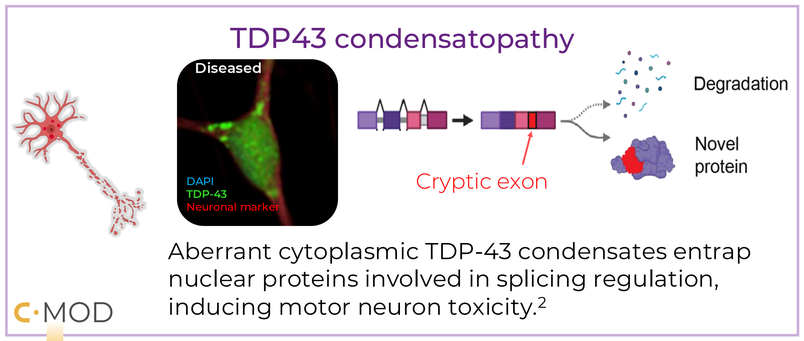
Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which results in loss of motor neurons. Given that the life expectancy of ALS patients is 3-5 years after diagnosis and that the current FDA-approved treatments have limited long-term efficacy, it is imperative to develop drugs that both improve patients' quality of life and extend their lifespan. To discover small molecule condensate modulators (c-mods) that correct a central node of dysfunction common in over 97 % of all ALS patients – aberrant cytoplasmic TDP-43 condensates, we have established a system that enables high-throughput drug screening and structure-activity relationship (SAR) efforts. We use ALS patient iPSC-derived motor neurons (iPSC-MN) as a cell model and cross-functional expertise across our organization. Our iPSC-MNs platform enables a wide range of phenotypic and functional assays, including high-content imaging (HCI), gene expression analyses, cell viability measurements, and neurite health assessment. In this poster, we present our optimized iPSC-MN workflow that drives optimization of the ALS pre-clinical candidate c-mods and provide examples on how these assays are applied as part of our weekly Design-Make-Test-Analyze (DMTA) cycle for small molecule drug discovery.

Background

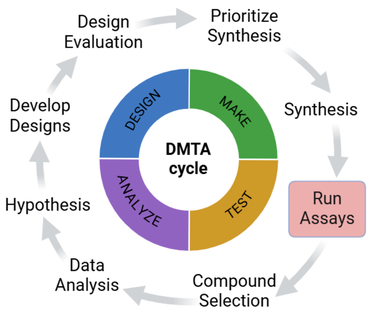
TDP-43 condensatopathy in ALS and therapeutic hypothesis



Aberrant cytoplasmic TDP-43 condensates entrap nuclear proteins involved in splicing regulation, inducing motor neuron toxicity.[2]

C-mods release TDP-43 from the aberrant cytoplasmic condensates, preventing formation of toxic aggregates and restoring the nuclear TDP-43 loss of function in splicing.

DMTA cycle for lead optimization

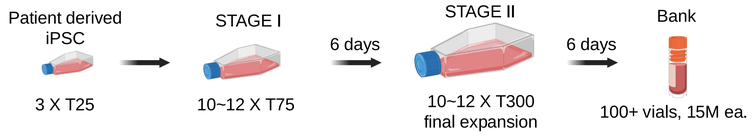


1. Timely execution of each step of the DMTA cycle is essential for the speedy optimization of potential chemotypes towards development candidate selection.
2. Therefore, establishing a systematic workflow for assays that support the DMTA cycle is critical to the delivery of data as test compounds are synthesized

Methods

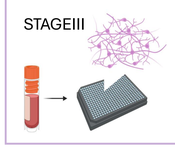
Motor neuron progenitor (MNP) generation and banking

Large-scale, single use cryovial stocks of ready-to-plate Stage II MNs are prepared by adapting typical iPSC-MN protocols to large scale expansion.[1] Patient derived iPSC are grown at small scale, followed by two rounds of expansion, each round of expansion lasting 6 days in two successive motor neuron differentiation media. The final expansion is harvested at day 12 and banked (100+ vials, 15M ea.).



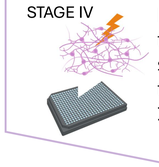
Workflow for DMTA support

Seeding MNPs



MNPs are seeded 2 weeks prior to the experiments and maintained with every other day media change.

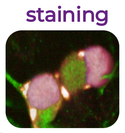
Treatment

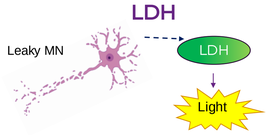


Mature MNs are treated with the stressor followed by test compounds for 24 hours.

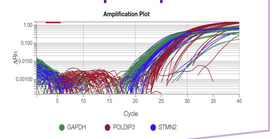
Assays

Immunofluorescence staining





Multiplex qPCR

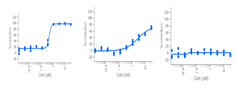


Data Analysis/Reporting

Harmony analysis

Segmentation of cytoplasmic condensates

Data upload/QC

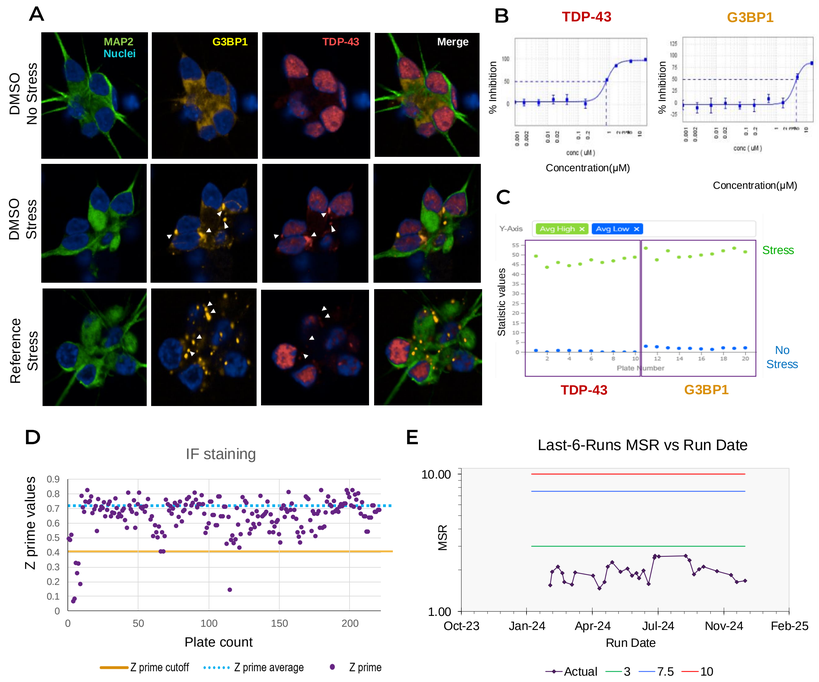


Data sharing with team

Data are shared with the chemistry & biology groups within a week for the next iteration of DMTA cycle.

Results

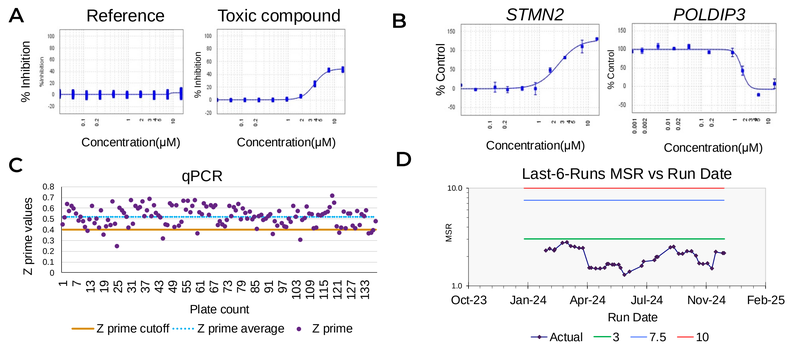
Reproducible and robust condensate assay by immunofluorescence (IF) staining in iPSC-MNs



(A) Immunofluorescence staining of iPSC-MNs detecting TDP-43 condensates and stress granules (G3BP1). **(B)** Concentration response curves (CRCs) based on quantified number of condensates to determine activity of reference compound. **(C)** Signal distance between positive and negative controls in 10 assay plates of an experiment. **(D)** Z- prime values of 225 plates assayed in the initial 6 months of the IF assay. **(E)** Reproducibility of the assays in efficacy and potency is expressed by MSR (minimum significant ratio) using results of reference compound from 40 experiments run in 2024. The overall values of MSR is 2.1.

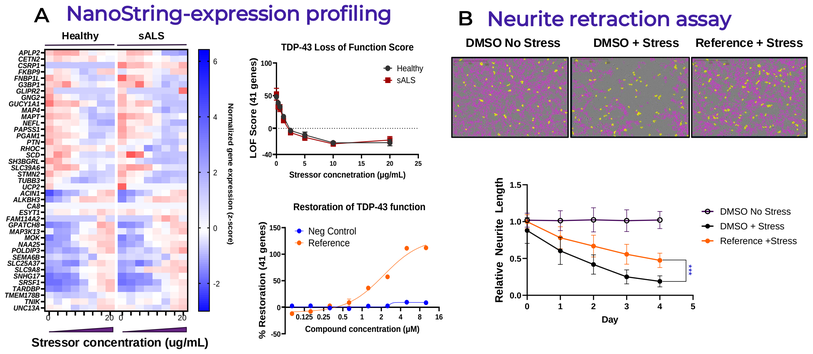
Results

Cytotoxicity measurement by LDH and multiplex qPCR for TDP-43 splicing target genes



(A) CRC of reference compound and a toxic compound from LDH assay. **(B)** Expression CRC of the two TDP-43 target genes determining the activity of reference compound. **(C)** Z- prime values of 140 plates assayed in the initial 6 months. **(D)** Reproducibility of the assay is expressed by MSR using results of 48 experiments run in 2024. The overall MSR for qPCR is 1.9.

Applications of iPSC-MN workflow for evaluation of c-mods by using additional functional analysis



(A) Expression of TDP-43-regulated genes was measured by NanoString and combined to a score to represent overall expression of the genes in a given condition. The expression measured by NanoString was correlated with RNA-seq data (r2= 0.67, data not shown). **(B)** Neurite length was measured by using IncuCyte. Images were taken at 10X magnification. Yellow, segmented cell bodies; pink lines, neurites.

Conclusions and future directions

1. We adapted a published protocol for human iPSC-MNs and established a method to generate MNPs at a large scale to be able to supply about 50 million MNPs weekly for multiple assays supporting DMTA cycle.
2. We optimized IF and qPCR assays in 384-well plates to determine activities of compounds in preventing TDP-43-loss of function due to stress, as well as LDH assay to evaluate cytotoxicity.
3. Our workflow enables weekly DMTA cycle for c-mod lead optimization and supports additional evaluation in functional assays such as neurite health and expression profiling in a disease-relevant *in vitro* model.
4. These assays are currently semi-automated and additional automated processes are in development to help further increase the throughput and reproducibility.

References

1. Workman MJ, et al. Large-scale differentiation of iPSC-derived motor neurons from ALS and control subjects. Neuron. 2023 Apr 19;111(8):1191-1204.

2. Huang WP, et al. Stress-induced TDP-43 nuclear condensation causes splicing loss of function and STMN2 depletion. Cell Rep. 2024 Jul 23;43(7):114421.

3. Mitrea, D.M. et al. Modulating biomolecular condensates: a novel approach to drug discovery. Nat Rev Drug Discov 21(11), 841-862 (2022).