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Webinar Q&A Report:

Using ALS Patient-Derived Motor Neurons to Study TDP-43 Mislocalization

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Is TDP-43 pathology present in all iPSC-derived motor neurons from sporadic ALS patients?

Two lines that we have tested showed the mislocalization of TDP-43. We do not know if this observation is applicable to all of the sporadic ALS patient derived motor neurons.

You mentioned that you see the TDP-43 phenotype in all the ALS lines you have looked at so far, is this always compared to the same control line, and/or have you included multiple control lines in this comparison?

We generally like to stick with one control line to be able to benchmark across experiments, but we have a number of in-house control lines that can be used upon request.

Can the same strategy be followed for staining TDP-43 in iPSC-motor neurons differentiated by other protocols?

We have only tested our in-house protocol for TDP-43 staining on cells differentiated using our proprietary method. We assume it can be used on cells acquired using other differentiation methods, but it would have to be tested in-house.

Have you looked at the spike activity by MEA in your differentiated neurons?

In case of SOD1 mutant lines, we have observed more robust network bursts in our MNs using an MEA assay compared to conventionally differentiated motor neurons. Indicating healthier development and potentially better maturation in our cells. The work has been published: https://www.frontiersin.org/journals/cellular-neuroscience/articles/10.3389/fncel.2020.604171/full

Do you provide calcium assays as a fee-for-service?

Yes

Do your neurons develop large pTDP-43 positive aggregates that are typically observed in ALS patient cortex?

Under the current culture conditions, we have not observed aggregates of pTDP-43, but we have observed mislocalization of pTDP-43 in our ALS patient derived lines.

Follow up - any data on aggregation of pTDP-43?

We have observed similar mislocalization of pTDP-43 in our ALS patient derived lines.

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For ALS characterization, did you measure any functional outcomes of TDP-43 mislocalization? Given you are strong in transcriptomic analysis, you may do this and see the difference in splicing in general in ALS samples. Otherwise did you check some known targets of TDP43 such as STMN2?

In the dataset presented, we focused on establishing TDP-43 mislocalization as a robust and pharmacologically responsive cellular phenotype. While downstream transcriptomic and splicing readouts were not assessed in these specific lines, we have evaluated TDP-43–related targets such as STMN2 in other lines. Expanding these analyses to additional lines is a clear and ongoing direction that aligns with our transcriptomic capabilities.

How many different patient lines do you have?

We currently have 6 ALS lines in-house.

What is the turn around time to test compounds? Number of lines, compounds and doses included in that timeline?

The turnaround time for testing compounds is typically 1 week. The number of lines, compounds, and doses depends on your project scope. You can schedule a free consultation to explore our capabilities and offerings.

What is the cost per experiment?

Costs depend on a number of variables, including project timelines, experimental design, sample type and quantity, assay complexity, and data analysis requirements. Because each study is tailored to the customer's objectives, pricing is determined after scoping.

Why did you chose to quantitate nuclear puncta rather than full nuclear signal?

The reason it is difficult to see descrete puncta in the nucleus is because of the overlap with the nuclear staining which is shown in cyan, which does not create a big contrast with the TDP-43 which is in green. Though we did not show it, quantifying puncta instead of total signal gives us some information on the size of the puncta distribution of aggregates. We are able to compare full signal if that is preferable when we design the assay.

Have you determined the genetic mutations in your sporadic and familial ALS lines?

Due to our licence agreement with the cell bank, we have not determined the genetic variations in the sporadic ALS lines, whereas we do know the mutation in the C9ORF72 line we tested.

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Have you looked at any other phenotypes?

In addition to TDP-43 localization, we have explored other phenotypic and functional readouts in this system, including measures of neuronal health and activity.

What is the TDP antibody that you use - is it seeing C terminal and or N-terminal TDP?

For this work, we use a validated internal antibody reagent. Specific antibody details are proprietary, but we confirm specificity and performance through internal QC and consistent biological behavior across experiments.

Have you looked at neuronal survival assays?

We do observe lower baseline viability in ALS motor neuron lines compared to healthy controls, which is expected given the disease context, but not to an extent that compromises the assay. Following 24-hour drug treatment, we see minimal changes in viability across conditions, likely reflecting the short treatment window rather than compound-related toxicity.

Can you purchase ALS iMNs?

Due to our licence agreement with the cell bank, we are unable to distribute cells themselves but able to share the data generated using those cells.

Have you tested some Stress Granule markers like TIA-1 to confirm the puncta?

No, we have not tested this.

How were the puncta quantified? Regarding ePHYS do you do it in house or have 3rd-party collaborators? Do you have MEA compatibility? Have you run any tests on MEA equipment to test for ePHYS differences?

We have capability to run MEA in house and done analysis using cholinergic neurons derived from hiPSCs carrying SOD1 mutation.

Which mutants were used to show cytoplasmic mislocalization of TDP-43? Do you see the same phenotype with different mutants?

We have seen it in motoneurons derived from ALS patient that carries C9ORF72 mutation and TARDBP mutation. Also we have seen it in sporadic ALS patient derived motor neurons.

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Have you tried culturing the ALS patient derived motor neurons longer? If yes, what were the results?

Yes, we have maintained these cultures for several weeks (approximately 3–4 weeks). Over this time frame, the TDP-43 mislocalization phenotype remains stable.

Have you seen differences in TDP-43 mislocalization across ALS disease subtypes?

Yes, we have observed TDP-43 mislocalization across both sporadic and familial ALS lines tested. While the magnitude can vary between lines, the presence of mislocalization is consistent across these disease subtypes, supporting the broader applicability of the model.

What is a reasonable throughput should one want to do compound lead optimization steps in this this system? How many compounds can be profiled per month to look at TDP43 localization and potentially collect enough RNA for qPCR measurement of downstream splicing events?

Our preference is in a 96-well format. Since the readout involves IF and image analysis and we have done 3-4 compounds per plate, 10-20 compounds could be screened per month.

Did your analysis include an assessment of changes in TDP-43 phosphorylation levels?

We have observed similar results using the antibody that detects phosphorylated TDP-43.

Have you performed longitudinal measurements of TDP-43 localization or function in this model to capture early versus later disease-associated changes, rather than one time point?

We have performed longer-term measurements, primarily in the context of treatment conditions rather than as a full longitudinal disease progression study. In those experiments, TDP-43 mislocalization remains stable over time, allowing us to assess how treatments modulate the phenotype across extended culture periods. A more detailed longitudinal characterization of early versus later disease-associated changes is a natural next step for the model.

As the motor neurons are differentiated by day 10, how long can you keep them going after?

These motor neurons can be maintained several weeks. Particulalry we have kept ones derived from a healthy donor for 6 months for an electrophisiological assay.

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Why did you focus on number of puncta for your analysis of TDP-43 mislocalization rather than on intensities in the cytoplasm vs. nuclei?

The mislocalization of TDP-43 was not bright enough to quantify based on the fluorescence intensity.

Do you worry you're bypassing key developmental stages required for the full complement of disease phenotypes?

That’s a fair point. This model is not intended to recapitulate every developmental stage, but it is designed to robustly capture core, disease-relevant phenotypes such as TDP-43 mislocalization. For the questions we are addressing, this balance between developmental detail and scalability is intentional and appropriate.

How important is background subtraction prior to quantifying TDP-43 mislocalization?

Since our analysis is not based on the mean fluorescence intensity, we have not subtracted the backgroud, or during the image segmentation, fluorescence above the threshold was selected.

Is it possible to cryopreserve post mitotic motor neurons and retain their functionality?

As far as we are concerned cryopreservation of fully differentiated neurons is very difficult.

Do you have any data on cryptic splicing or on synaptic function?

We have confirmed the expression of STMN2a in some of the ALS patient derived motor neurons not presented in this webinar. We have done MEA analsysis using cholinergic neurons carrying SOD1 mutation.

Have you done any downstream analysis like missplicing readouts?

In this study, we focused on TDP-43 mislocalization as a robust, quantitative disease-relevant phenotype. Downstream missplicing analyses were not included here, but they represent a clear and logical extension of the model.

Did you see reduced cytosplasmic localization of any other protein post-treatment?

So far our focus is testing TDP-43 only so other proteins were not tested.

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Will you be studying the mechanism for TDP-43's prion-like behavior?

It is out of our current scope. However, we are willing to work with partners who have such interest.

What kind of imaging (confocal, etc) was used to acquire the images shown?

The imaging was based on confocal microscopy.

For the imaging-based analysis of TDP-43 mislocation, which type of plate was used for cell culture (96 or 384 well?), and which magnification will be better for this type of immunofluorecence image (4x, or 10x, or 20x)?

We have used a 96-well plate format with a 60x objective lens.

What is the range of healthy cell's TDP43 localization index? Seems like the value is bit higher in the drug testing example than the initial characterization example.

We do see some variability in baseline TDP-43 localization in healthy controls across experiments. In the drug-testing dataset, the healthy cells were DMSO-treated and cultured one day longer, which likely contributed to the slightly higher values. These differences fall within normal variability and are always interpreted relative to the matched ALS lines.

Have you tried to look for additional endpoints in the media, such as Nf-L concentrations?

We have not measured secreted biomarkers such as Nf-L in the media in the work presented. However, this is a relevant complementary readout and represents a logical extension of the model for future studies.

Can this system be used for chronic treatment? Can this system be used for other therapeutic modalities (ASO, siRNA, Mab, etc)?

Yes, the system can support chronic treatment paradigms, and we have maintained cultures for multiple weeks, allowing for repeated or extended dosing as needed. In addition to small molecules, the platform is compatible with other therapeutic modalities such as ASOs, siRNA, and biologics, with assay conditions adapted to the modality being tested.