Development of a robust and scalable iPSC platform for predictions of efficacy and in vivo toxicity of RNA therapeutics early in the drug discovery pipeline

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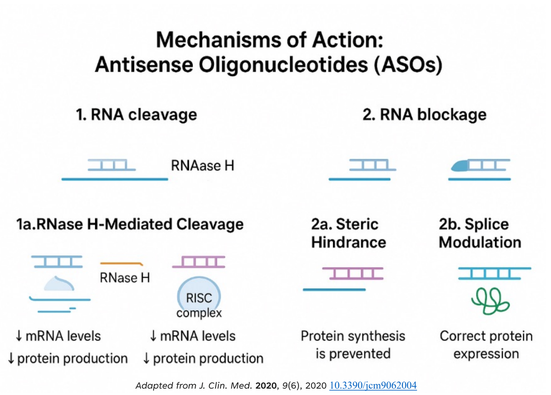
Background

RNA therapeutics, especially Antisense Oligonucleotides (ASOs), have huge potential to modify cellular pathways by inducing decay, steric blockage, or altered splicing of the target mRNA. These innovative molecules provide precise control over gene expression. Being able to predict acute side effects early in drug development facilitates the confident selection of candidates, saving both time and valuable resources.

Human induced pluripotent stem cells (hiPSCs) have become a powerful and versatile tool for drug discovery. They bring unprecedented opportunities for directly assessing human-specific toxicity and efficacy in physiologically relevant cell types, improving translational accuracy.

Ncardia developed two robust platforms using its hiPSC-derived neuronal cell models to screen both for efficacy and neurotoxicity of ASOs:

- Cortical neurons (hiPSC-CNs) to study effects on target knockdown by RT-qPCR in a fully automated experimental setting including cell seeding, maintenance, ASO treatment, and RT-qPCR, which enabled the development of a highly reproducible and sensitive assay with both intra- and inter-plate variation (%CV) of <5%.
- CNS cultures (hiPSC-CNS) to assess acute ASO neurotoxicity through quantification of intracellular calcium fluxes, providing an efficient and human-relevant approach for neurotoxicity screening in early discovery.



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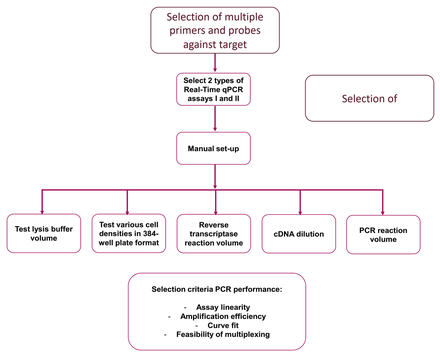
Designed to bind complementary RNA targets, ASOs can modulate RNA processing to reduce, restore, or modify protein expression through several distinct mechanisms. These include promoting RNA degradation via RNase H, altering splicing patterns, or blocking translation, thereby enabling precise and targeted regulation of gene expression at the RNA level.

Automated platform for ASO screening

A



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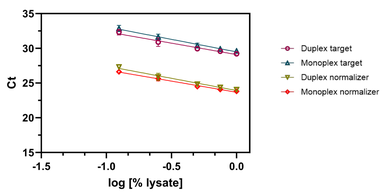
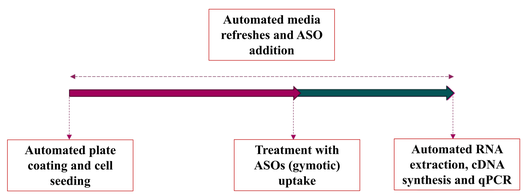


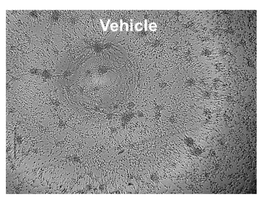
Prior to assay automation, two types of Real-Time qPCR assays were evaluated in a manual setup for a side-by-side comparison. We optimized the number of cells seeded per well in 384-well plate format, volume of lysis buffer as well as reverse transcriptase, cDNA dilution and PCR reaction volumes based on the PCR performance (linearity, amplification efficiency, curve fit, delta Ct) as illustrated in (A) in lysates from untreated cells. (B) shows the linear regression curves of the selected condition in monoplex and duplex PCR reactions per sample concentration (mean ± SD).

A



B

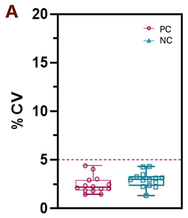
C



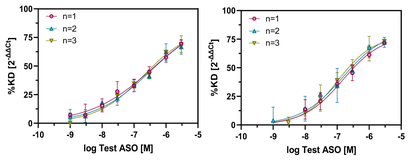
(A) illustrates the assay setup from cell seeding until downstream assay. All steps were performed using a cell culture grade fully automated liquid handling system as shown in (B). No significant morphological changes were visualized in vehicle controls as illustrated by phase contrast imaging (C). Neurons can be maintained in culture for extended periods.

Automated platform for ASO screening

A



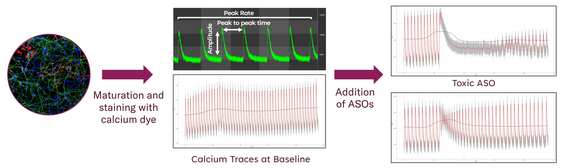
B Dose response curves in biological duplicate plates of a test ASO



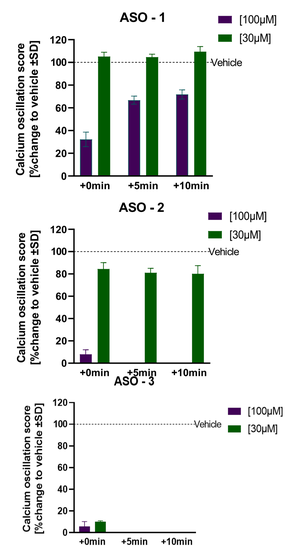
(A) shows the mean of negative control conditions across 14 cell plates. Each cell plate was run as technical PCR triplicate. In both cases, the inter-plate variability in positive or inter-plate variability between technical replicates was below 5% and the inter-plate variation among biological replicates was 2.8% for vehicle and 3.9% for the control, concluding a robust assay performance.

(B) shows an exemplary dose response of a test ASO in biological replicate. Data were plotted as %KD and fitted as non-linear, 4 parameter sigmoidal curve ± SD. Each line represents the fit of a technical PCR triplicate.

In vitro safety assessment of ASOs



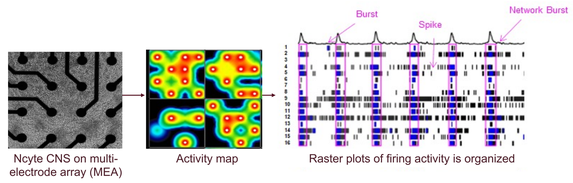
Exemplar calcium traces from Ncyte CNS cultures treated with toxic (upper trace) and non-toxic ASOs (lower trace). Toxic ASOs cause the disruption of spontaneous calcium activity

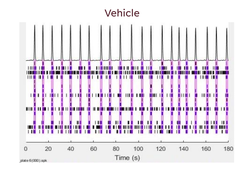


Graphs show illustrations demonstrating how the optimized calcium transient assay was applied to evaluate ASO-induced effects across a range of concentrations.
This sensitive assay enables clear differentiation between:

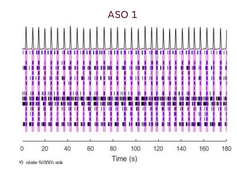
- ASO 1 shows an example of a putative “safe” ASO, which does not alter calcium transient amplitude, frequency, or kinetics compared to untreated controls, indicating preserved neuronal function.
- ASO 2 depicts an ASO that appears acutely safe at lower concentrations but begins to show changes in calcium signaling parameters as the concentration increases, suggesting a narrow safety window.
- ASO 3 demonstrates a “toxic” ASO, which causes marked disruptions in calcium transient patterns such as reduced amplitude, irregular frequency, or complete loss of activity consistent with impaired neuronal viability or excitability.

In vitro safety assessment of ASOs

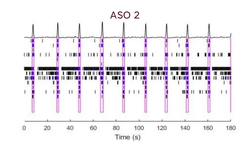




Illustrate representative outcomes from the MEA assay used to evaluate the effects of ASOs. This assay enables us to functionally assess neuronal activity and detect potential cytotoxic effects.
In vehicle treated wells, spikes and network bursts are strong and at regular intervals



In neuronal co-cultures treated with ASO-1, a significant decrease in amplitude with an increase in event frequency was observed, suggesting an overall excitatory or hyperactive neuronal profile.



ASO 2 demonstrates a “toxic” ASO, which causes marked disruptions in electrical activity such as reduced amplitude and network burst frequency, consistent with impaired neuronal excitability.

Collectively, these examples highlight how MEA can be applied as a sensitive, functional screen to discriminate between safe and neurotoxic ASOs.

Conclusions

- Fully automated RT-qPCR assay established to assess ASO efficacy in human iPSC-derived neurons, delivering highly robust data (<5% inter-plate variability) and applicable across multiple iPSC-derived cell types.
- High-throughput calcium transient assay using Ca^{2+} imaging in hiPSC-derived CNS cultures to evaluate dose-dependent acute neurotoxicity of ASOs.
- MEA (microelectrode array) assay developed to assess ASO-induced effects on neuronal network activity, capturing changes in firing rate, burst frequency, and synchrony.
- These assays are available on-demand to support candidate prioritization before in vivo studies, reducing animal use and increasing confidence in translational success.

Ncardia’s hiPSC-derived neuronal cell models enable early screening for ASO efficacy and neurotoxicity, providing a powerful platform for improving drug development efficiency.

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