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

- 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.

1. Automated platform for ASO screening

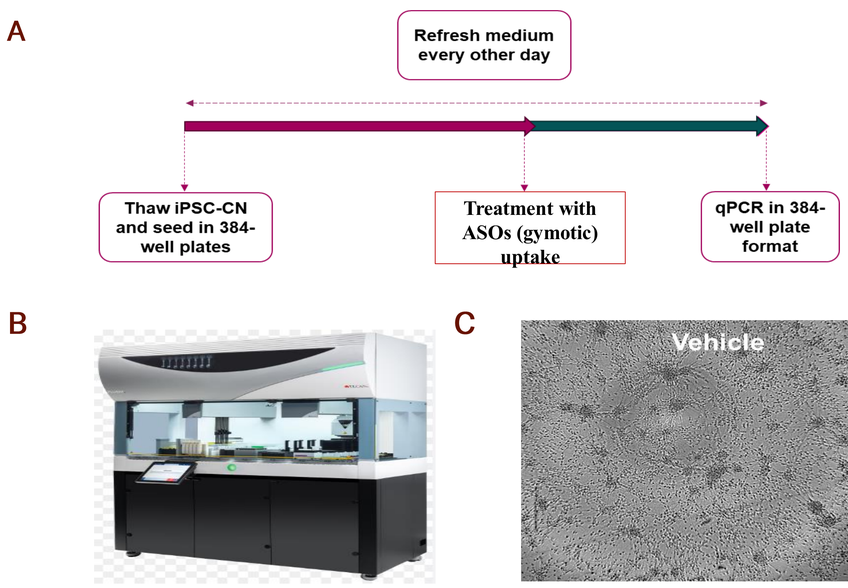
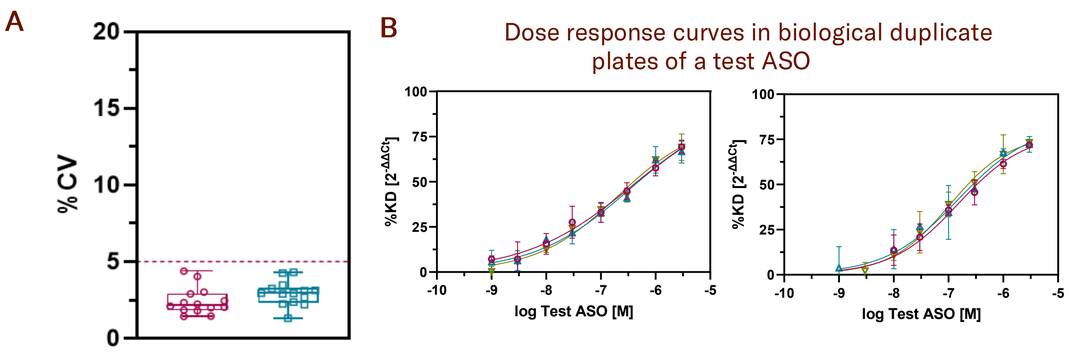


Figure 1: Prior to assay automation, we optimized factors such as cell number, volume of lysis buffer as well as reverse transcriptase, cDNA dilution and PCR reaction volumes. Based on the PCR performance (linearity, amplification efficiency, curve fit, delta Ct) a single workflow was automated. (A) 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 > 14 days.

2. Automated platform for ASO screening



(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.

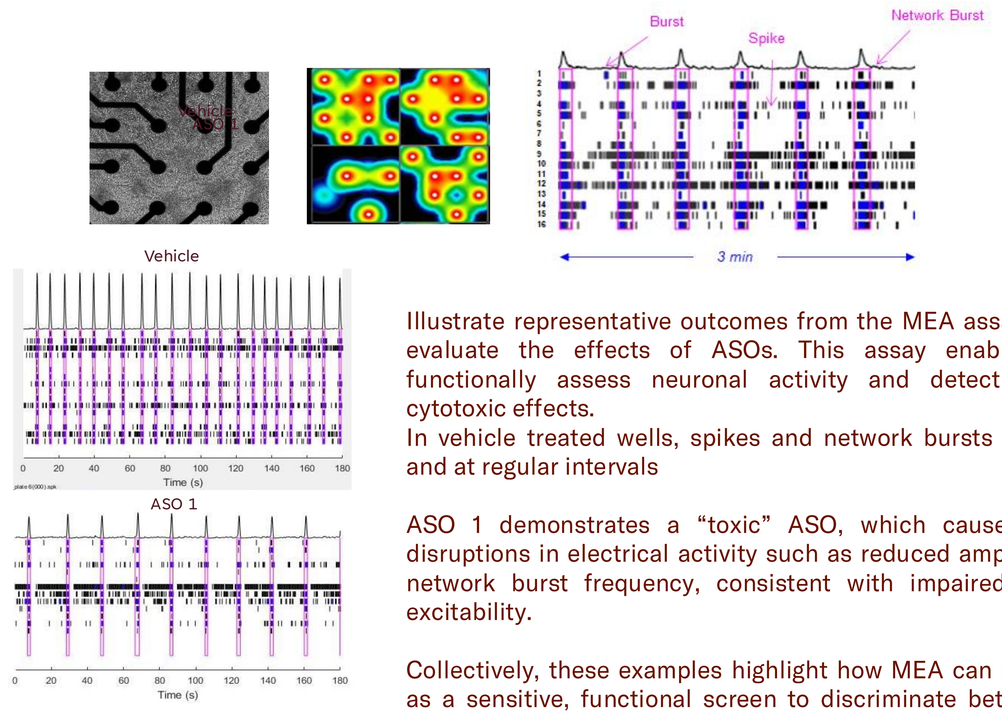
(B) shows an exemplary dose response of a test ASO in biological replicate. Data were plotted as %KD and fitted as non-linear, 4 parameter sigmoidal curve ± SD. Each line represents the fit of a technical PCR triplicate.

3. In vitro safety screening of ASOs – Calcium

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

- 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 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.

4. In vitro safety screening of ASOs - MEA



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

ASO 1 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.

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