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. Being able to predict acute side effects early in drug development facilitates the confident selection of candidates saving time and resources.

Human induced pluripotent stem cells (hiPSCs) have become a powerful tool for drug discovery. They bring unprecedented opportunities for directly assessing human specific toxicity and efficacy.

Ncardia developed two robust platform using two Ncardia's 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 robust assay with both intra- and inter-plate variation (%CV) of <5%.

- CNS cultures (hiPSC-CNS) to assess acute ASO neurotoxicity by quantification of intracellular calcium fluxes.

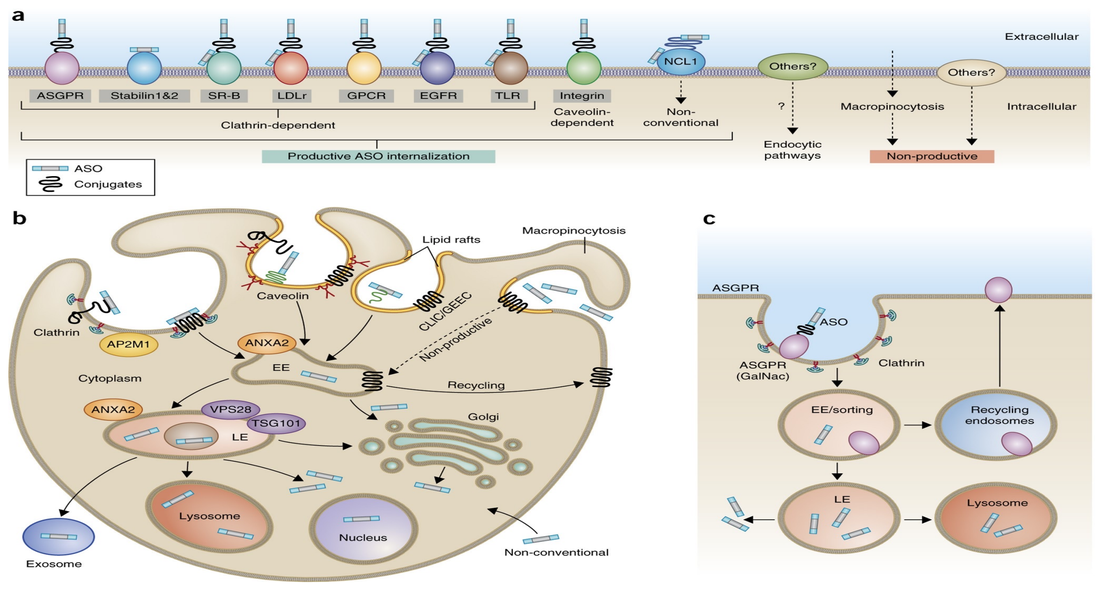
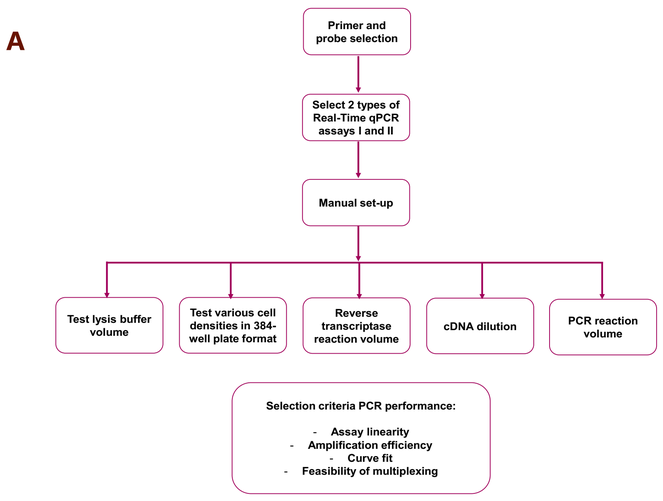


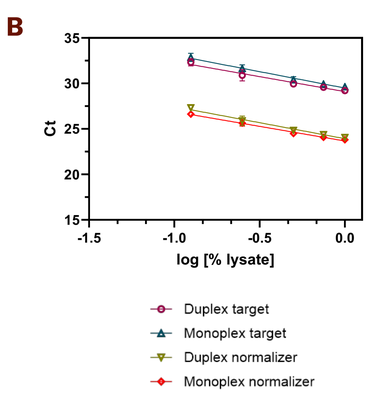
Figure 1. Cellular uptake of antisense oligonucleotides (ASOs) modified with phosphorothioate (PS) linkages and different 2′ modifications
- Enter cells without additional modification or formulation
- PS-ASOs function in both the cytoplasm and nucleus, localization to different subcellular regions can affect their therapeutic potency
- Cellular uptake and intracellular distribution of are mediated by protein interactions.

Crooke, S., Wang, S., Vickers, T. et al. Cellular uptake and trafficking of antisense oligonucleotides. Nat Biotechnol 35, 230–237 (2017). https://doi.org/10.1038/nbt.3779

Automated platform for ASO screening

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).





Automated platform for ASO screening

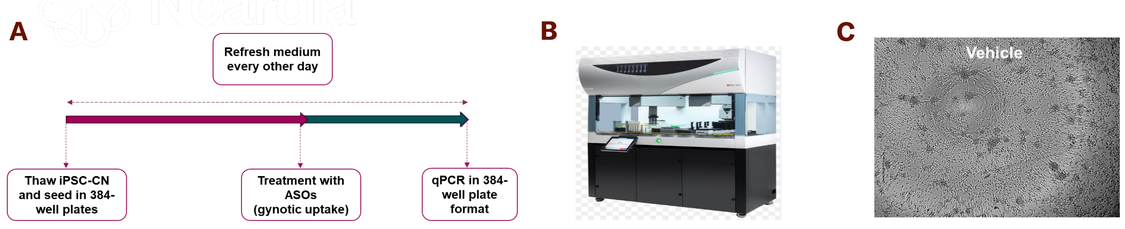


Figure 2. (A) illustrates the assay setup from cell seeding until downstream assay. All steps were performed using a cell culture grade fully automated liquid handling system as shown in **(B)**. No significant morphological changes were visualized in vehicle controls as illustrated by phase contrast imaging **(C)**.

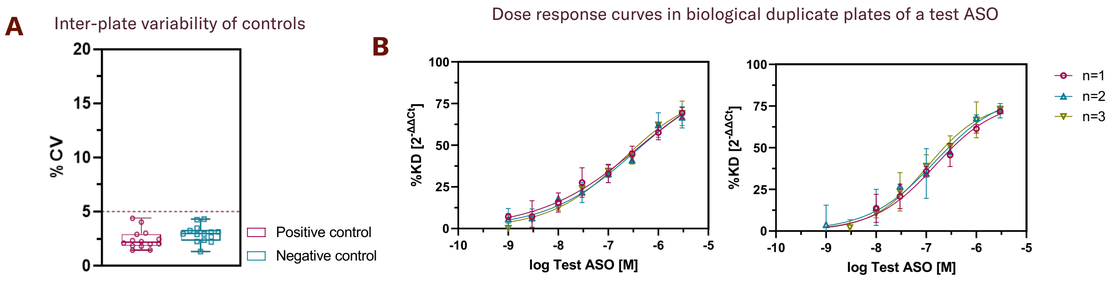
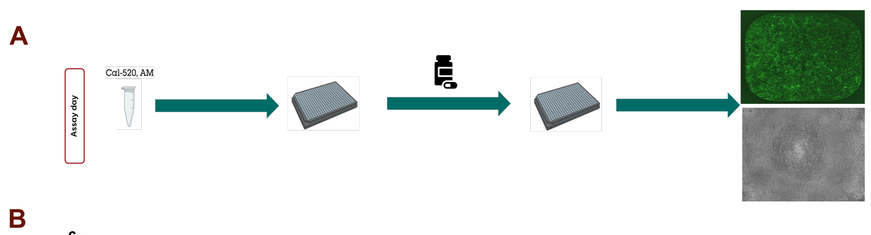


Figure 3. **(A)** shows the mean of negative control conditions across 14 cell plates. Each cell plate was run as technical PCR triplicate. In both cases, the inter-plate variability in positive or inter-plate variability between technical replicates was below 5% and the inter-plate variation among biological replicates was 2.8% for vehicle and 3.9% for the control, concluding a robust assay performance.

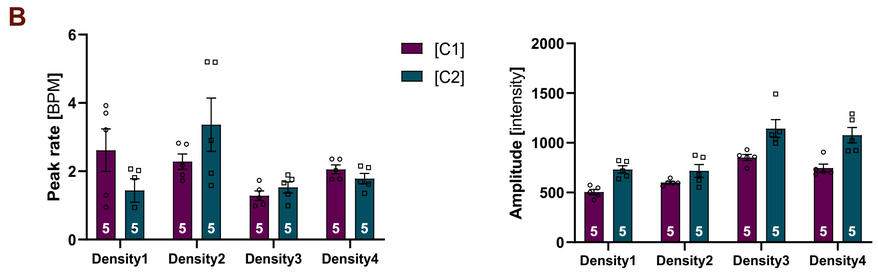
(B) An exemplary dose response of a test ASO in biological replicate is shown in Figure C. Data were plotted as %KD and fitted as non-linear, 4 parameter sigmoidal curve ± SD. Each line represents the fit of a technical PCR triplicate.

In vitro assessment of ASOs

Some oligonucleotides can produce acute, neurobehavioral side effects after intracerebroventricular (ICV) dosing. We have developed a human in vitro Ca2+ assay (outlined below) in iPSC co-cultures of neurons and astrocytes that can be used as a tool to prioritize ASO candidates prior to in vivo safety testing minimizing the number of animals required and minimizing suffering (Hagedorn et al, 2022). Our mature co-cultures demonstrated spontaneous calcium oscillations at sufficient frequency and amplitude for quantification of ASO toxicity.



(A) Cal-520 dye is added to the cells, and after acclimatization and baseline calcium transient recordings, compounds are added with continuous recording.



(B) The peak rate [BPM] of the Ncyte CNS cultures at different densities and concentrations of Cal-520 dye. Graphs show the peak amplitude and rate of CNS cultures at different densities and different concentrations of Cal-520 dye.

In vitro assessment of ASOs

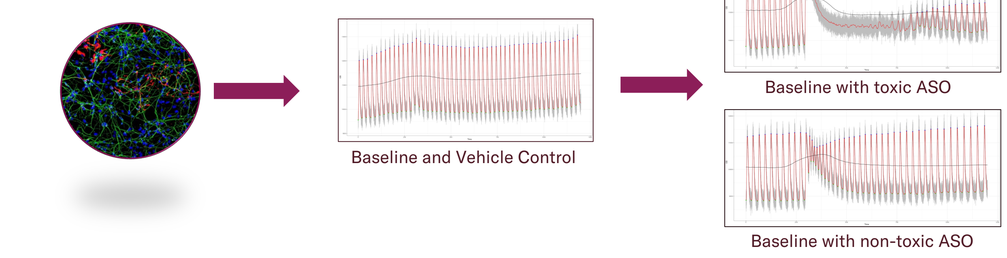


Figure 4. Exemplar calcium traces from Ncyte CNS cultures treated with toxic (upper trace) and non-toxic ASOs (lower trace). Toxic ASOs cause the disruption of spontaneous calcium activity

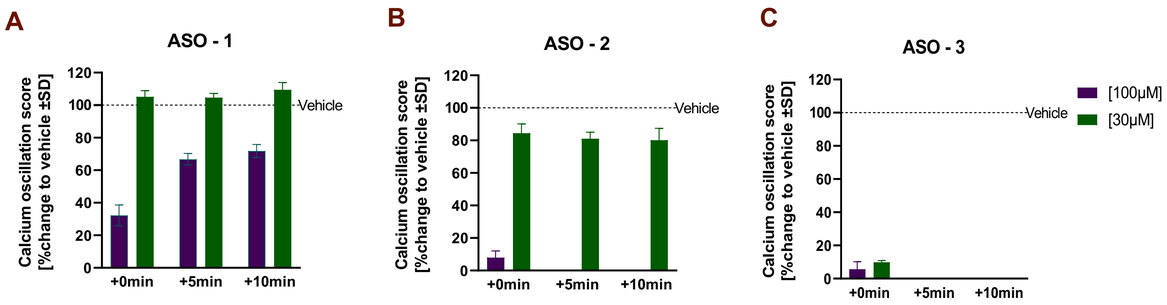


Figure 5. (A), (B) and (C) show illustrations of how we have used the optimized calcium transient assay to test ASOs at various concentrations. We can identify putative "safe" ASOs (A) acute, safe ASOs at lower concentrations (B) and "toxic" ASOs (C).

Conclusions

- We have established a fully automated RT-qPCR assay to study the efficacy of ASOs in human iPSC-derived neurons providing highly robust data (inter-plate variability below 5%). The process is applicable to a wide variety of iPSC derived cells.
- We have established a high throughput, highly robust calcium transient assay, by employing Ca2+ imaging in hiPSC-derived CNS cultures to determine dose-dependent acute neurotoxicity of ASOs.
- These assays are available on-demand to help drug developers to prioritize candidates before moving into in vivo toxicity studies, reducing the need for animal models and increasing the confidence of future success

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

Questions? contact us at: support@ncardia.com, www.ncardia.com

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