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

Jessica Koepke, Kimberley Riegman, Benoit Samson-Couterie, Katerina Pitsa, Shushant Jain
Ncardia Services BV Leiden, The Netherlands, support@ncardia.com, www.ncardia.com

Background

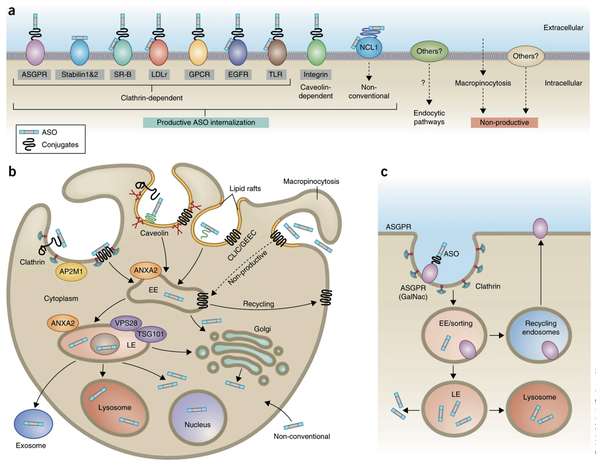
RNA therapeutics, especially Antisense Oligonucleotides (ASOs), are a relatively new modality for nucleotide-based therapeutics. They offer a huge potential to specifically modify cellular pathways by reducing target protein expression through RNA cleavage, altered splicing or blocking translation.

Being able to predict acute side effects early in the development process facilitates the confident selection of candidates with higher chances of success in preclinical and clinical stages, saving time and resources.

Human induced pluripotent stem cells (hiPSCs) have become a powerful tool for drug discovery. With their unlimited proliferation capacity, potential to be differentiated into any cell type and their representation of donor's genetic background they bring unprecedented opportunities for directly assessing human specific toxicity and efficacy.

Ncardia developed two robust assays 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.



Debbie Maizels-Springer Nature

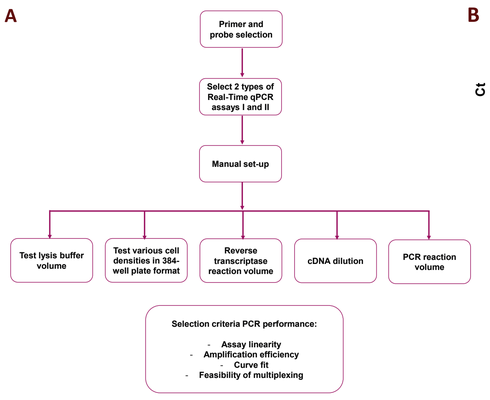
Cellular uptake of antisense oligonucleotides (ASOs) modified with phosphorothioate (PS) linkages and different 2' modifications

- 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.
- Main proteins and intracellular sites involved in these processes have been identified

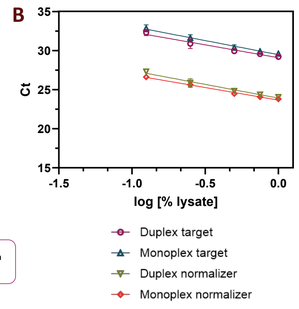
REF: Crooke, S., Wang, S., Vickers, T. et al. Cellular uptake and trafficking of antisense oligonucleotides. Nat Biotechnol 35, 230–237 (2017). https://doi.org/10.1038/nbt.3779

1. Assay optimization (RT-qPCR)

A



B



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 **Figure A** in lysates from untreated cells.

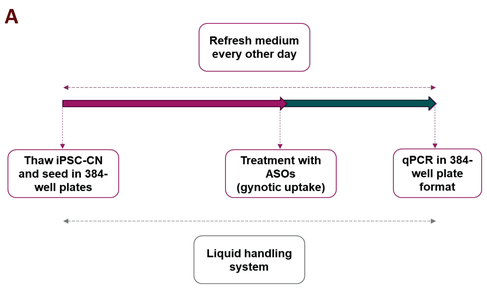
Figure B shows the linear regression curves of the selected condition in monoplex and duplex PCR reactions per sample concentration (mean ± SD).

None of the test conditions with the qPCR kit I kit showed satisfactory PCR performance of either target or normalizer in mono- or duplex setup whereas the performance with qPCR kit II demonstrated amplification efficiencies ~100% in mono- and duplex setup, R2 of 0.96 and a ΔCt [target – normalizer] of 0.3. Together with the feasibility of assay miniaturization and automation of qPCR kit II, we continued with the next phase of assay development by running a fully automated experiment as shown in Section 2.

qPCR kit II showed best performance for selected targets

2. Assay setup (RT-qPCR)

A



B

C

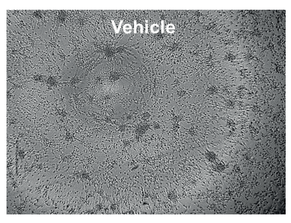
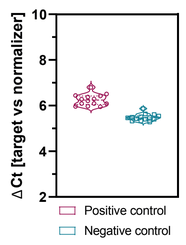


Figure A illustrates the assay setup from cell seeding until downstream assay. All steps were performed using a cell culture grade fully automated liquid handling system as shown in **Figure B**. No significant morphological changes were visualized in vehicle controls as illustrated by phase contrast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

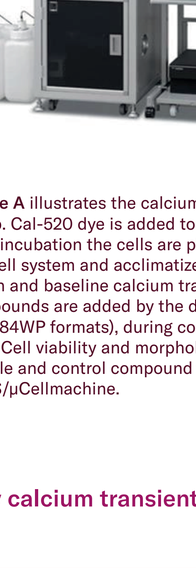
Automated cell and assay handling showed viable, healthy neuronal cultures

3. Automated HTS assay performance (RT-qPCR)

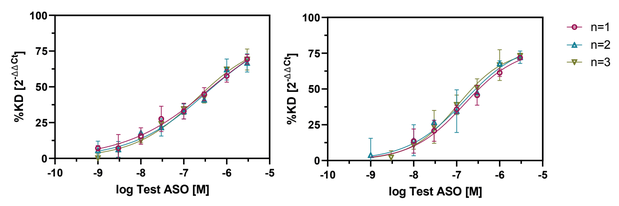
A **Inter-plate variability of controls**



B **Inter-plate variability**



C **Exemplary dose response curves in biological duplicate plates of a test ASO**



Figures A and B show the delta of target and normalizer (A) and the mean of inter-plate variability (B) in positive or negative control conditions across 14 cell plates. Each cell plate was run as technical PCR triplicate. In both cases, the 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.

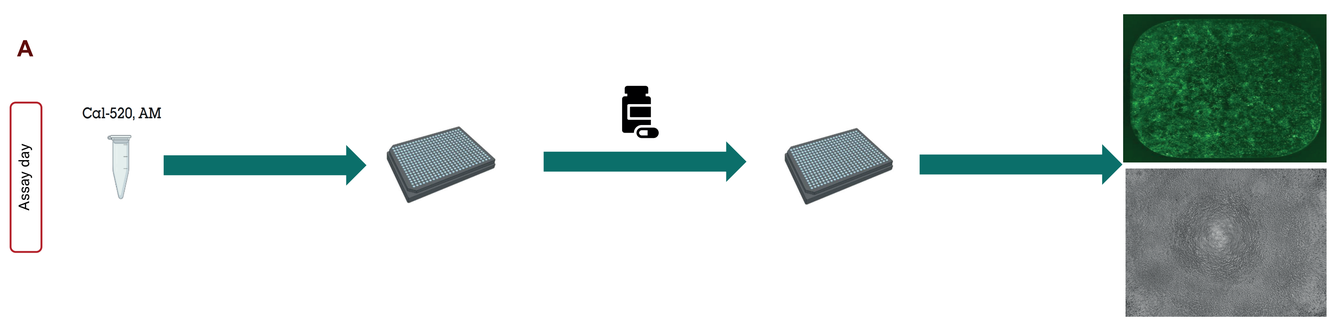
An exemplary dose response of a test ASO in biological replicate is shown in **Figure C**. Data were plotted as %KD and fitted as non-linear, 4 parameter sigmoidal curve ± SD. Each line represents the fit of a technical PCR triplicate.

Robust assay performance with inter-plate variability below 5% between technical and biological copy plates and highly reproducible dose response curves

4. Assay setup (Calcium transient)

Some oligonucleotides can produce acute, non-hybridization dependent, neurobehavioral side effects after intracerebroventricular (ICV) dosing in mice. 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; 10.1089/nat.2021.0071). Our mature co-cultures demonstrated spontaneous calcium oscillations at sufficient frequency and amplitude for quantification of ASO toxicity.

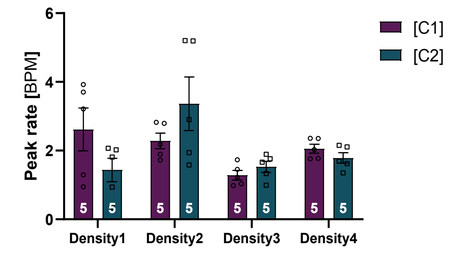
A



B

Figure A illustrates the calcium transient assay setup. Cal-520 dye is added to the cells, and after incubation the cells are placed in the FDSS/µCell system and acclimatized. After acclimatization and baseline calcium transient recordings, compounds are added by the dispensing head (96/384WP formats), during continuous recording. Cell viability and morphology was good in vehicle and control compound wells. **Figure B** FDSS/µCellmachine.

C



D

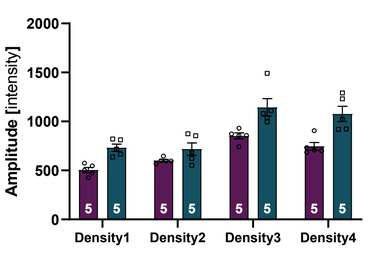


Figure C demonstrates the peak rate [BPM] of the Ncyte CNS cultures at different densities and different concentrations of Cal-520 dye. **Figure D** demonstrates the peak amplitude [intensity] of the Ncyte CNS cultures at different densities and different concentrations of Cal-520 dye.

FDSS/µCell system allows high throughput and automated testing of compounds for toxicity as assessed by calcium transients

5. Exemplary screening results (Calcium transient)

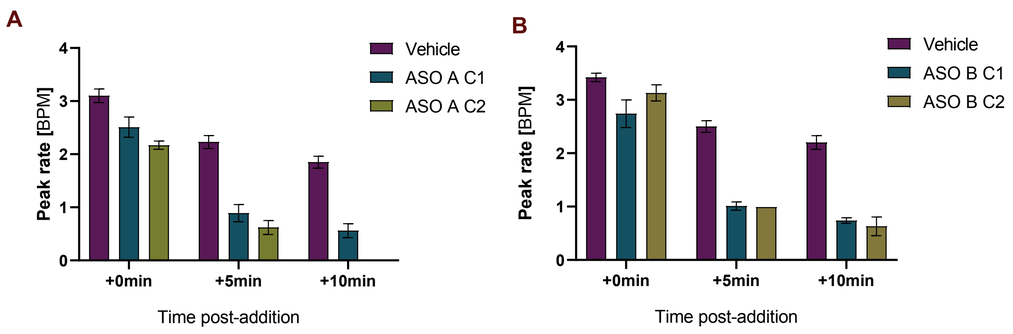


Figure A and B show illustrations of how we have used the optimized calcium transient assay to test ASOs at various concentrations. We demonstrated that we can identify acute and less acute neurotoxicity of ASOs and are able to identify dose-dependent differences in neurotoxicity profiles.

Robust assay performance detecting dose-dependent toxicity of ASOs on calcium transients

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%).
- We have established a 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 select the best candidates before moving into in vivo toxicity studies, reducing the need for animal models and increasing the confidence of future success.

Scan to download the poster

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