# EVALUATING THE TRANSDUCTION EFFICIENCY OF DIFFERENT AAV SEROTYPES ON HUMAN IPSC-DERIVED CARDIOMYOCYTES

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## Background

Gene therapy holds great promise for the treatment of a wide range of genetic disorders. Adeno-associated viruses (AAVs) have emerged as versatile and efficient vectors for gene delivery due to their ability to transduce a variety of cell types with minimal immunogenicity. In recent years, the integration of AAV-based gene therapy with human induced pluripotent stem cell (hiPSC) technology has unlocked new possibilities for personalized medicine and disease modeling.

The identification of AAV vectors with the highest transduction efficiency, lowest toxicity and best efficacy in ameliorating disease-specific phenotypes, is crucial for efficiently selecting which vectors pass on subsequent preclinical and clinical studies. hiPSC derived cell models allow us to assess all three of these key aspects.

## Experimental approach

**Equipment:** ImageXpress Micro Confocal High-Content Imaging System, Molecular Devices

**Scale:** 96 well plates

**Parameters assessed:**

- Cell viability (transduced cells/control*)
- Transduction efficiency (GFP+ cells/total cells)
- Average Fluorescence Intensity of GFP signal

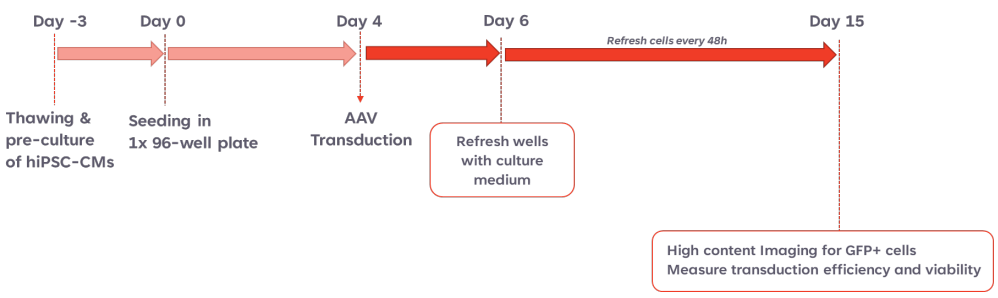
**control: cells treated with 0.1% AAV Buffer (buffer used to dilute AAV stocks)*

**Cell system:** Ncyte® vCardiomyocytes

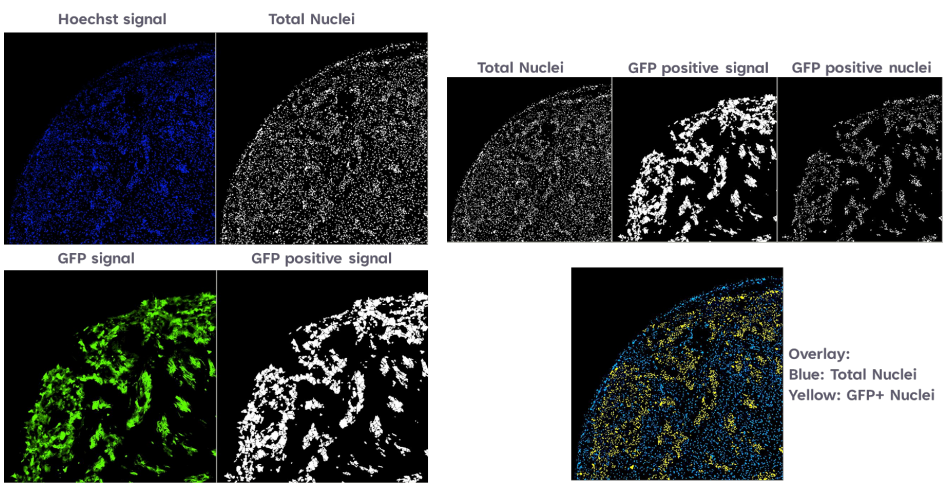
**Vectors:** AAV1-, AAV4-, AAV5-, AAV6-, AAV8-, AAV9- eGFP (Promoter: CMV) from Vector Builder

**MOIs:** 1000/3000/10000/30000/50000 (n= 3 wells/MOI)

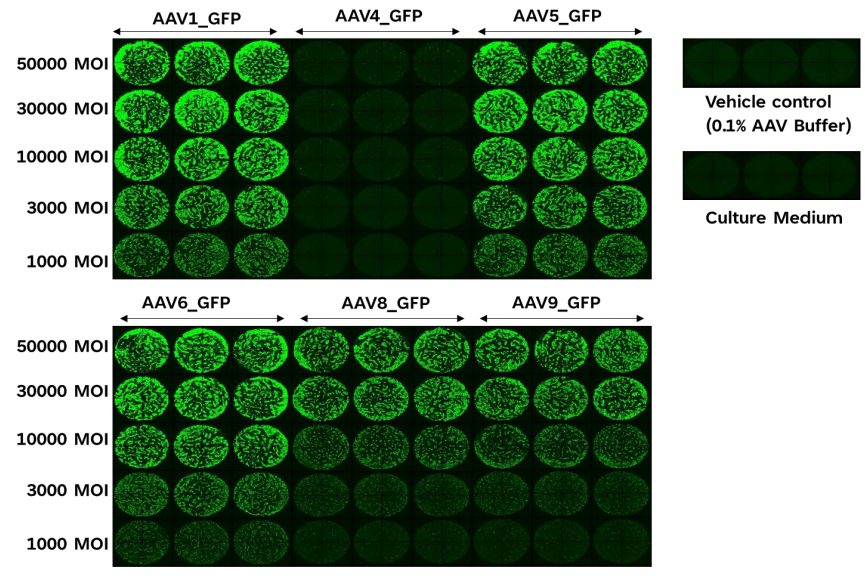
## Experimental timeline




## Example of image analysis with HCI software

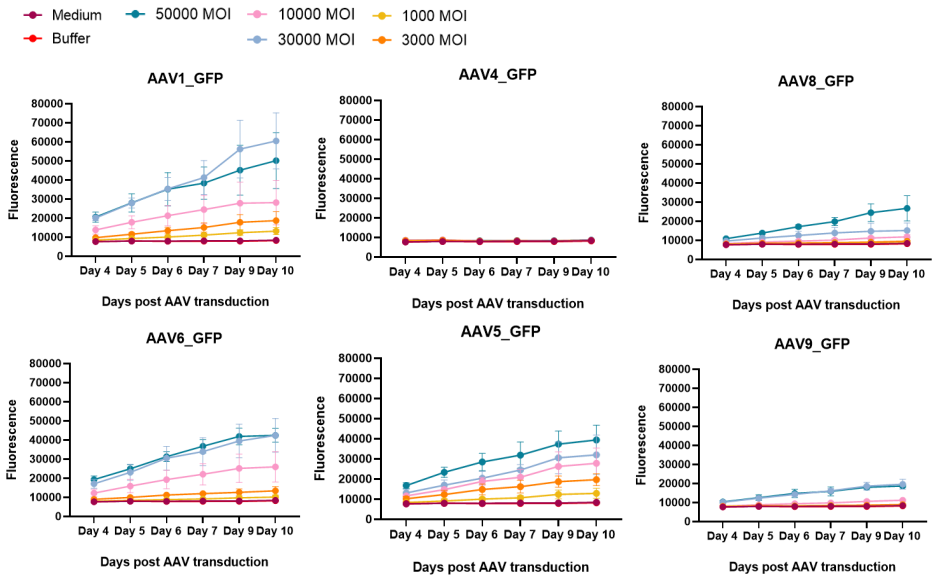



## Overlay of the GFP signal




This poster shows how Ncardia utilized high content screening as a readout to assess the transduction efficiency and cytotoxicity of a panel of different serotype AAVs in our bioreactor generated hiPSC derived cardiomyocytes

## Fluorescence over time

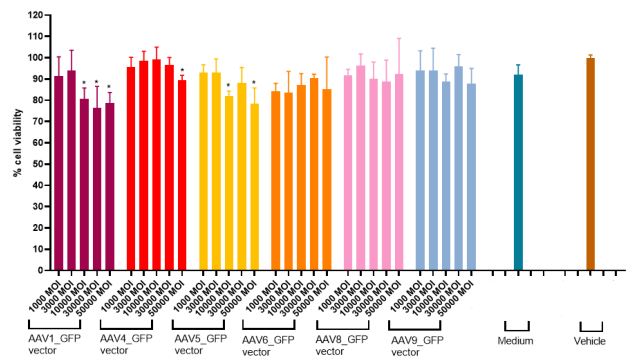



Fluorescence intensity levels for GFP were recorded daily for a period of 10 days post transduction using a microplate reader.

A MOI-dependent increase in fluorescence for all the vectors was observed ,except for AAV4_GFP.

All the vectors showed a plateau phase of GFP fluorescence 9 days after transduction except for AAV1_GFP in which fluorescence kept increasing for the higher MOIs

## Cell Viability

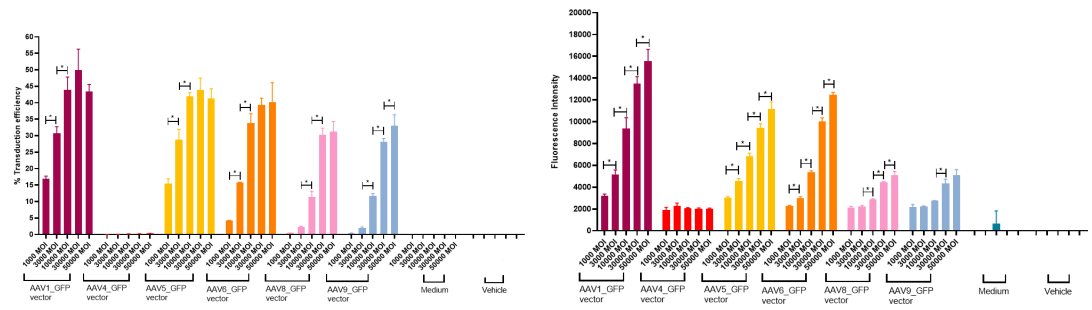



Cell viability was reduced by ~15-25% in the cells that were transduced with AAV1_GFP vector, AAV4_GFP and AAV5_GFP at higher MOIs

No significant difference in cell viability was observed for all other conditions

Statistical analysis: Dunnett's multiple comparison test (One way ANOVA), * p value<0.05

## Transduction efficiency




The transduction efficiency and fluorescence intensity increased with increasing MOIs for several AAV serotypes

AAV1_GFP and AAV5_GFP showed the highest transduction efficiency of ~45-50% at 30000 MOI

AAV4_GFP vector showed no transduction

Tukey's multiple comparison test (One way ANOVA), * p value<0.05

## Conclusions

The most efficient transductions were achieved using AAV1 and AAV5 vectors. The only vector that failed to transduce hiPSC-CMs was AAV4. For all other vectors transduction efficiency increased with increasing MOIs as expected. Cytotoxicity was only observed at higher MOIs (>10.000) for several vectors.

Most AAV serotypes at their respective MOIs, had an acceptable GFP signal (2-7x fluorescence intensity above local background).

We have established a human-based platform to evaluate the transduction efficiency and cytotoxicity of AAVs with high translatability.

This platform helps identifying the most suitable AAV serotypes for transducing our Ncyte vCardiomyocytes, and facilitates the assessment of AAVs supplied by our clients as well as the development of similar platforms for other cell types.

Additional post-transduction functional analysis are also available.