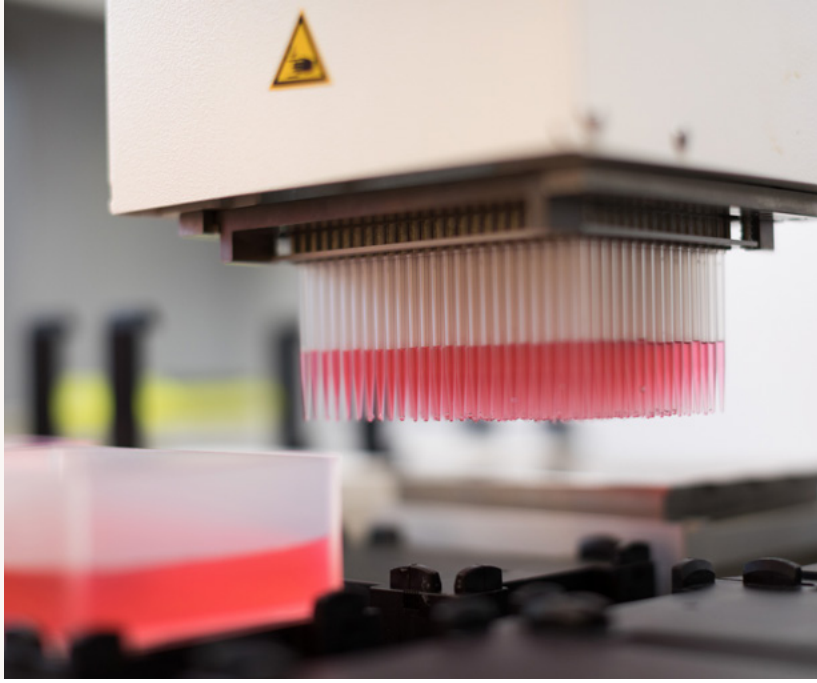
Ncardia
Stem cell experts

Case study

Development of a cardiac hypertrophy iPSC-derived disease model



Background

Cardiovascular disease is a foremost cause of mortality worldwide, with an estimated 17.3 million deaths per year. A major obstacle for efficient drug discovery and development is the absence of physiologically relevant and predictive cell-based assays.

With the aim to improve the drug discovery and development process, using cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC) we develop disease relevant phenotypic assays with a clear link to human disease, in combination with functional readouts closely related to the desired clinical readout in patients, that can be utilized for high-throughput screening. Human iPSC-derived cardiomyocytes recapitulate physiological features of mature cardiomyocytes, allow screening in a patient genetic background, and can be produced in the large quantities required for high throughput screening platforms.

In the current study we have developed an innovative high throughput drug efficacy screening application in a model of hypertrophic cardiomyopathy (HCM), utilizing hiPSC-derived cardiomyocytes.

Disease modeling

Familial HCM is an autosomal dominant disease of the cardiac sarcomere, mainly due to mutations in the MYH-7 protein, which results in abnormal thickening of the left ventricular myocardium and is associated with elevated circulating levels of the biomarker NT-proBNP in patients. HCM was induced by Endothelin-1 (ET-1, a potent pro-hypertrophic peptide produced by endothelial and smooth muscle cells) in bioreactor-derived cardiomyocytes. Overnight exposure of bioreactor-derived iPSC-CMs to ET-1 results in production of BNP as measured by immunofluorescence microscopy.

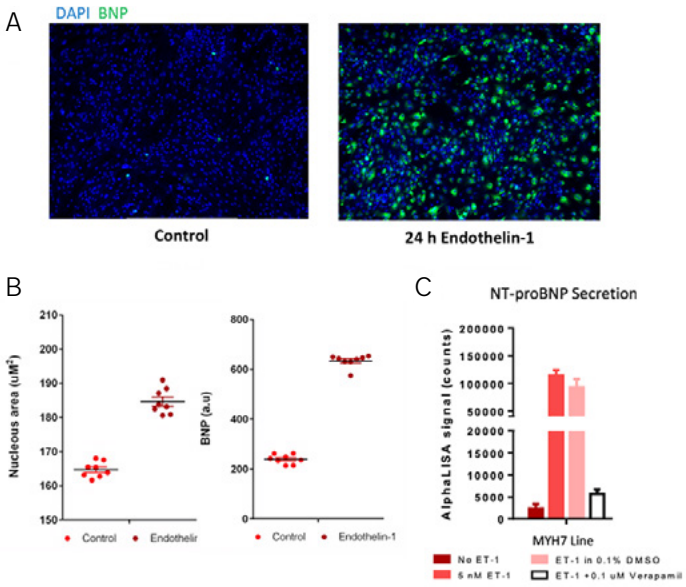


Figure 1: A: Control or ET-1 treated cardiomyocytes were stained for BNP (green) and nuclei (blue). B: Quantification of high content images showing total nuclear area (left) or BNP expression (right) in the presence or absence of ET-1. C: ET-1 induced NT-proBNP secretion, measured by AlphaLISA, was rescued by treatment with Verapamil.

Manufacturing

Cardiomyocytes were produced at large scale using controlled stirred tank bioreactors. Our Fluent® workstation allowed full automation of cell culturing in 384-well plates. We operate enough bioreactors for full factorial design = 2n , where n = the number of factors having impact on the process. These factors include:

- Stirring speed
- pH
- Inoculum size
- Chemical concentrations
- Media refreshment pattern

Consecutive iPSC-CM lots were manufactured in an ISO 9001:2015-certified facility. Automated bioprocessing and integrated quality control help to reduce batch variability as evidenced by consistent cTNT expression and batch sizes.

Assay development

Hypertrophy was assessed by measuring secretion of NT-proBNP using the AlphaLISA assay. The developed assay resulted in robust and reproducible assay quality. An increase in fluorescence units (AlphaLISA counts) in response to 5 nM ET-1 was observed.

Verapamil, an inhibitor of L-type calcium channels that prevents (here, pathological) calcium-induced calcium release, was used as a positive control in this and subsequent assays. Verapamil was able to fully rescue ET-1 induced NT-proBNP release/BNP expression and was the positive comparator on every plate.

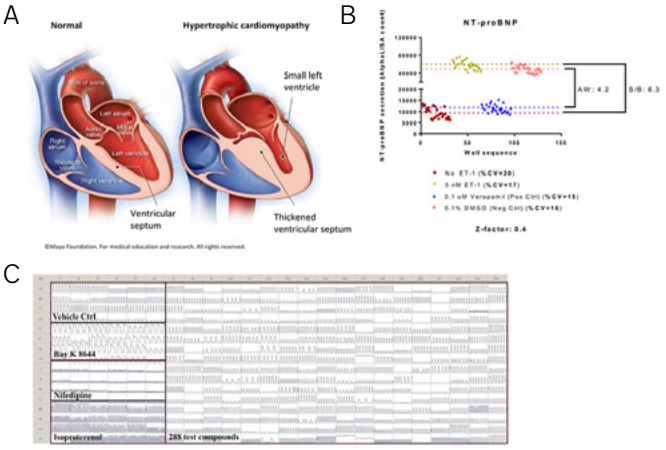


Figure 2: A: Pathological hypertrophy is characterized by asymmetric hypertrophy of the left ventricular wall, typically accompanied by contractile functional deficit, fibrosis, and cardiomyocyte disarray. B: Endothelin-induced secretion of NT-proBNP, a clinical marker of cardiac injury/hypertrophy, was rescued by verapamil treatment in iPSC-derived cardiomyocytes. C: Functional activity was assessed by calcium transient screening in 384 well format.

High Throughput Screening

Validation screening from duplicate plates as well as in a patient derived MYH7 mutated line confirmed the reproducibility of the assays (Pearson Correlation > 0.6). After successful validation of the assays we screened ~5000 data points including >1800 approved drugs, and a thousand further compounds with known mechanism of action. Compounds with percent inhibition (PIN) > 40% were confirmed as hits. Hits were confirmed via three distinct assays; NT-proBNP AlphaLISA assay, AlphaLISATruHits assay (deselect false positives), and intracellular expression assessed by a high content imaging secondary assay.

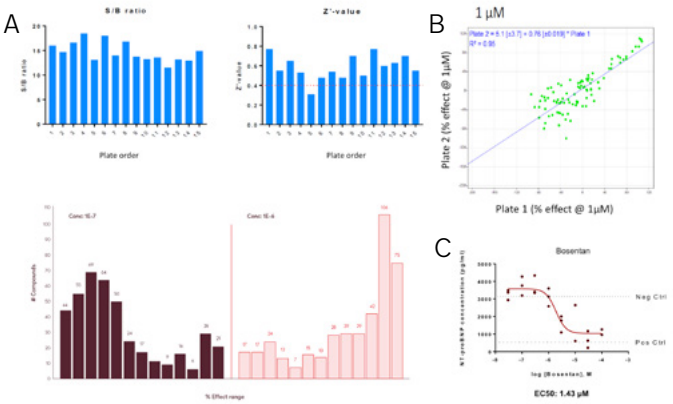


Figure 3: A: Assay performance from 15 x 384 well plates screened in succession. B: Hits grouped by % effect (rescue of hypertrophy) from >3000 compound screened. C: Dose response curve for Bosentan, one of the validated hits identified from the primary screen.

Finally, to determine compound efficacy, potency analyses with 8 point dose response curves were generated, and hits with various potencies identified (i.e. low potency EC50 > 10 uM, moderate potency EC50 > 1 uM and high at nM to sub-nM potency). Many confirmed hits have lead properties (sub-nM potency, favorable DMPK properties, good safety profile, etc.)

Get in contact with us

Ncardia has over 10 years of experience in cardiovascular drug discovery research, based on hiPSC-derived disease models. We have the expertise to generate and co-culture a range of cardiac cell types, creating representative disease models to deliver a more physiologically relevant solution, compared to animal models.

Our team of specialized scientists understands your challenges in drug discovery and development for cardiac diseases. Contact us to discuss your project plans, and find out how we can help you accelerate your research.

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