I am delighted to welcome you to Cologne to Ncardia’s Application Workshop 2017, where I am sure you will enjoy an interesting and exciting few days with us.

The various departments within our company work collectively in developing, producing and commercializing highly-predictive human cellular assay systems for safety and efficacy testing. This collaborative effort is the core of Ncardia, which combines creativity with our lifeline, innovation, in order to provide our broad portfolio of products and services. Our goal is to be the trusted global leader in the hiPSC drug discovery and development field so that Ncardia is the partner of choice for scientists operating in cardiovascular and neural safety and efficacy projects.

At Ncardia we know that successful teams are built on personal relationships and interest in each other. Furthermore, we believe that continuous learning is one of the most important parts of our business approach. These aspects of our company enforce why we have assembled here today: to get to know each other better and be at the forefront of stem cell technology that will help to get better medicines to patients faster.

On behalf of the entire Ncardia team I would like to sincerely thank you all for joining.

Best wishes,
Stefan
# Wednesday
**November 29**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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</thead>
<tbody>
<tr>
<td>12:00 noon</td>
<td>Arrival at Ncardia (BioCampus main building)</td>
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<tr>
<td></td>
<td><strong>LUNCH</strong></td>
</tr>
<tr>
<td>01:00 PM</td>
<td>Stefan Braam, PhD (Ncardia) “Welcome”</td>
</tr>
<tr>
<td>01:25 PM</td>
<td>Prof. Michael Schneider, PhD (Imperial College London) “Cardiac muscle cell death as a druggable target: Human iPSC-derived cardiomyocytes for target validation and compound development”</td>
</tr>
<tr>
<td>01:55 PM</td>
<td>Mohamed Kreir, PhD (Janssen Pharmaceutica) “In vitro cell-based assays to predict drug-induced seizure liability using hiPSC-derived neurons (hiPSC-neurons)”</td>
</tr>
<tr>
<td>02:20 PM</td>
<td>Ryan McGarrigle, PhD (Luxcel Biosciences) “Cardiomyocyte function characterised through combined analysis of metabolic flux, beat rate and cellular oxygenation and its application in in vitro ischemia reperfusion modeling”</td>
</tr>
<tr>
<td>02:45 PM</td>
<td><strong>Coffee break / Poster session</strong></td>
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<tr>
<td>03:30 PM</td>
<td><strong>Live Demonstrations</strong> For full details regarding the Live Demonstrations (including your specific participation), please refer to your Personalised Demo Plan inlay, as well as the projections during break sessions</td>
</tr>
<tr>
<td>05:00 PM</td>
<td>Shuttle bus to hotel (Motel One)</td>
</tr>
<tr>
<td>07:00 PM</td>
<td>Dinner at Brauhaus Sünner im Walfisch (please refer to page 18 for the address and relevant details)</td>
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# Thursday
**November 30**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>08:45 AM</td>
<td>Arrival at Ncardia (BioCampus main building)</td>
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<tr>
<td>09:00 AM</td>
<td>Said El Haou, PhD (Metrion Biosciences) “Validating human iPSC cardiomyocytes for CiPA cardiac safety testing – from electrophysiology to phenotypic assays”</td>
</tr>
<tr>
<td>09:25 AM</td>
<td>Mark Bryant, PhD (Clyde Biosciences) “Optical measurements of electrical and mechanical function of Cor.4U® cells using the CellOPTIQ® System”</td>
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<tr>
<td>09:50 AM</td>
<td>Stephane Bedut, PhD (Scientific service provider on site at VWR Catalyst) “High throughput optical recording of voltage and calcium signals of human iPSC-derived cardiomyocytes for drug screening and cell lines characterization”</td>
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<tr>
<td>10:15 AM</td>
<td><strong>Coffee break / Poster session</strong></td>
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<tr>
<td>10:45 AM</td>
<td>Silvia Caimarca, PhD (Axxam) “iPSC-derived cells going high throughput: new strategies for ion channels drug discovery”</td>
</tr>
<tr>
<td>11:10 AM</td>
<td>Christian Holz (Molecular Devices) “Phenotypic assays for compound effects using imaging-based morphological characterization of 3D neuronal cultures and calcium transients in beating cardiomyocytes”</td>
</tr>
<tr>
<td>11:35 AM</td>
<td>Linn Schneider, PhD (Bayer HealthCare) &amp; Benjamin Wolters, PhD (Ncardia) “Opening the optogenetic toolbox for iPSC-derived Cardiomyocytes”</td>
</tr>
<tr>
<td>12:00 noon</td>
<td>Lunch break / Poster session</td>
</tr>
<tr>
<td>01:00 PM</td>
<td>Prof. Christine L. Mummary, PhD (Leiden University Medical Center) KEYNOTE: “Advancing disease modeling using hiPSC”</td>
</tr>
<tr>
<td>01:40 PM</td>
<td>Caroline Archer, PhD (AstraZeneca) “Validation and utility of human iPSC-vascular smooth muscle cells as an in vitro vascular model”</td>
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<tr>
<td>02:05 PM</td>
<td>Prof. Philipp Sasse, PhD (University of Bonn) “The enlightened heart: Optogenetic methods to investigate and treat cardiac arrhythmia”</td>
</tr>
<tr>
<td>02:30 PM</td>
<td>Elena Dragicevic, PhD (Nanion Technologies) “HTS automated patch clamp takes cardiac safety testing to the next level”</td>
</tr>
<tr>
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</tr>
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<td><strong>Live Demonstrations</strong> For full details regarding the Live Demonstrations (including your specific participation), please refer to your Personalised Demo Plan inlay, as well as the projections during break sessions</td>
</tr>
<tr>
<td>04:45 PM</td>
<td>Shuttle bus to hotel (Motel One)</td>
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</table>
08:45 AM  Arrival at Ncardia (BioCampus main building)

09:00 AM  Thomas Licher, PhD (Sanofi)
"High" throughput assay applications with iPSC-derived cardiomyocytes: Ca\textsuperscript{2+}-oscillations and beyond"

09:25 AM  Georg Rast, PhD (Boehringer Ingelheim)
"hiPSC-derived cardiomyocytes: diversity in a dish"

09:50 AM  Tessa de Korte (Ncardia)
"Multiparametric assessment of the effects of chemotherapeutic drugs on the (electro)physiology of Pluricyte\textsuperscript{®} Cardiomyocytes"

10:10 AM  Coffee break / Poster session

10:40 AM  Live Demonstrations
For full details regarding the Live Demonstrations (including your specific participation), please refer to your Personalised Demo Plan inlay, as well as the projections during break sessions

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**Posters**

<table>
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<th>No.</th>
<th>Title</th>
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<tbody>
<tr>
<td>1</td>
<td>&quot;Multiparametric Assessment of Cardiotoxicity of Chemotherapeutic Drugs in Human Induced Pluripotent Stem Cell (hiPSC)-derived Cardiomyocytes&quot;&lt;br&gt;P. Mulder, F. Famili, T. de Korte, C. Hechard, S.R. Braam, M.L.H. Vlaming (Ncardia)</td>
</tr>
<tr>
<td>2</td>
<td>&quot;Cytotoxicity Monitoring For Safety Assessments: iPSC Cardiomyocytes and More&quot;&lt;br&gt;K. Juhasz (Nanion Technologies; Nanion Technologies, Inc.)</td>
</tr>
<tr>
<td>3</td>
<td>&quot;Cardioxic Classification of 85 Compounds Based On Statistical Learning Applied To Impedance Responses&quot;&lt;br&gt;L. Batista, T. Bastogne, F. Altenzor, A. Delaunois, and J.-P. Valentin (Cybernano)</td>
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<tr>
<td>4</td>
<td>&quot;High-Throughput Pharmacology of Cardiac L-type Ca\textsuperscript{2+} Channels in Overexpressing Cell Lines and iPSC-Cardiomyocytes&quot;&lt;br&gt;E. Dragicevic, C. Haarmann, T. A. Goetzte, M. Rapedius, N. Brinkwirth, I. Rinke, N. Brinkwirth, S. Fris, S. Sbtilze-Fixe, A. Brüggemann, M. George, N. Fertig (Nanion Technologies)</td>
</tr>
<tr>
<td>5</td>
<td>&quot;Application of Human-Induced Pluripotent Stem Cell Derived Cardiomyocytes in High-Throughput Screening Assays for Drug Safety and Efficacy Testing&quot;&lt;br&gt;P. Nacken\textsuperscript{1}, J.M. D’Angelo\textsuperscript{1}, T. de Korte\textsuperscript{1}, R. Wilbers\textsuperscript{1}, F. Famili\textsuperscript{1}, S.R. Braam\textsuperscript{1}, M.L.H. Vlaming\textsuperscript{1} (Ncardia; \textsuperscript{2}Hamamatsu Photonics)</td>
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<td>6</td>
<td>&quot;Coupled Impedance &amp; Field Potential Data Analysis of in vitro Cardiomyocyte Assays&quot;&lt;br&gt;L. Batista, L. Doerr, M. Beckler, N. Fertig, and T. Bastogne (Cybernano)</td>
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<td>8</td>
<td>&quot;Studying Inotropic Compound Effects in Relatively Mature Human iPSC-derived Cardiomyocytes using 2D and 3D Models&quot;&lt;br&gt;T. de Korte\textsuperscript{1}, L. Mannhardt\textsuperscript{1}, A. Hansen\textsuperscript{1}, U. Saleem\textsuperscript{1}, T. Eschenhagen\textsuperscript{1}, C. Denning\textsuperscript{1}, S.R. Braam\textsuperscript{1}, M.L.H. Vlaming\textsuperscript{1} (Ncardia; \textsuperscript{2}University Medical Center Hamburg-Eppendorf; \textsuperscript{3}University of Nottingham)</td>
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<td>9</td>
<td>&quot;A Data-Driven Modeling Method to Analyze Cardiomyocyte Impedance Data&quot;&lt;br&gt;L. Batista, T. Bastogne, F. Altenzor, A. Delaunois, and J.-P. Valentin (Cybernano)</td>
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<tr>
<td>10</td>
<td>&quot;Development of Native Neuron and iPSC-derived Electrophysiology Assays for Neurotoxicology Screening and Translational Drug Discovery&quot;&lt;br&gt;A.M. Rush, L. Webbale, M. Rogers (Metrion Biosciences)</td>
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<tr>
<td>11</td>
<td>&quot;High-Content Assay for Morphological Characterization of Neuronal Development in 3D Matrix Using Human iPSC-derived Neuronal Cultures&quot;&lt;br&gt;O. Sirenko\textsuperscript{1}, G. Chandy\textsuperscript{1}, G. Thompson-Steckel\textsuperscript{1}, R. Bucerus\textsuperscript{1}, T. Palm\textsuperscript{1}, F. Haniel\textsuperscript{1}, B. Simon\textsuperscript{1}, V. Millers\textsuperscript{1} (Molecular Devices, LLC; \textsuperscript{2}Laboratory of Biosensors and Bioelectronics, ETH Zurich; \textsuperscript{3}Ncardia; \textsuperscript{4}Ectica Technologies)</td>
</tr>
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### Abstracts

**MAP4K4 mediates human cardiac muscle cell death: Human pluripotent stem cell-derived cardiomyocytes for target validation and drug development**

Prof. Michael Schneider, PhD, MD  
Imperial College London

Heart disease is a paramount cause of global death and disability. Although cardiomyocyte death plays a causal role and its suppression would be logical, no clinical counter-measures target the responsible intracellular pathways. Therapeutic progress has been hampered by lack of pre-clinical human validation. Mitogen-activated Protein Kinase Kinase Kinase Kinase-4 (MAP4K4) is activated in failing human hearts and relevant rodent models. Using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) and MAP4K4 gene silencing, we demonstrate that death induced by oxidative stress requires MAP4K4. Consequently, we developed a novel small-molecule inhibitor, DMX-5804, that rescues cell survival, mitochondrial function, and calcium cycling in hiPSC-CMs. In addition to protection in conventional 2D cultures, we demonstrate rescue in 3D human engineered heart tissue. As proof-of-principle that drug discovery in hiPSC-CM may predict efficacy in vivo, DMX-5804 reduces ischemia-reperfusion injury in mice by more than 50%. We implicate MAP4K4 as a well-posed target toward suppressing human cardiac cell death, and highlight the utility of hiPSC-CMs in drug development to enhance cardiomyocyte survival.

**In vitro cell-based assays to predict drug-induced seizure liability using HiPSC-derived neurons (hiPS-neurons)**

Mohamed Keir, PhD, Greet Teuns, PhD, Hua Rong Lu, PhD, David J. Gallacher, PhD  
Janssen Pharmaceutica

Drug-induced seizures contribute to the high attrition rate of pharmaceutical compounds in development. The assessment of drug-induced seizures mostly occurs in the later phases of the drug discovery process using low throughput and intensive in vivo assays, in species that are not necessarily the most predictive of human. We therefore evaluated the potential of an in vitro assay using hiPS-neurons to detect potential drug-induced seizures risk. Cultured hiPS-neurons exhibit spontaneous electrical activity that can be modulated by chemical stimulations and can be monitored over time using Multi-electrode arrays (MEAs). Firstly, we characterized the hiPS-neurons by investigating the neuronal activity after exposure to different neurotransmitters (NTs). The data showed that hiPS-neurons are affected by a range of NTs indicating that a wide variety of most functional neuronal receptors are present. These functional data are matching well to the gene expressions of these neuronal receptors. Secondly, we evaluated the effects of reference drugs, known to have a clinic seizurogenic risk. Two compounds showed discrepancies between clinical data and the hiPS-neurons but overall responded well to a wide range of compounds despite hiPS-neurons having a substantially low network activity. It is apparent that this approach can provide sensitive endpoints to detect drug-induced seizures in vitro, and that the hiPS-cell based assay could potentially lead to a better prediction of the concomitant human drug-induced seizurogenic events in the future.

**Cardiomyocyte function characterised through combined analysis of metabolic flux, beat rate and cellular oxygenation and its application in vitro ischemia reperfusion modeling**

Ryan McGarrigle, PhD  
Luxcel Biosciences

Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are finding increased use as an in vitro model in disease research and for the assessment of drug-induced toxicities. However, fully exploiting the potential of this cell model requires a set of tools that comprehensively and conveniently assess altered cell metabolism in a manner that can be co-

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| 12  | “Comparison of Compression Solutions for Impedance and Field Potential Signals of Cardiomyocytes”  
P. Guyot, L. Batista, E. Djermoune, J.-M. Moureaux, L. Doerr, M. Beckler, and T. Bastogne (Cybernano) |
| 13  | “CardioExcyte 96 meets CellDrum - True Contractility in High Performance”  
M. Gossmann (mnoViro; Nanion Technologies; Institute for Bioengineering, Aachen University of Applied Sciences) |
| 14  | “Unblinded: Responses to CiPA 28 Compounds in Cor.4U Cardiomyocytes”  
R. Kettenhofen, G. Luerman, H. Bohlen, S.R. Braam (Ncardia) |
| 15  | “CardioExcyte 96 CiPA Study Recordings with Different iPSC-derived Cardiomyocytes”  
| 16  | “Neurotransmitter Screening via Dynamic Calcium Flux and MEA-based Seizure Liability Assessment in Human iPS Neurons”  
D. Hess, R. Kettenhofen, G. Luerman, H. Bohlen, S.R. Braam (Ncardia) |
| 17  | “Opening the Molecular Toolbox: Co-transfection Of KCNJ2 and Channelrhodopsin2 mRNAs for Precise Control of Cardiac Myocytes Pacinig and Functionality”  
B. Wolters, M. Pourrier, Y. Dou, G. Luerman, R. Bucerius, H. Bohlen (Ncardia; IonsGate) |
| 18  | “Antiarrhythmic Effects of Carvedilol and Flecaainide in Cardiomyocytes Derived From Catecholaminergic Polymorphic Ventricular Tachycardia Patients.”  
R.P. Pölönen, K. Penttinen H. Swan, K. Aalto-Setälä  
Faculty of Medicine and Life Sciences and BioMediTech Institute, University of Tampere, Tampere, Finland;  
Helsinki University Central Hospital, Helsinki, Finland;  
Heart Center, Tampere University Hospital, Tampere, Finland |
| 19  | “Comparison of Compression Solutions for Impedance and Field Potential Signals of Cardiomyocytes”  
P. Guyot, L. Batista, E. Djermoune, J.-M. Moureaux, L. Doerr, M. Beckler, and T. Bastogne (Cybernano) |
| 20  | “Modifications of Stem Cell-Derived Cardiomyocytes Using Non-Integrating RNA: Applications for Optogenetics, siRNA Knockdown and Dominant Negative Expression of Mutated Transcripts”  
B. Wolters, M. Pourrier, Y. Dou, G. Luerman, R. Bucerius, H. Bohlen (Ncardia; IonsGate) |
| 21  | “Characterization of Human iPSC-derived Cardiomyocytes (Cor.4U®) on an Automated Planar Patch Clamp Set Up (QPatch)”  
D.R.P. Sauter, K. Boddm, K. Tsurudome, R.B. Jacobsen, G. Mattsson (Sophion Bioscience) |
bined with established measurements of cardiac function. Here we describe specific multiplexable cardiomyocyte-compati-
bile fluorescence-based microplate assays for the measurement of mitochondrial function (MitoXpress® range) and glycolysis (pHXtra™ range) and outline how these measurements can be combined with other functional analyses (e.g. beat rate) to more comprehensively characterise cellular response to drug treatment or putative therapeutic intervention. We also describe how a fluorescence-based intracellular oxygen probe (MitoXpress® Intra) can be used to provide real-time monitoring of cardio-
myocyte oxygenation and how, when combined with plate reader atmospheric control (CLARIOstar® ACU), can provide a controllable programmable model for in vitro modelling of ischemia reperfusion.

Validating human iPSC cardiomyocytes for CiPA cardiac safety testing – from electrophysiology to phenotypic assays
Said El Haou, PhD
Metron Biosciences
The FDA’s Comprehensive in vitro Prearrhythmia Assay (CiPA) initiative aims to provide a thorough preclinical cardiac safety profile of new chemical entities to enable prediction of human clinical proarhythmia risk. To allow the successful utilisation of commercial human iPSC-derived cardiomyocytes iPSC-CM as models of human cardiomyocytes in the CiPA safety para-
digm, their biophysical and pharmacological profile needs to be fully characterised. Here we will highlight our work to assess the utility of Ncardia vCor.4U® iPSC-CM for CiPA-relevant cardiotoxicity screening.

Using the gold standard of manual patch clamp, the action potential (AP) phenotype was assessed using compounds from the CiPA validation toolbox as well as pharmacological agents to discriminate between atrial and ventricular profiles. Collect-
ively, these data confirmed the predominantly ventricular phenotype of vCor.4U cells, and the functional expression of core cardiac currents including IfNa, L-type Ica and hERG (Ikr). The integrated response of cardiomyocytes to CiPA relevant com-
ounds were then determined using plate-based phenotypic recordings of extracellular field potentials (EFP) on a multi-elect-
rode array (Axion Maestro). Finally, the dual readout CardioExcyte96 (Nanion) platform was used to evaluate acute drug effects on contractility and excitability, as well as evaluate chronic (> 72 h) cardiotoxicity effects of a variety of compounds including known cardiotoxic anti-cancer drugs. Our data demonstrate the utility of Ncardia vCor.4U cells in cardiac ion channel drug discovery and toxicology screening. In the light of CiPA guidelines, these cells represent a suitable model for in vitro preclinical cardiac safety evaluation and prediction of potential human proarhythmic risk.

Optical measurements of electrical and mechanical function of Cor.4U® cells using the CellIOPTIQ® System
Mark Bryant, PhD
Clyde Bioscience
This talk will describe the CellIOPTIQ® system that provides a medium through-put assay system of electrical, intracellular Ca2+ and mechanical function from Cor.4U® cells. Data from pharmaceutical validation studies will be presented.

High throughput optical recording of voltage and calcium signals of human iPSC-derived cardiomyocytes for drug screening and cell line characterisation
Stephane Bedut, PhD
VWR International
Human iPSC-derived cardiomyocytes (hiPSC-C) represent a great opportunity for basic sciences and drug discovery by combining the level of information of cardiomyocytes with the handling of cultured cells. They allow the work in multi-well format and thus the high-throughput generation of high-content data. The optical recording with a fast imaging plate reader leads to the recording from 96 to 384 of simultaneous voltage and calcium signals with dedicated fluorescent dyes. This kind of assay could be very promising for drug characterization (including for safety purpose as investigated in the CiPA initiative) or phenotypic screening. The presentation will describe the main technical aspects of such assay, compare the signals with MEA recordings and be illustrated with some examples of results.

hiPSC-derived cells going high throughput: new strategies for ion channels drug discovery
Silvia Cainarca, PhD
Axxon
The ion channel drug discovery process needs to rely on HTS assays and electrophysiology. The merge of optogenetics and iPSC-based technologies provides informative and cost-effective drug screening tool to achieve fast control of specific cell events. The project goals are to generate iPSCs with a genetically encoded Ca2+ sensor suitable for HTS, to adapt iPSC-de-

Phenotypic assays for compound effects using imaging-based morphological characterization of 3D neuronal cultures and calcium transients in beating cardiomyocytes
Christian Holz
Molecular Devices
Imaging-based morphological characterization of 3D neuronal cultures Development of more complex, biologically relevant, and predictive cell-based assays for compound screening is one of the main challenges in drug discovery and so there is an increasing interest in using three-dimensional (3D) cultures for better resemblance of the characteristics of human tissues. Hydrogels are widely used as an artificial extracellular matrix to grow neural cells in a 3D environment. Fully synthetic hydrogels are available pre-casted in a 96-well plate, featuring an in-depth surface density gradient to promote the infiltration in 3D of cells that are deposited on the hydrogel surface. The platform offers high simplicity of use and high compatibility to automation for neurodegenerative and neurotoxicology screens. We have tested concentration-response effects of a series of selected compounds that are known neurotoxicants. Cells used for the assay were CNS.4U™, an iPSC-derived cell mix of neurons and astrocytes. We will demonstrate how our ImageXpress Micro Confocal imaging system and analysis software were used for the quantitative characterization of the extent and viability of complex neural networks.

Calcium transients in beating cardiomyocytes The assessment of cardiotoxicity is important in the early stages of drug discovery to enable elimination of potentially toxic compounds from further development. There is a growing need for highly predictive in vitro cardiotoxicity assays that use biologically relevant cell-based models and are suitable for high-throughput screening. iPSC-derived cardiomyocytes are especially attractive cell models because they represent gene expression profiles as well as having phenotypic characteristics similar to native cardiac cells. In our study, we tested compounds with known therapeutic effects as well as cardiotoxic effects on Cor.4UTM cardiomyocytes. We measured the beat rate and oscillation pattern on our SpectraMax Reader and FLIPR Tetra system by using the calcium sensitive dye available in our EarlyTox Cardiotoxicity kit.

Phenotypic assays for compound effects using imaging-based morphological characterization of 3D neuronal cultures and calcium transients in beating cardiomyocytes
Linn Schneider, PhD1, Benjamin Wolters, PhD2
1Bayer HealthCare; 2Ncardia
Light-sensitive proteins allow precise manipulation and monitoring of specific cellular processes. Within recent years, genetically introduced light-gated ion channels have been widely applied as optical actuators. Expressing those Channelrhodopsins in cardiomyocytes enables for non-invasive control of action potentials with high temporal resolution and precision. Here we show a transient transfection method for iPSC-derived cardiomyocytes and the application in high-
throughput screening (HTS) assay formats.
Advancing disease modeling using hiPSC
Prof. Christine Mummery, PhD
Department of Anatomy and Embryology, Leiden University Medical Centre

Derivation of many different cell types from human pluripotent stem cells is an area of growing interest both for potential cell therapy and as a platform for drug discovery and toxicity. Most particularly, the recent availability of methods to introduce specific disease mutations into human pluripotent stem cells and/or to derive these cells as hiPSC cells by reprogramming from any patient of choice, are creating unprecedented opportunities to create disease models “in a dish” and study ways to treat or slow down its rate of development. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to their derivatives and mimicking these in defined culture conditions in vitro is now essential for moving the field forward. We have used these methods to produce isogenic pairs of hiPSC lines to compare diseased and corresponding control cardiomyocytes and vascular endothelial cells and identify disease related phenotypes and mechanisms. The use of isogenic pairs has proved crucial since variability between “healthy control” hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. We have also examined drug responses of hESC-derived cardiomyocytes to a variety of cardiac and non-cardiac drugs and shown that iPS/C-derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients. The development of appropriate bioassays in which to measure disease phenotypes which may be highly cell type specific dependent. For heart cells, this might be electrical activity or contractions force.

Validation and Utility of human iPS-vascular smooth muscle cells as an in vitro vascular model for cardiovascular safety assessment
Caroline Archer, PhD, Milka Budnik-Zawiliska, PhD, Kelly Gray, PhD, Amy Pointon, PhD
AstraZeneca

Drug-induced changes in blood pressure are a common and undesirable side effect of novel compounds. There are currently limited in vitro cellular phenotypic models available to predict vasoactivity applicable to early safety screening in drug discovery and development. Cellular approaches have been hampered by a lack of consistency between human vascular smooth muscle cells (VSMC) with contractile phenotype. In collaboration with Ncardia, we have developed a human induced pluripotent (iPS)-derived VSMC population, exhibiting a stable synthetic phenotype subsequently eradicating donor-donor variability, thereby providing a cellular model amenable for in vitro vascular safety assays.

Here we describe the validation and characterisation of the hiPS-VSMC. We confirm that hiPS-VSMC express vascular smooth muscle cell specific proteins and respond to well-defined vasoconstrictors in calcium transient assays. This cell model has been further validated against a larger panel of vasoactive and non-vasoactive compounds and the expression of targets defined through analysis of gene profiling. We also discuss preliminary work investigating the development of multicellular vascular models that confer physiological relevance.

The enlightened heart: optogenetic methods to investigate and treat cardiac arrhythmia
Prof. Philipp Sasse, PhD
Institute of Physiology, University of Bonn

Optogenetic methods take advantage of light-sensitive proteins to manipulate cells and organs by light with great advantages over electric or pharmacological stimulation. Here we provide an overview of our contributions to cardiac optogenetics in cardiomyocytes in vitro and hearts in vivo. We will show how Channelrhodopsin-2 can be used to stimulate human cardiomyocytes (Cor4L®, Ncardia) in the culture dish and to pace the heart of transgenic mice. Furthermore, we have proven that optogenetic defibrillation is highly effective to terminate ventricular tachycardia and atrial fibrillation and could represent an alternative approach to painful electrical shocks. With the aim of clinical applications, we have developed a gene transfer strategy by systemic adeno-associated virus injection, which enabled reliable optogenetic pacing and defibrillation of wild-type mouse hearts. Side effects on cardiac ion channels causing lethal arrhythmias are one major reason for drug withdrawing from the market. We have recently developed technologies to combine field potential measurements with optogenetic stimulation. Therefore, a commercially available field potential recording system (CardioExcyte96, Nanion Technologies) was modified for LED stimulation. This enabled reliable pacing of Cor4L® cardiomyocytes (Ncardia) by light flashes at physiologically relevant heart rates and the automated frequency-dependent analysis of drug effects on Na+, Ca2+ and K+ channel function.

In summary, we successfully applied optogenetic methods for electrical stimulation of cardiomyocytes in vitro and the heart in vivo and provide an outlook for treating cardiac arrhythmia and for cardiotoxic drug screening.

HTS automated patch clamp takes cardiac safety testing to the next level
Elena Dragicevic, PhD
Nanion Technologies

Patch clamp electrophysiology remains the gold standard for studying ion channels. Although yielding information rich data, conventional patch clamp is technically demanding and not suitable for high throughput screening efforts. Over the last two decades, a number of automated patch clamp (APC) devices employing a planar approach have successfully been developed. These devices are capable of measuring either single cells, or multiple cells simultaneously, whilst maintaining high-giga-Ohm seal quality data. Importantly such devices offer the possibility for recording multiple cells in parallel, the highest throughput so far recording from 768 cells simultaneously and therefore addressing the needs of high throughput screening laboratories. However, there is an increasing demand not only for higher throughput, but also for more technically demanding features such as temperature control, internal solution exchange, current clamp, dynamic clamp and optogenetics. Here we focus on the Patchliner and SyncroPatch 384/768PE, two APC devices recording from 8 or 384/768 cells simultaneously, respectively. In the light of the comprehensive in vitro pro-arrhythmic assay (IPA) initiative, features such as temperature control and current clamp recordings of stem cell-derived cardiomyocytes on medium and high throughput APC devices have become important. Here, we show temperature-controlled measurements of NERG and other relevant cardiac currents, as well as action potentials, recorded from diverse heterologous cell lines and stem cell-derived cardiomyocytes on the Patchliner and SyncroPatch 384/768PE. Additionally, we were able to manipulate the duration of those action potentials on the Patchliner, by introducing virtual IKI. Finally, the light activated action potentials using Cor4L® cardiomyocytes transfected with channelrhodopsin (ChR2), will be shown using the SyncroPatch 384/768PE. In conclusion, we demonstrate that the newest features of automated patch clamp have important implications for advancing high throughput drug discovery and safety pharmacology screening.

“High” throughput assay applications with iPSC-derived cardiomyocytes: Ca2+-oscillations and beyond
Thomas Licher, PhD
Sanofi

This abstract will be available from the Ncardia website after the Applications Workshop has ended: www.Ncardia.com

hiPSC-derived cardiomyocytes: diversity in a dish
Georg Rast, PhD
Boehringer Ingelheim Pharma GmbH & Co. KG

hiPSC-derived cardiomyocytes are in routine use for electrophysiological drug safety profiling, however, subtle differences exist between cardiomyocytes from different providers and little is known about the diversity between individual cells and the consequences of such diversity. The interdependence of spontaneous beating rate and duration of repolarization will serve as an example for the diversity between providers and will demonstrate which questions need to be asked to understand the responsible mechanisms.
Multiparametric assessment of the effects of chemotherapeutic drugs on the (electro) physiology of Pluricyte® Cardiomyocytes

Tessa de Korte
Ncardia

Cardiotoxicity is a major cause for the attrition of pharmaceutical drugs and the failure of compounds during drug development. In order to improve the outcome of toxicity screening, preclinical drug assays ideally would be performed on adult human cardiomyocytes. This has not been feasible in early stage of drug discovery due to the difficulties in obtaining human heart tissues, and to propagate them in vitro. However, with the introduction of human induced pluripotent stem cell (hiPSC) technology, hiPSC-derived cardiomyocytes are being applied to model heart diseases, to screen for new drugs, and to test candidate drugs for cardiotoxicity.

We have developed fully functional hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) which resembles to mature human cardiac cells in many aspects. This was confirmed by an increased contraction profile, a highly organized sarcomere organization, as well as improved electrophysiological properties (negative resting membrane potential, well defined action potential plateau and rapid depolarization).

It has been shown that several chemotherapeutic agents including Tyrosine Kinase Inhibitors (TKIs) or anthracycline drugs could potentially induce cardiotoxicity in vivo. Therefore, developing in vitro tools to assess the potential cardiotoxicity becomes essential to evaluate new drug candidates. To this end, we developed a high-throughput and multi-parametric platform in which various mechanisms of drug-induced cardiotoxicity can be assessed. We treated Pluricyte® Cardiomyocytes with a set of chemotherapeutic drugs to measure the acute and chronic toxicity of compounds on electrophysiology (MEA assay), contractility (Ca-transient assay), viability (ATP assay) and biomarker detection (Troponin I release assay).

In conclusion, high-throughput application of Pluricyte® Cardiomyocytes in various (electro) physiology-based assays, is an unique model to predict cardiotoxicity profile of compounds at different cellular levels during drug discovery.
ACEA BIOSCIENCES, INC., ACEA Biosciences, Inc., founded in 2002, is a privately-owned biotechnology company with headquarters in San Diego, California. ACEA’s mission is to transform cell analysis by providing innovative products and solutions to the research and drug discovery community. ACEA’s xCELLigence® impedance-based, label-free, real-time cell analysis system and NanoCyte™ flow cytometers are used in pre-clinical drug discovery and development, toxicity, safety pharmacology, and basic academic research. In 2014 ACEA inroduced its Second-Generation cardiac system, the xCELLigence® RTCA CardioECR, to meet the longstanding need for more predictive preclinical assays of cardiac liability.

AXION BIOSYSTEMS is the leader in microelectrode array (MEA) data acquisition and analysis. With the Maestro MEA platform, functional activity assays on neural or cardiomyocyte cell networks are easily performed on the benchtop facilitating investigations in neurotoxicology, cardiac safety, in vitro disease modeling, and drug discovery. High-throughput optogenetic stimulation (Lumos) is an available option to add to your Maestro.

BMG LABTECH is a leading global developer and manufacturer of innovative, high-quality, and reliable microplate reader instrumentation. BMG LABTECH has been committed to producing microplate readers for more than twenty-five years. By focusing on the needs of the scientific community, the company’s microplate readers have earned the company the reputation of being a technology leader in the field. BMG LABTECH has developed a wide range of dedicated and multi-mode microplate readers for life sciences applications and high-throughput screening. All BMG LABTECH microplate readers are “Made in Germany” and are conceived, developed, assembled, and tested entirely at BMG LABTECH headquarters in Germany.

HAMAMATSU PHOTONICS is a world-leading manufacturer of optoelectronics, providing solutions for a wide range of applications including drug screening and assay development. The FDSS series of whole plate readers from Hamamatsu are fast, reliable, cost-effective and highly sensitive imaging based systems in a flexible, modular format, ideal for all your drug screening needs. From the beginning, the FDSS systems have been developed to provide you with the best dispensing and imaging instruments to answer your needs. All of our systems include one or multiple injection heads, can be used with different plate formats. The modularity of our systems makes each one completely unique and their adaptability ensures they will evolve with your changing needs towards new applications such as high-speed cardiomyocyte in-vitro toxicity assays and electric-field stimulation of cardiomyocytes.

LUXCEL BIOSCIENCE has created a range of in-vitro cell-based assay kits, targeting Cell Metabolism, Drug Safety Toxicology and Hypoxia / Oxidative Stress for the Life Sciences market. These 96- and 384-well microtitre-based fluorescence assays are configured for use on standard fluorescence plate readers and provide simple solutions for research, drug discovery and safety toxicology.

NANNION TECHNOLOGIES supplies leading technologies for ion-channel drug discovery and screening. Our portfolio comprises patch clamp technology for sophisticated and high throughput applications (Port-a-Patch, PatchLiner and SyncroPatch384/768PE), impedance and extracellular field recordings (CardioExcyte 96), parallel biayer recordings (Orbit 16 and Orbit mini), and membranes transporter protein recordings (SURFETR™ N1 and SURFETR™ 96SE).

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# Partners

HORIZON 2020 SME INSTRUMENT Ncardia is supported by the European Union’s Horizon 2020 research and innovation programme through an SME Instrument grant. This grant gives Ncardia the opportunity to further develop and improve its safety pharmacology assays as well as developing novel cardiac disease models and high-throughput screening assays for cardiovascular drug discovery.

HORIZON 2020 FAST TRACK TO INNOVATION Three European technology SME’s, Luxcel Biosciences Ltd, Ncardia and BMG LABTECH GmbH, together with two internationally recognised research institutions, Oxford University and Imperial College London, have been awarded funding from the European commission under the H2020 Fast Track to Innovation (FTI) pilot, to develop and launch a cell metabolism analysis platform (MetaCell) that is predicted to be part of routine in vitro cell biology and drug development.

EUROSTARS In HeartCHIP, supported by the Eurostars Programme, Ncardia will leverage its unique expertise in cardiac disease modeling and work in close collaboration with the German company ibidi GmbH, a leading supplier of cell-based assays, and the Erasmus University Medical Centre Rotterdam, located in The Netherlands. The partners within the HeartCHIP consortium will combine their unique proprietary technologies to develop extensive new knowledge and co-develop a breakthrough cardiac phenotypic screening platform.

FP7 Plurimes combines the expertise of eight academic and two industrial partners to bring together stem cell experts, genetic engineers, developmental biologists, cell therapy pioneers, bioengineers and specialist SMEs in a cross-disciplinary collaborative effort. Plurimes is supported through the European Commission’s Framework 7 HEALTH research programme and Coordinated by Professor Austin Smith from the Wellcome Trust – Medical Research Council Cambridge Stem Cell Institute at the University of Cambridge.

ECSEL.JU In the InForMed project an integrated pilot line for medical devices will be established, covering the complete innovation chain from technology concept to system qualification. It will include micro-fabrication, assembly and even the fabrication of smart catheters. Ncardia is active in the development of demonstrator products within the consortium, in particular demonstrator 2: Advanced devices for electrophysiology. InForMed is an ECSEL JU project and is co-funded by grants from Belgium, Finland, France, Germany, Great Britain, Ireland, the Netherlands, Spain, Sweden and Switzerland.

Ncardia is involved in the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, led by the US FDA, Safety Pharmacology Society (SPS), Cardiac Safety Research Consortium (CSRC) and Health & Environmental Sciences Institute (HESI). This project aims to improve current regulatory guidelines by introducing predictive technologies, including human stem cell-derived cardiomyocytes, into preclinical cardiac safety assessment.

Ncardia, in a consortium with 4 academic partners and one SME, won the CRACK IT InPulse challenge for development of a cardiac contractile assay. A panel of experts from GSK, AstraZeneca, NC3Rs and renowned academic institutions selected the consortiums solutions from a strong line-up of competing technologies. The consortium will develop an assay to detect cardiac contractility effects at an early stage of drug development.
Location

Cologne Bonn airport

Shuttle bus (free)

**Wednesday, Nov 29**

05:00 PM  from  BioCampus  to  Hotel Motel One

**Thursday, Nov 30**

08:00 AM  from  Hotel Motel One  to  BioCampus

04:45 PM  from  BioCampus  to  Hotel Motel One

**Friday, Dec 1**

08:00 AM  from  Hotel Motel One  to  BioCampus

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We are shaping the future of drug discovery & development.