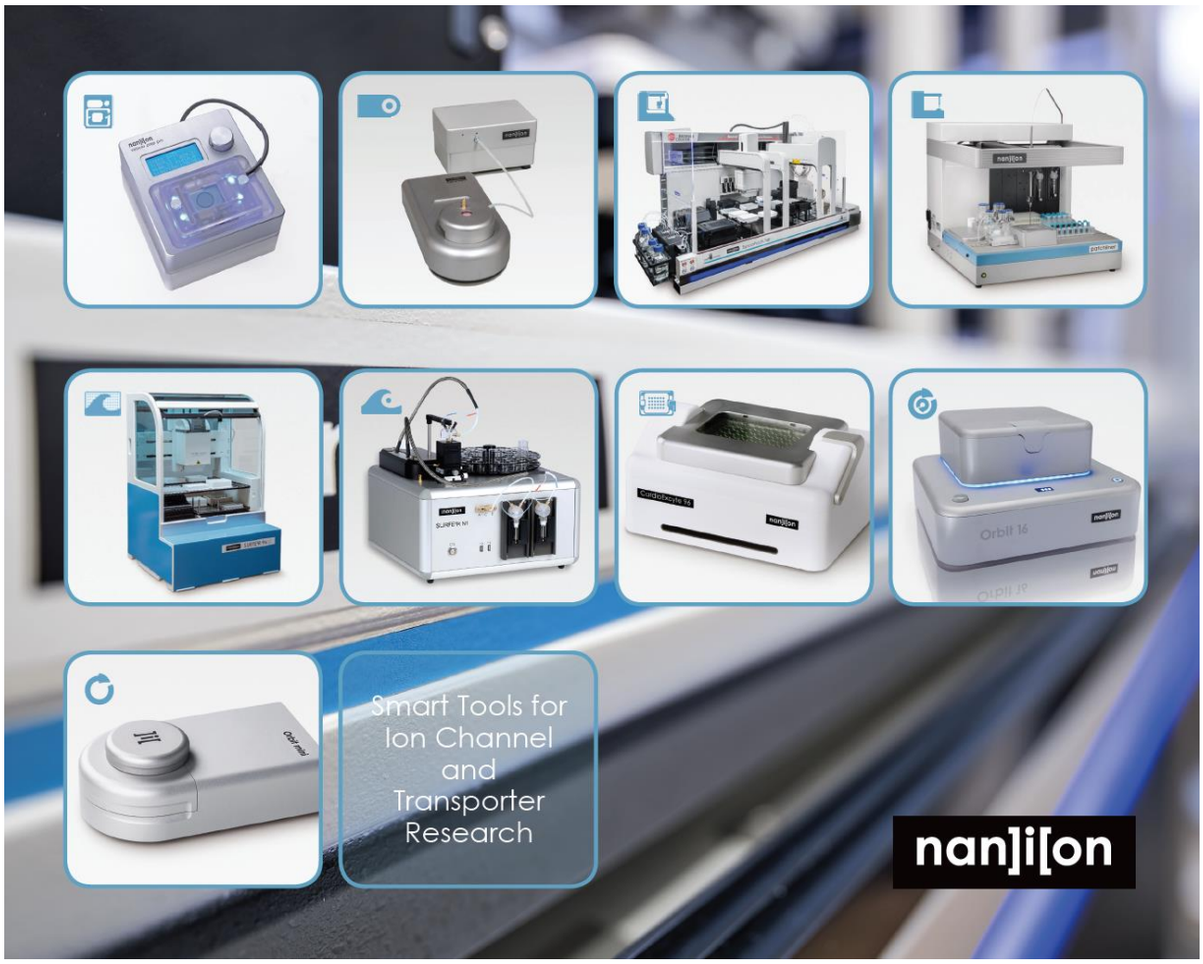
Nanion's Annual User Meeting USA



2018

Welcome to Nanion's Annual User Meeting USA 2018

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User Meeting Venue

Hyatt Regency Hotel
575 Memorial Dr.
Cambridge MA 02139, USA

Date

June, 21. – 22. 2018

Thursday June 21

7:30 – 8:30 AM ▸ **Arrival & Breakfast Charles View Ballroom 16th Floor / Poster Set Up Foyer Area 16th Floor**

8:30 – 8:45 AM ▸ **Welcome and introduction**
Rodolfo Haedo & Niels Fertig, Nanion Technologies


Thursday Morning Session 1:

8:45 – 9:30 AM ▸ **Keynote:**
Targeting Potassium Channels in Rheumatoid Arthritis
Prof. Dr. Christine Beeton, Baylor College

9:30 – 10:00 AM ▸ **Molecular Mechanisms for the Activation and Block of TRPA1 Channel**
Dr. Jun Chen, Genentech

10:00 – 10:30 AM ▸ **Are two channels enough? The search for the missing piece**
Prof. Dr. Jürgen Bosch, Case Western University

10:30 – 11:00 AM ▸ **Coffee Break / Poster Presentations: Foyer Area 16th floor**

Thursday Morning Session 2

11:00 – 11:30 AM ▸ **Decrypting Variants of Unknown Significance in the Channelopathies**
Prof. Dr. Al George, Northwestern University

11:30 – 11:45 PM ▸ **Genome-Editing of Isogenic hiPSC to Establish Pathogenicity of Genetic Variant of Unknown Significance**
Prof. Dr. Björn C. Knollman, Vanderbilt University Medical Center

11:45 – 12:00 PM ▸ **Using the CE96 for Investigation of Contractile and Electrophysiological Properties of Optically Stimulated Academically Generated hiPSC-CMs**
Shan Parikh, Vanderbilt University Medical Center

12:00 – 1:00 PM ▸ **Lunch: 16th floor**

Thursday Afternoon Session 1

1:00 – 1:30 PM ▸ **Targeting hERG K+ Channel Intracellular Domains to Manipulate Channel Function and Cardiac Repolarization**
Prof. Dr. David Jones, University of Michigan

1:30 – 2:00 PM ▸ **Characterization of the Curious Pores of the Fluc Fluoride Channel with the Orbit mini**
Dr. Nicholas Last, HHMI Brandeis

2:00 – 2:30 PM ▸ **Coffee Break / Poster Presentations: Foyer Area 16th floor**

Thursday June 21

Thursday Afternoon Session 2

2:30 – 3:00 PM ▸ **iPSC Derived Cells in Drug Discovery and Development**
Dr. Stefan Braam, Ncardia

3:00 – 3:30 PM ▸ **Evaluation of MYH7-R403Q Patient-Derived iPSC Cardiomyocytes as an In-Vitro Model of Hypertonic Cardiomyopathy**
Dr. Scott MacDonnell, Regeneron Pharmaceuticals

3:30 – 3:45 PM ▸ **Closing Remarks**
Tim Strassmaier, Nanion

4:15 – 4:30 PM ▸ **Gather at the Hyatt Lobby to take Shuttle for Dinner Cruise**

Friday June 22

8:00 – 9:00 AM ▸ **Arrival and Breakfast Charles View Ballroom 16th Floor**

9:00 – 9:15 AM ▸ **Introduction**
Andrea Brüggemann, Nanion Technologies

Friday Morning Session 2

9:15 – 10:00 AM ▸ **Keynote:**
Precision Medicine Targeting Pain: Progress Finding Treatments for Specific Nav1.7 Mutations
Dr. Mark Estacion, Yale University

10:00 – 10:30 AM ▸ **Antiarrhythmic Potential of Antimalarial Aminoquinolines**
Prof. Dr. Sami Noujaim, University of South Florida

10:30 – 11:00 AM ▸ **Finding Nav1.1 Enhancers on the SyncroPatch 384PE**
Dr. Stephen Hess, Evotec

11:00 – 11:30 PM ▸ **CardioExcyte and SyncroPatch 384PE High Throughput Assessment of Functional Abnormalities of Patient-Specific iPSC-CMs Harboring Multiple Mutations**
Dr. Gary Aistrup, Masonic Medical Research Institute

11:30 – 12:00 PM ▸ **Applications of the SyncroPatch 384PE: Safety Pharmacology and more**
Dr. Andrea Brüggemann, Nanion Technologies

12:00 – 1:00 PM ▸ **Lunch: 16th floor**

Friday June 22

Workshop SyncroPatch 384/786PE

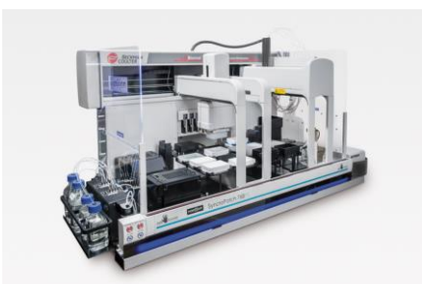
1:00 – 3:00 PM ▸ Workshop Cambridge Room 2nd Floor

SyncroPatch 384/768PE: HTS Planar Patch Clamp

Meet the SyncroPatch 384/786PE Team:

Andrea Brüggemann
CSO

Søren Friis
Director Global
Customer Relations



In this workshop you will learn from Søren Friis tips and tricks on assay development for specific ion channels.
Andrea will present new data on ion channel assays and will give an overview on new features.

Workshop CardioExcyte 96

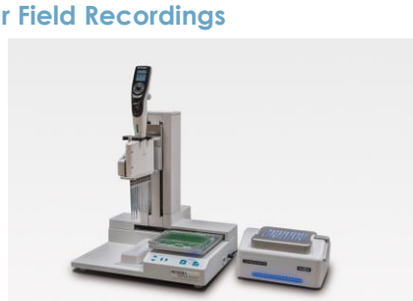
1:00 – 3:00 PM ▸ Workshop Cambridge Room 2nd Floor

CardioExcyte 96:
Label-free Impedance and Extracellular Field Recordings

Meet our CardioExcyte 96 Team:

Corina Bot
Senior Application Scientist
Lab Operations Manager

George Okeyo
Product Manager



In this workshop you will learn from George Okeyo tips and tricks and troubleshooting on cell handling.
Corina Bot will present new data on CiPA studies and will give an overview on new features.

Abstracts Oral Presentations:

Thursday
8:45 – 9:30 AM ▸ **Targeting Potassium Channels in Rheumatoid Arthritis**

Prof. Dr. Christine Beeton, Baylor College

Research Interest: Our current work revolves around two main topics: targeting potassium channels for the treatment of chronic diseases (multiple sclerosis, rheumatoid arthritis, and type 1 myotonic dystrophy) and using antioxidant nanomaterials for the treatment of T lymphocyte-mediated autoimmune diseases (multiple sclerosis and rheumatoid arthritis).
Christine participated in the design and characterization of ShK-186, a Kv1.3 channel blocker with immunomodulatory properties in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. In addition to her continuing work on Kv1.3 in T lymphocytes, Dr. Beeton's group identified KCa1.1 (BK) channels as potential therapeutic targets on fibroblast-like synoviocytes in rheumatoid arthritis. She is leading efforts to define its roles in regulating cell pathogenesis and to develop selective blockers as potential therapeutics for rheumatoid arthritis.

Thursday
9:30 – 10:00 AM ▸ **Molecular Mechanisms for the Activation and Block of TRPA1 Channel**

Dr. Jun Chen, Genentech

Content: TRPA1 is an irritant sensor and an important therapeutic target. We have identified many modulators and mapped their respective binding sites to various domains of channel protein, hence revealing the mechanisms for TRPA1 activation and block.

Thursday
10:00 – 10:30 AM ▸ **Are two channels enough? The search for the missing piece**

Prof. Dr. Jürgen Bosch, Case Western University

Research Interest: Dr. Bosch's primary research interest is focused on the Plasmodium parasite, the causative agent of Malaria, leading to 200 million infections per year and more than 0.5 million deaths worldwide. Using a targeted structure-based drug design approach, we employ X-ray crystallography, virtual library screening (VLS), and surface plasmon resonance (SPR) methods to study important key players, with the ultimate aim to develop novel, drug-like compounds for future therapeutic use.

Thursday
11:00 – 11:30 AM ▸ **Decrypting Variants of Unknown Significance in the Channelopathies**

Alfred L. George, Jr., M.D., Department of Pharmacology, Northwestern University
Feinberg School of Medicine, Chicago, IL

Abstract: Genetic testing has become standard-of-care for many diseases including channelopathies affecting cardiac rhythm such as the congenital long-QT syndrome (LQTS). However, interpreting genetic test results is often confounded by the discovery of 'variants of unknown significance' for which there is insufficient data to establish whether or not a particular variant is pathogenic. For channelopathies, in vitro functional assessments have been valuable for determining the potential pathogenicity of variants discovered in the research setting but the value of this approach for variant classification in the clinical setting

has not been evaluated. Manual patch-clamp recording is the gold standard in assessing the likely pathogenicity of ion channel variants, but the extreme time and labor intensity of this approach is insufficient to tackle the thousands of known human variants. We implemented the Syncropatch 768PE to enable functional evaluation of ion channel variants at an unprecedented scale. Data from our studies of KCNQ1 variants will illustrate the power of this approach.

Thursday
11:30 – 11:45 PM ▸ **Genome-Editing of Isogenic hiPSC to Establish Pathogenicity of Genetic Variant of Unknown Significance**

Prof. Dr. Björn C. Knollman, Vanderbilt University Medical Center

Research Interest: My laboratory is interested in the biology of cardiac arrhythmias. Using genetically-altered mice as model systems, ongoing research examines several key pathways of arrhythmias and sudden death in humans: 1) Impaired cardiac calcium cycling and mutations in cardiac Ca^{2+} handling proteins, 2) Troponin T mutations associated with hypertrophic cardiomyopathy and 3) Mutations associated with the congenital long QT syndrome. In neither case the mechanisms that lead to sudden cardiac death are fully understood. The laboratory performs comprehensive studies from the molecular level to the whole animal in each mouse model in order to better understand the mechanisms of arrhythmogenesis. For example, single cell patch-clamp, intracellular calcium and cell shortening measurements, whole-heart electro-physiology and contractility measurements, and in vivo electrocardiogram and hemodynamic studies are performed. This research may identify therapeutic strategies, which then can be tested in the same model system prior to human studies.

Thursday
11:45 – 12:00 PM ▸ **Using the CE96 for Investigation of Contractile and Electrophysiological Properties of Optically Stimulated Academically Generated hiPSC-CMs**

Shan Parikh, Vanderbilt University Medical Center

Content: Shan is completing his graduate studies in the laboratory of Dr. Bjorn Knollmann where he uses hiPSC-CM for modeling cardiac disease. Specifically, Shan is enamored by the potential of high-throughput technologies for studying cardiac disease. Today he will be discussing the lab's progress on the use of the optical pacing feature on the CardioExcyte96.

Thursday
1:00 – 1:30 PM ▸ **Targeting hERG K+ Channel Intracellular Domains to Manipulate Channel Function and Cardiac Repolarization**

Prof. Dr. David Jones, University of Michigan

Abstract: The hERG PAS domain serves to suppress current, and disruption of the PAS domain enhances current in a manner that may have therapeutic potential in treating cardiac arrhythmias. We have developed two anti-PAS antibodies that interfere with PAS action and enhance current amplitude. To understand the underlying mechanism, we tested the effect of the anti-PAS antibodies on movements of the voltage sensor domain (VSD). Working at physiological temperature (36 ± 1°C), we measured VSD activation and deactivation by recording gating currents from -120 mV and +50 mV holding potentials, respectively, from HEK293 cells stably expressing hERG. In controls, VSD deactivation displayed a double Boltzmann and a Vmedian that was 16 mV more negative than the Vmedian of activation, a reflection of VSD "relaxation" at positive voltages. The antibodies converted VSD deactivation to a single Boltzmann and hyperpolarized the Vmedian compared to controls by approximately 20 mV. Conversely VSD activation was unaffected by either antibody. Thus, the effect of the antibodies on the PAS domain is to stabilize the VSD relaxed state and in this way promote channel open probability, consistent with their effects on cardiac IKr amplitude (Harley et al., 2016). These effects on the VSD are not reflected in the conductance-voltage relationship of the ionic current, suggesting that the antibody reduces coupling of VSD movement during closure of the pore gate. Our findings support a conclusion from a previous study, done at room temperature, that the PAS domain

promotes VSD and pore gate coupling (Tan et al., 2012). Our findings also explain the mechanism of action by which manipulation of the PAS domain may provide therapeutic action in the setting of prolonged action potential duration and arrhythmia.

Thursday
1:30 – 2:00 PM ▸ **Characterization of the Curious Pores of the Fluc Fluoride Channel with the Orbit mini**

Dr. Nicholas Last, HHMI Brandeis

Abstract: The Fluc family of membrane proteins are highly selective fluoride channels with an unusual topology: they exist as antiparallel homodimers within the membrane. These channels lack a clear central aqueous pore, but instead show fluorides bound off-center to highly conserved phenylalanines. We demonstrate that these bound fluorides demark out two independent antiparallel pores, which are symmetry clones of each other due to the channel's topology. Fluoride conduction requires the presence of weakly polar interactions at the core of the protein, which can surprisingly be provided either by the canonical phenylalanine or methionine. These unexpected characteristics demonstrate the evolutionary difficulty in selectively and rapidly conducting fluoride.

Thursday
2:30 – 3:00 PM ▸ **iPSC Derived Cells in Drug Discovery and Development**

Dr. Stefan Braam, Ncardia

Abstract: With the advent of more physiological cellular tools, drug development is becoming focused more on a phenotypic approach rather than conventional target-based drug discovery. We will show several studies which utilize human induced pluripotent stem cell-derived cardiomyocytes, neurons, and novel tools within this field. These systems offer a flexible and more predictive cellular environment than, for example, immortalized cell lines.

Thursday
3:00 – 3:30 PM ▸ **Evaluation of MYH7-R403Q Patient-Derived iPSC Cardiomyocytes as an In-Vitro Model of Hypertonic Cardiomyopathy**

Scott MacDonnell, Hannah Fandl*, Ted Kaplan, Michael Dunn, Lori Morton

Cardiovascular Research TFA, Regeneron Pharmaceuticals, Inc., Tarrytown, NY.
*University of Colorado, Boulder.

Abstract: Hypertrophic cardiomyopathy (HCM) affects 1 in 500 people worldwide, leading to reduced left ventricle chamber size, impaired relaxation, reduced cardiac output, fibrosis, arrhythmia and failure. Up to 35% of HCM cases are linked to missense mutations in the *MYH7* gene. One mutation, R403Q, is associated with a high incidence of sudden cardiac death. A lack of viable cardiomyocytes to study HCM has led to the development of induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs). The purpose of this study was to profile the phenotype of iPS-CMs from a healthy adult and a patient with the MYH7-R403Q mutation. **Methods:** iPS-CMs from both a heathy(C) and MYH7-R403Q(E) patient were obtained from Cellular Dynamics International and profiled using the Nanion CardioExcyte96. Impedance measurements to assess contractile function and extracellular field potential (EFP) to evaluate action potential duration were collected. FLIPR was used to profile Ca^{2+} dynamics, gene expression was assessed using NGS, and BNP levels determined using high content imaging. **Results:** Reduced impedance amplitude ($p<0.01$) and lower intrinsic beat rate ($p<0.01$) were observed in R403Q vs. healthy. Reduced contraction rise time ($p<0.01$) and increased upstroke velocity ($p<0.01$) were observed in R403Q vs. healthy. R403Q cells demonstrated increased fall time ($p<0.01$) and beat width ($p<0.01$) vs. healthy and increased EFP duration ($p<0.01$). Increased BNP expression and longer Ca^{2+} peak decay times were observed in R403Q vs. healthy. Increased gene expression of both SERCA2A and ACTC1 were observed in R403Q vs. healthy. **Conclusions:** Distinct differences were observed between healthy and MHY7-R403Q iPS-CMs. Reduced rise time and increased

upstroke velocity suggests increased contractility while increased contraction beat width and fall time suggest impaired diastolic performance and SR Ca^{2+} uptake. Increased expression of hypertrophic genes and elevated BNP level suggest these cells are in a compensated state. These data suggest that distinct difference in iPSC from healthy and diseased patients can be observed *in-vitro* helping define mechanisms involved in HCM.

Friday
9:15 – 10:00 AM ▸ **Precision Medicine Targeting Pain: Progress Finding Treatments for specific Nav1.7 Mutations**

Dr. Mark Estacion, Yale University

Abstract: Rare genetically heritable pain syndromes have identified critical genes involved in pain. Multiple distinct pain syndromes such as inherited erythromelalgia (IEM), paroxysmal extreme pain disorder (PEPD), and congenital insensitivity to pain (CIP) have all mapped to SCN9A, the gene that encodes the voltage-gated sodium channel Nav1.7. For patients that suffer from IEM, they report warmth-induced burning pain episodes that are localized to the feet and hands with associated redness of the painful areas. Unfortunately, IEM patients are generally resistant to non-opioid drugs and are in dire need of effective therapies. Our lab has characterized a number of Nav1.7 mutations in part by creating "pain in a dish" model systems in which we can heterologously express mutant human Nav1.7 channels in acutely dissociated rodent DRG neurons and quantify excitability. Starting with the V400M mutation, which was found in a family which reported a positive response to carbamazepine (CBZ), we used molecular modeling and mutant cycle analysis to identify an IEM mutation that is spatially close and energetically coupled for activation voltage-dependence. We hypothesized that this additional mutation (S241T) may also be sensitive to CBZ. We demonstrated in vitro that the Nav1.7-S241T channel was similarly responsive to CBZ by both voltage-clamp assays and current-clamp assays. These bench results encouraged us to design and perform a clinical trial to test for a clinical response in IEM patients carrying the S241T mutation. Our trial documented many ways in which CBZ appears to be clinically effective for the S241T patients. This specific bench to bedside precision medicine success gives us hope that we will be able to use our pain in a dish assays to find effective therapies for additional IEM mutations and thus help additional IEM families.

Friday
10:00 – 10:30 AM ▸ **Antiarrhythmic Potential of Antimalarial Aminoquinolines**

Prof. Dr. Sami Noujaim, University of South Florida

Research Interests: We focus on the mechanistic understanding of cardiac normal and abnormal electrical impulse propagation. We strive to further explore, from the molecular, to the whole organ level, the determinants of cardiac excitability and arrhythmias. Along with our collaborators, our scientific investigations employ multidisciplinary approaches that include molecular and cellular biology, cellular and whole organ electrophysiology, and structural biology of ion channels, in order to investigate the mechanisms and therapeutics of cardiac pathoelectrophysiology. Our areas of scientific interest include:

1- The mechanisms of arrhythmogenesis in pathological conditions associated with intracellular Ca overload.
2- The role of the intrinsic cardiac nervous system in the generation of abnormal heart rhythm.
3- The role of the inward rectifier potassium channels in atrial and ventricular tachyarrhythmias.

Dr. Noujaim's general research interests focus on cardiac arrhythmias, with a special emphasis on the mechanisms and therapeutics of atrial fibrillation (AF), the most common arrhythmia seen in the clinic. The Noujaim laboratory at the University of South Florida, Tampa, strives to gain a better mechanistic understanding of AF in order to ultimately develop more effective therapeutics.

Friday
10:30 – 11:00 AM ▸ Finding Nav1.1 Enhancers on the SyncroPatch 384PE

Dr. Stephen Hess, Evotec AG

Content: The Nanion SyncroPatch 384PE has supported Hit-finding, Hit-to-Lead, and Lead Optimization campaigns on a variety of Ion Channel targets at Evotec. Identification of NaV opener molecules will be highlighted in the presentation.

Friday
11:00 – 11:30 PM ▸ CardioExcyte and SyncroPatch 384PE High Throughput Assessment of Functional Abnormalities of Patient-Specific iPSC-CMs Harboring Multiple Mutations

Jacqueline A. Treat, Alida R. Cooke, Robert J. Goodrow, Sarah Tahir, Tim Strassmaier, Corina T. Bot, Jonathan M. Cordeiro and Gary L. Aistrup
Masonic Medical Research Institute, Utica, NY

Abstract: Considerable cell-to-cell variability was found in our traditional (low-throughput) confocal calcium transient and cellular electrophysiology functional assessments of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from a patient presenting with repeated syncope, had several relatives implanted with ICDs, and for which genetic screening indicated mutations in RyR2, ANK2, KCNA5, ABCC9, PKP2, and HCN2 of varying predicted severity. To provide a more concise assessment of the prevalent functional manifestation of this ipPSC-CM cell-line, we employed both the CardioExcyte 96 and SynchroPatch 348PE, to conduct high-throughput extracellular field potential, impedance and patch clamp assessments. Results and interpretations will be discussed.

Friday
11:30 – 12:00 PM ▸ Applications of the SyncroPatch 384PE: Safety Pharmacology

Andrea Brüggemann[1], Sonja Stölzle-Feix[1], Claudia Haarmann[1], Alison Obergrussberger[1], Markus Rapedius[1], Tom Götze[1], Søren Friis[1,2], Nina Brinkwirth[1], Ilka Rinke-Weiß[1], Michael George[1], Tim Strassmaier[3], Rodolfo Haedo[3], Niels Fertig[1]

[1]Nanion Technologies GmbH, Ganghoferstr. 70A 80339 Munich, Germany, [2]Department of Veterinary Clinical and Animal Science, University of Copenhagen, Copenhagen, Denmark, [3]Nanion Technologies Inc., 1 Naylon Place, Livingston, NJ, 07039, USA

Abstract: Drug induced arrhythmia was one major causes for the removal of drugs from the market. In the beginning of 2002 Step2 of the S7B – ICH Guideline was approved. It described the Non-clinical Testing Strategy; the in vitro IKr and in vivo QT assay. Since then no drugs were removed from the market due to Torsades-de-Pointes.
Today mutations in at least 15 different genes are described to cause a LQT syndrome. Most of them are encoding ion channels or their auxiliary subunits. In addition there are also drugs on the market that are IKr inhibitors, but show a low Torsade risk due to an additional inhibition of inward currents like L-Type Calcium currents.
For this reason the FDA started to direct a new initiative: The Comprehensive in Vitro Proarrhythmia Assay (CIPA). This initiative is focused on proarrhythmia (not QT prolongation) to improve specificity compared to in vitro hERG and in vivo QT studies.
Here we are describing the CIPA initiative and present some first results. Details of the experimental designs will also be discussed.

Abstracts Poster Presentations:

Poster #1:

"Accurate phenotyping of extracellular field potentials in iPS-cardiomyocytes with the CardioExcyte96"

Andrew Glazer[1], Joe-Elie Salem[1], Shan Parikh[1], Nikhil Chavali[1], Laura Short[1], Marcia Blair[1], Lynn Hall[1], Bjorn Knollmann[1], and Dan Roden[1,2,3]

Departments of Medicine[1], Pharmacology[2], and Bioinformatics[3], Vanderbilt University Medical Center

Induced pluriopotent stem cell-derived cardiomyocytes (iPS-CMs) are a promising model for assessing drug response and modeling cardiac disease. High-throughput tools such as the Nanion CardioExcyte96 can measure extracellular field potentials (EFPs) in order to characterize depolarization and repolarization dynamics of iPS-CMs. However, accurate and consistent phenotyping methods for characterizing EFPs are necessary. Here we present EFP Analyzer, an interactive online tool for analysis of EFP traces. We used the tool to analyze over 900 EFP traces from 13 control lines on the CardioExcyte96. We analyzed spontaneously beating iPS-CMs, as well as iPS-CMs treated with AAV-channelrhodopsin, which enabled optical pacing. A wide variation in EFP morphology was observed between and within lines. We present data and guidelines for which morphologies result in high-quality, reproducible data. With proper guidelines for selecting "high-quality" EFP traces, Field Potential Duration can accurately measured in patient-derived lines, including measurements of drugs and mutations that affect action potential duration. We also demonstrate a "tangent method" for assessing Field Potential Duration, analogous to a similar method used for measuring the QT interval in ECGs. The EFP Analyzer tool and the phenotyping guidelines presented here represent progress towards a broader use of iPS-CMs as a high-quality model of adult cardiomyocytes.

Poster #2:

"High Throughput 384-Well Anisotropic, Physiologically-Relevant hiPSC-Cardiomyocyte Cultures Enable More Resolution Over Profiling Compounds With Known Cardiotoxic Mechanisms Of Action."

R. Contu, R. Padilla, W. Si, S. Spangenberg , B. Van Hese, A. Fanton, A. Witty F. Zanella

Stemionix Inc.

Drug removal from the clinical market, as well as late-stage failures in clinical trials, are often linked to unforeseen cardiac toxicity. hiPSC-CMs are an integral component of a new paradigm, the Comprehensive in vitro Proarrhythmia Assay (CiPA) Initiative, through which panels of compounds with known mechanism of cardiotoxicity are being evaluated in hiPSC-CM platforms across independent test sites and through cutting-edge technologies. Key challenges under consideration for the hiPSC-CM system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization, and electro-physiological maturity. Bioengineering approaches developed to enhance hiPSC-CM maturity have shown improvements in aspects of hiPSC-CM physiology, however those approaches have limited scalability and thus are not amenable to high throughput screening. hiPSC-CMs cultures plated on a high throughput platform which passively promote cardiomyocyte alignment have been shown to display physiologically-relevant features, including more physiological cellular geometry, coherent unidirectional contraction, cardiac cell junction re-modeling, and improved calcium handling. To evaluate whether the changes induced by this platform translated into differential responses to cardio-active compounds, high throughput calcium flux assays were performed on hiPSC-CMs cultured in standard high throughput screening cell cultureware or anisotropic 384-well plates and subsequently interrogated with the 28 compounds included in the CiPA initiative. Interestingly, differential responses were observed in nearly 60% of the compounds tested. Specifically, compounds in the high risk category showed a dose-dependent

progression in the severity of the pro-arrhythmic phenotypes in anisotropy. This was associated with a higher severity of early afterdepolarizations (EADs). Six out of eleven compounds in the intermediate risk category showed a more sensitive response in anisotropy. No EADs were observed in either control or anisotropic conditions treated with low risk compounds. Altogether, anisotropic high throughput hiPSC-CM cultures formatted in the platform employed in this study showed better resolution over the progression and severity of pro-arrhythmic events.

Poster #3:

"High Throughput Investigation of Contractile and Electrophysiological Properties of Optically Stimulated hiPSC-CM Monolayers."

Shan Parikh[1], Nikhil Chavali[1], Andrew Glazer[2], Christian Shaffer[1], Marcia Blair[3], Dan Roden[3], Bjorn Knollmann[2].

[1]Vanderbilt University, Nashville, TN, USA, [2]Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA, [3]Vanderbilt University Medical Center, Nashville, TN, USA.

The accelerated utilization of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) for drug screening and disease modeling requires adaptation of high-throughput technology for more efficient assessment. Multiple technologies have emerged including single/multi-electrode array for extracellular field potential (EFP) detection, and voltage sensitive dyes and automated patch-clamp for action potential measurement. EFP assessment to date is predominantly performed on spontaneously beating hiPSC-CM, however, as rate significantly influences EFP morphology at baseline and in response to pharmacological stimulation, our work focused on developing the use of optical pacing in academically generated patient specific hiPSC-CM. In this study, we provide a physiological and pharmacological assessment of optically stimulated hiPSC-CM using the CardioExcyte96 (Nanion), which allows for rapid 96-well evaluation of action potential and contractility correlates. HiPSC-CM were transduced with an adeno-associated virus (AAV) encoding a non-selective cation channel (channelrhodopsin2) and seeded on a matrigel coated sensor plate for ten days. An efficient workflow with criteria for the generation of high quality hiPSC-CMs, data conversion tools, and criteria for EFP and IMP analysis were developed. Using this workflow, our study provides a detailed characterization of the use of AAV-ChR2 infected hiPSC-CM for measuring field potential duration (a correlate of action potential duration) and impedance (contractility correlate). Optical pacing increased reliability of waveform detection, enhanced synchronization of cardiomyocyte depolarization, and allowed for frequency dependent assessment. High-throughput assessment of EFP and IMP using optical stimulation will greatly enhance our ability to model cardiomyopathy and drug response using hiPSC-CM.

Poster #4:

"High Throughput Ion Channel Screening and Cardiotoxicity Investigations in iCell Cardiomyocytes2 and Neurons."

Corina T. Bot[2], Sonja Stölzle-Feix[1], Nadine Becker[1], Krisztina Juhasz[1], Claudia Haarmann[1], Alison Obergrussberger[1], Tom Götze[1], Sarah Tahir[2], Leo Doerr[1], Markus Rapedius[1], Matthias Beckler[1], Michael George[1], Andrea Brüggemann[1], Rodolfo J. Haedo[2], Niels Fertig[1]

[1]Nanion Technologies, Ganghoferstraße 70a - D-80339 Munich, Germany,
[2]Nanion Technologies Inc., 1 Naylon Place, Livingston, NJ, 07039, USA

iCell® neurons and iCell® Cardiomyocytes2 have proven to represent a relevant human in vitro system for modeling and interrogating complex biological processes, phenotypic profiles and disease models. We present automated patch clamping recordings in both iCell® Cardiomyocytes2 and iCell® neurons using Nanion's Patchliner system. Recordings were performed in both voltage clamp and current clamp modes, and confirmed manual patch clamping results. Furthermore, ligand-gated ion channel responses in iCell®

neurons will be presented. In light of the new Comprehensive in Vitro Proarrhythmia Assay (CiPA), a FDA directed initiative to improve guidelines and standardize assays and protocols, the use of hiPSC-CMs may become critical in determining the proarrhythmic risk of potential drug candidates. The CardioExcyte 96 is a hybrid screening instrument that combines impedance with MEA-like extracellular field potential (EFP) recordings. In accordance with the CiPA guidelines, we present pharmacological investigations of short- and long-term effects of compounds in iCell® Cardiomyocytes2. This approach strengthens the importance of testing compounds in assays complementary to patch clamp electrophysiology, to provide a more complete safety profile.

Poster #5:

"Introducing Stimulated IK1 into Human iPSC-cardiomyocytes Using Dynamic Clamp on an Automated Patch Clamp Setup"

Corina Bot[1], Nadine Becker[2] , Birgit Goversen[3], Sonja Stoelzle-Feix[2], Alison Obergrussberger[2] , Toon A.B. van Veen[3] , Niels Fertig[2], Teun P. de Boer[3].

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Dynamic clamp is a powerful tool involving injection of real-time simulated membrane currents into patch clamped cells. This technique has been employed in conventional patch clamp electrophysiology to introduce inward rectifier I_{K1} current into human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). I_{K1} is expressed at low levels in these cells. For this reason, hiPSC-CMs display a more depolarized membrane potential than adult cardiomyocytes, limiting their use in safety pharmacology. Therefore, introducing simulated IK1 into hiPSCCMs may improve maturity of these cells and ensure that they represent a viable alternative to the scarcely available dissociated adult human cardiomyocytes. Indeed, they are attractive cells types because of their unlimited availability and human origin. In this study, we combined dynamic clamp with an automated patch clamp platform to demonstrate that IK1 conductance can be added to hiPSC-CMs on this platform. Our results show that IK1 can be successfully added to hiPSC-CMs to up to 4 cells simultaneously and that this results in a more stabilized and hyperpolarized resting membrane potential. Action potential (AP) shape also changes when I_{K1} is added. We have used with different amounts of IK1 (100-2000 pS/pF) and show that increasing I_{K1} results in AP shortening and an acceleration of the upstroke. We could measure native Ba^{2+}-sensitive I_{K1} in voltage clamp mode in approximately 50% of these cells, but I_{K1} was small, on average 1.98±0.42 pA/pF (mean ± SEM). Adding a Ca^{2+} channel activator (BayK 8644), or blocker (nifedipine) caused an increase and decrease of the AP duration, respectively. In conclusion, combining dynamic clamp with automated patch clamping results in an enhanced, medium-throughput platform for safety pharmacology.

Poster #6:

"High Throughput Automated Patch Clamping and Cardiotoxicity Investigations For Cardiac Safety: CiPA Compound Effects in Cardiomyocytes"

Corina T. Bot[1], Sonja Stölzle-Feix[2], Krisztina Juhasz[2], Leo Dörr[2], Matthias Beckler[2], Claudia Haarmann[2], Alison Obergrussberger[2], Markus Rapedius[2], Tom Götze[2], Marius Vogel[2], Michael George[2], Andrea Brüggemann[2], Rodolfo Haedo[1], Niels Fertig[2]

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Drug induced arrhythmia is one of the most common causes of drug development failure. Human induced pluripotent stem cell-derived cardiomyocytes (iPSCMs) show great promise for cardiovascular research and predictive in-vitro cardiac safety screening. In the light of the new Comprehensive in Vitro Proarrhythmia Assay (CiPA), a FDA directed initiative to improve guidelines and standardize assays and protocols, the use of hiPSC-CMs may become critical in determining the proarrhythmic risk of potential drug candidates. Human stem cell-derived cardiomyocytes (hIPSC-CMs) have recently proven to recapitulate

key features of human cardiac electrophysiology in vitro. Patch clamp assays, the gold standard of ion channel research, are distinguished by high complexity. Conventional patch clamp is technically demanding and is unsuitable for highthroughput screening (HTS) experiments. Chip-based approaches allow parallel patch clamp recordings without compromising data quality or technical sophistication. We present highthroughput voltage and current clamp recordings of CiPA reference compounds. Since drug efficacies may vary with temperature, we present recordings at room and at physiological temperatures. In addition to patch-clamping experiments, we present hybrid impedance (cell contractility) with MEA-like extracellular field potential (EFP) recordings. Pharmacological effects of a number of CiPA reference compounds including those designated high risk(e.g. Dofetilide), intermediate risk (e.g. Cisapride) or low risk (e.g. Verapamil) were evaluated and will be presented. Experiments were complemented with optical stimulation of monolayers of hiPSC-CMs expressing the lightgated cation channel Channelrhodopsin2. This approach allows frequency-dependent drug screening and detection of potential side effects on Na+-, Ca2+- and repolarizing K+ channels. Furthermore, investigations of potential breaks in excitation-contraction coupling can accompany the ion channel screening with the final aim to enable a reliable automatized cardiac toxicity screening platform.

Poster #7:

"Automated Patch Clamp studies of Ca activated Cl channel TMEM16A (ANO1) using the SynchroPatch384PE"

Nina Brinkwirth[1], Søren Friis[1], Tom Goetze[1], Markus Rapedius[1], James Costantin[2], Andrea Brüggemann[1], Michael George[1], Niels Fertig[1].

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TMEM16A/Anoctamin1 and TRPC5 are ion channels activated by elevated cytosolic calcium concentrations and as both fulfill central physiological functions their electrophysiological characterization is of great interest in the pharmaceutical industry.
As a calcium-activated chloride channel, TMEM16A has a broad functional spectrum in processes like trans-epithelial ion transport, olfaction, photo-transduction, smooth muscle contraction, nociception, cell proliferation and control of neuronal excitability. TRPC5 on the other hand is a calcium-permeable cation channel predominantly expressed in neuronal cells, but also in the kidney and the cardiovascular system. It plays an important role in calcium flux and its biological function ranges from neurotransmission and control of axon guidance to vascular smooth muscle cell migration and contractility. Changing the intracellular calcium concentration to initiate channel activity during the course of a patch clamp experiment is usually challenging and therefore often done in inside-out Patches. The current amplitude can limit the use of this method as well as run down that often is more pronounced in excised patches.
Here we present data of whole cell measurements where the intracellular solution is exchanged in a very robust manner. At the same time the extracellular solution is still accessible for compound application. The 384-well format of the patch clamp platform allows measurements in a highly parallel manner. This allows the measurement of your target cell lines and controls to be performed in one run. The data shown will summarize our results on Calcium sensitivity and pharmacological modulation.

Poster #8:

"EAAT3 Investigated Using SSM-Based Electrophysiology"

Maria Barthmes, Andre Bazzone, Stephan Holzhauser, Michael George, Niels Fertig, Andrea Brüggemann

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The excitatory amino acid transporter 3 (EAAT3) is involved in the neuronal re-uptake of glutamate and plays a central role in the regulation of excitatory neurotransmission and synaptic plasticity. EAAT3 also transports cysteine, necessary for the synthesis of glutathione and GABA. EAAT3 is expressed not only throughout the brain, but in many organs such as intestines, liver and heart. Here it seems to provide the

main pathway of aspartate. Several connections of EAAT3 to severe neuronal disorders like epilepsy and schizophrenia have been described, as well as to metabolic disturbances concerning in the maintenance of aspartate and cysteine levels. This makes EAAT3 not only an important target for functional research, but also a potential drug target. Here we introduce a novel assay on EAAC1, a mouse homologue of EAAT3, using SSM (solid supported membrane)-based electrophysiology. SSM-based electrophysiology is a label-free electrical measuring method with very high sensitivity which enables the resolution of low turnover transport and even binding-events. Using the purified membrane of EAAC1 expressing CHO cells, we were able to determine substrate affinities and their interaction and to compare the effect of six known inhibitors directly with each other. We evaluated the assay stability and success rate. Furthermore, we were able to resolve substrate binding and to confirm the described anion conductance of the transporter. Implementing SSM-based electrophysiology we were able to generate an efficient, robust and very flexible assay with a good throughout, which is an ideal tool for the biophysical and pharmacological characterization of EAAC1 and even suitable for drug screening approaches.

Poster #9:

"Investigations into idiosyncratic drug induced hepatotoxicity and chronic proliferation of cancer cells using a label free method"

Corina T. Bot[2], Sonja Stölzle-Feix[1], Elena Dragicevic[1], Krisztina Juhasz[1], Leo Doerr[1], Matthias Beckler[1], Michael George[1], Andrea Brüggemann[1], Rodolfo Haedo[2], Niels Fertig[1]

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Hepatic toxicity has accounted for 15 of the 47 drugs withdrawn from the market in the last two decades. More specifically, Drug Induced Liver Injury (DILI) is the major cause of acute liver failure in the USA and Europe and is one of the main reasons for regulatory actions. DILI is classified as intrinsic (or dose-dependent) or as idiosyncratic. A prominent example of idiosyncrasy is acetaminophen (paracetamol), with a variable time of onset and not directly dependent on dose. We present a non-invasive DILI assay approach based on impedance measurements in monocyte-derived hepatocyte-like (MH) cells from MetaHeps®. Although improvements have been made to cellular and animal models to predict intrinsic (dose-dependent) DILI, it is almost impossible to predict idiosyncratic DILI. MH cells have been developed as a tool to investigate long-term hepatotoxicity, metabolism and drug interactions. Furthermore, patient-derived MH cells could provide a tool for diagnosis or exclusion of idiosyncratic DILI, and provide the causative agent in polymedicated patients. MH cells were used on a 96-well screening system that monitors changes in impedance (or cell monolayer resistance). Once the monolayer is exposed to a cytotoxic agent, the impedance changes and measures of toxicity can be quantified long-term. Cells are monitored under physiological conditions for temperature, humidity and CO_2. We investigated the hepatotoxic effects of paracetamol on MH cells when exposed for 24 and 48 hours. In agreement with other standard toxicity assays, such as the lactate dehydrogenase release assay, low doses of paracetamol caused transient toxicity and 'adaptation' was observed. At higher doses, hepatotoxic effects of paracetamol could be reversed upon washout after 24 hours, but continued exposure caused increased hepatotoxicity. In addition to hepatotoxicity, another validation of the principle is shown for chronic proliferation of cancer cells. Traditional cell proliferation assays involve labeling cells of interest with compounds that become reduced in the environment of metabolically active cells, or by incubating cells with radioactive labels. In this study, murine mammary carcinoma cells (H8N8 and H8N8 T3.2) were used and changes in impedance, and therefore confluency, were used as a measure of toxicity. Intrinsic (dose-dependent) effects of the standard clinical treatment regimen cyclophosphamide, doxorubicin and 5-fluouracil could be identified consistent with other methods of live cell analysis systems. Therefore, the utilized 96-well impedance system in combination with murine mammary carcinoma cells provides a novel tool for investigating therapy resistance of cancer cells in vitro.

Thank you for attending our User Meeting!

Rodolfo Haedo
Senior Vice President USA

George Okeyo,
Product Manager USA

Tim Strassmaier,
Senior Application Scientist USA

James Costantin
Director of Customer Relations USA

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Your Nanion Team

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