<table>
<thead>
<tr>
<th>Event</th>
<th>Ncardia Workshop</th>
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<tr>
<td>Presenter</td>
<td>Tessa de Korte, MSc</td>
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<tr>
<td>Date</td>
<td>December 1, 2017</td>
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<td><strong>Ncardia’s hiPSC-CMs and applications</strong></td>
<td><strong>Assessment of functional toxicity</strong></td>
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Ncardia’s hiPSC-CMs and assays
Human iPSC-derived cardiomyocytes

Fully functional human iPSC-derived cardiomyocytes
• obtained through *in vitro* differentiation of transgenic hiPSCs and puromycin selection technology
• obtained without genetically modification or purification/selection procedures
✓ Showing the essential human physiological conditions and pharmacological responses (3R’s benefit)

Pluricyte® Cardiomyocyte Kit
Cor.4U® Cardiomyocyte Kit
CardioPlate™

Alpha actin (green)
Myosin heavy chain 7 (red)
Supported applications for Pluricyte® and Cor.4U® Cardiomyocytes

MEA:
- MCS 96
- Axion Maestro
  - Classic 48 & 96, E-Stim 48, Lumos 48

MEA/Impedance:
- xCELLigence Cardio 96
- xCELLigence CardioECR 48
- CardioExcyte 96

Calcium-flux:
- FDSS/µCELL 96 & 384
- FLIPR Tetra 96 & 384

Biomarker assays:
- Pro-BNP
- ATP
- Troponin release
- Metabolite biomarkers
Assessment of functional toxicity

MEA assays
Assessment of functional toxicity of Pluricyte® Cardiomyocytes using MEA technology

**Functional toxicity:** To predict the potential for drug-induced ECG abnormalities.

Alterations in the **sodium-spike amplitude**
*Axion Maestro system*

![Graph showing Na⁺ Channel block]

Alterations in **FPD** and occurrence of **arrhythmia**
*CardioECR instrument – ECR*

![Graph showing ECG changes with drug concentrations]

**Chronotropic and inotropic effects**
*CardioECR instrument – Impedance*

![Graph showing heart rate and contractility changes with Isoproterenol and E4031]
3 independent testing sites confirmed the predictivity of Cor.4U® Cardiomyocytes as a human cellular model.

Electrophysiological analysis of Cor.4U® on the MEA System

<table>
<thead>
<tr>
<th>Beat period</th>
<th>FPD</th>
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<tr>
<td><strong>Site 1</strong></td>
<td><img src="Image1" alt="Graph" /></td>
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<td><strong>Site 2</strong></td>
<td><img src="Image2" alt="Graph" /></td>
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<tr>
<td><strong>Site 3</strong></td>
<td><img src="Image3" alt="Graph" /></td>
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Pharmacological analysis of Cor.4U® on the MEA System

Comparison of different testing sites per compound

![Graphs](Images)
Assessment of structural toxicity

Troponin I Release assays
Assessment of structural toxicity of Pluricyte® Cardiomyocytes using Troponin I release assays

**Structural toxicity**: morphological damage to cardiomyocytes, changes to intracellular organelles, or loss of cardiomyocyte viability
Troponin I release assay

- The troponin complex consists of three subunits: troponin C, troponin I, and troponin T, located on the myofibrillar thin (actin) filament of striated (skeletal and cardiac) muscle

- In some diseases (e.g. pulmonary embolism), transient leakage of cytosolic TnI into the blood stream has been reported.

- During myocardial infarction which results in serious myocyte damage rapid release of TnI into the blood steam occur and can last up to 10-21 days

- The same holds true in case of cardiotoxicity of drugs e.g in chemotherapy

- Therefore, cTnI detection in blood is an important diagnostic marker for several cardiovascular diseases
Cardio-oncology: the intersection of cardiovascular disease and cancer

- Incidence of drug-induced structural cardiotoxicity → associated with the use of anthracycline anti-cancer drugs, but has also been shown to occur following treatment with the new generation of targeted anti-cancer agents: tyrosine-kinase inhibitors.

- Prognosis for many cancers improving + aging of the population → increased the focus on the cardiovascular risks of cancer patients (Sanjeev A. et al, 2015, Albini et al, 2010).

- Cardiotoxicity associated with anticancer drugs:
  - Direct cytotoxic effects and associated cardiac systolic dysfunction
  - Left ventricular (LV) dysfunction
  - Cardiac ischemia
  - QT-prolongation
  - Thrombosis
  - Metabolic abnormalities
  - Arrhythmias
  - Pericarditis
  - Repolarization abnormalities
cTnI release assay to assess potential structural toxicity of TKI’s

Setup:
- Specimen → Pluricyte® Cardiomyocytes on Matrigel coated plates (384 W plates)
- Compounds → lapatinib, sunitinib and staurosporine (10, 5, 2.5, 1, 25, 0.6 and 0.1 µM)
- Harvest → 16 and 24 hr post-treatment
- Readout → cTnI release assay

Timeline:

D0 → D1 → D3 → D6 → D7 (16hr and 24 hr)

Seed PCs in PCM → Med ref → Med ref → Med ref + Cpd addition → Harvest sup + Lyse cells → cTnI release assay
Sunitinib and staurosporine induce structural toxicity in Pluricyte® Cardiomyocytes after long-term incubation
4

Multiplex assays

Functional and structural toxicity assessment
Multiplexing cTnI release assay with CardioECR assay (Impedance and ECR) to assess potential structural and functional toxicity

Setup:
- Specimen → Pluricyte® Cardiomyocytes on Fibronectin coated E-plates (48 W plates)
- Compounds → Ponatinib, doxorubicin, lapatinib, nilotinib (0.3, 3 and 10 µM) and nitrendipine (0.01, 0.1 and 1 µM)
- Harvest → Acute, 1, 16, 24, 40, 48 and 64 hr post-treatment
- Readout → cTnI release and CardioECR (Impedance and ECR)

Timeline:
- D0: Seed PCs in PCM
- D1/D3/D5: Med ref
- D8: Cpd addition
- Harvest sup
- D11 (64hr)
- D10 (40hr and 48hr)
- D9 (16hr and 24hr)
- cTnI release assay
Multiplexing Impedance/MEA and Troponin I release

**CI over time**
- Ponatinib
- Doxorubicin
- Nitrendipine

**Troponin I release**
- Ponatinib
- Doxorubicin
- Nitrendipine

**Impedance amplitude**
- Ponatinib
- Doxorubicin
- Nitrendipine

Cl/Impedance peak amplitude
- well-connected monolayer of cells → High impedance
- Disturbed cell monolayer → deattachment and cell loss → Low impedance

**Cl/Impedance over time**
e.g. drug addition
Multiplexing Impedance/MEA and Troponin I release

CI over time

Troponin I release

Impedance and ECR

Lapatinib

0 µM lapatinib

0 µM Nilotinib

Nilotinib

10 µM Nilotinib

10 µM lapatinib

Counts

Counts
Concluding remarks

- Troponin I Immunoassay allows us to detect small amount of cTnI (around 10 pg/ml) using small sample volume (5 µl)
- 48-384W formats

- It is possible to perform kinetic measurement of cTnI release in a 384W format and in combination with other functional assays
- Multiplexing the cTnI release with impedance and MEA technology leads to better prediction of drug-induced cardiotoxicity
- Short term vs long term, structural vs functional toxicity could be assessed simultaneously from a single well
Safety Services

• Integrated cardiovascular services to enhance the assessment of cardiac safety and toxicity of drug candidates.
• Based on fully functional and validated hiPSC-derived cardiomyocytes that are manufactured under strict quality assurance.
  ➢ Cardio.Acute
  ➢ Cardio.Long
  ➢ **Cardio.Plex**
  ➢ Cardio.Effect
  ➢ Cardio.Force
  ➢ Cardio.Flux
  ➢ **Cardio.Tox Troponin I**
  ➢ Customized Project Service

Benefits of Ncardia’s safety services:
✓ **High quality** – our safety pharmacology experts perform the studies on validated platforms
✓ **Save time** - no assay optimization and data analysis needed – let our experts do this for you
✓ **Reliable** – assays are based on fully functional and validated hiPSC-derived cardiomyocytes
✓ **Versatile** – we offer a broad range of optimized assays on various platforms
✓ **Flexible** – we will make sure to meet your specific needs

www.Ncardia.com
Thank you!