Introduction
Cardiac liability remains a major cause of drug withdrawal. Today, testing is mainly focused on NERG screening (biochemical or non-cardiac/cytotoxic cell based assays) and costly in vivo tests. Predictive in vitro electrophysiology as well as cellular toxicity assays are missing due to lack of relevant cell models.

We have used mouse ESC-derived Cor.AT® cardiomyocytes in a set of 4 assays to comprehensively assess cardiac liability:

- Changes in electrophysiological properties (Microelectrode Array, MEA);
- Beatting frequency and rhythmicity (cELЛИgence RTCA 96w Cardio instrument);
- Cardiomyocyte specific cytotoxicity (NtU test);
- Mitochondrial impairment (MitoxPress kit).

Results

- In the MFA (Fig. 1), 59 compounds (7 with dual activities) were tested; all positive and negative compounds were identified correctly. Moreover, the assay provides insight into the MOA of a compound.
- 24 compounds were tested in the RTCA Cardio instrument (Fig. 2), resulting in 1 false-negative due to species specific differences.
- In the NtU test (Fig. 3), 46 compounds (21 positive, 25 negative, all blinded) were tested on cardiomyocytes and mouse fibroblasts as control. Data evaluation revealed 2 false positives and 2 false negatives, resulting in >90% predictivity.
- 5 compounds (2 positive, 3 negative) were tested in the MitoxPress test (Fig. 4); all of them were identified correctly.

Fig. 1: Micro-Electrode Array (MEA) analysis
For the MFA measurements, ESC-derived Cor.AT® cardiomyocytes were thawed and pre-cultured for 2-3 days on fibronectin coated cell culture flasks. Cells were then dissociated and seeded in a 24 well plate containing 2.4 µl wells on the fibronectin-coated cell culture area of a MEA (Multichannel Systems, Reutlingen, Germany) subsequently covered with 1 ml of Cor.AT® culture medium. Measurements were performed after overnight culture. After baseline measurement, cumulative dosing was performed by replacing half of the medium with a 5x concentrated compound solution (0.1% DMSO in and buffer). Each dose was run for 15 s and 3 measurements were performed within each treatment period, followed by a washout period.

Fig. 2: Analysis of beating pattern in the RTCA Cardio device
For measurements in the RTCA Cardio device, ESC-derived Cor.AT® cardiomyocytes were thawed and cultured on fibronectin-coated 96 plate Cor.AT® culture medium, and mouse fibroblasts as control. Data evaluation revealed 2 false positives and 2 false negatives, resulting in >90% predictivity.

Fig. 3: Detection of cardiomyocyte-specific cytotoxicity
For the Cor.AT® Tox test, ESC-derived Cor.AT® cardiomyocytes were thawed and seeded at 3x10^3 cells per well on fibronectin coated 96 well (25µl/pit) plates. The human embryonic fibroblasts (MEF) on non-specific control were seeded at 5x10^3 cells per well at same conditions. Cells were pre-cultured for 3 days in Cor.AT® culture medium, and MEF medium was changed after 24h. For the MitoXPress assay, ESC-derived Cor.AT® cardiomyocytes and MEF were thawed and pre-cultured for 2-3 days on fibronectin coated E-Plates Cardio 96 (Roche, Penzberg, Germany) at a density of 4x10^4 cells/well. After 1,000,000 CFU were added per well, and medium was changed after 24h. For measurements in the RTCA Cardio instrument, ESC-derived Cor.AT® cardiomyocytes were thawed and cultured on fibronectin-coated 96 plate Cor.AT® culture medium, and mouse fibroblasts as control. Data evaluation revealed 2 false positives and 2 false negatives, resulting in >90% predictivity.

Fig. 4: Detection of mitochondrial impairment
For the MitoxPress assay, ESC-derived Cor.AT® cardiomyocytes were thawed and seeded at 3x10^3 cells per well on fibronectin coated 96 plate Cor.AT® culture medium, and MEF medium was changed after 24h. For the MitoXPress assay, ESC-derived Cor.AT® cardiomyocytes and MEF were thawed and pre-cultured for 2-3 days on fibronectin coated E-Plates Cardio 96 (Roche, Penzberg, Germany) at a density of 4x10^4 cells/well. After 1,000,000 CFU were added per well, and medium was changed after 24h. For measurements in the RTCA Cardio instrument, ESC-derived Cor.AT® cardiomyocytes were thawed and cultured on fibronectin-coated 96 plate Cor.AT® culture medium, and mouse fibroblasts as control. Data evaluation revealed 2 false positives and 2 false negatives, resulting in >90% predictivity.

In summary, our results strongly indicate that ESC-derived Cor.AT® cardiomyocytes in combination with advanced methodology are a predictive and convenient tool for early assessment of cardiac liability.