Cell based high throughput assay to evaluate cardiovascular safety profile of newly synthesized compounds to be nominated for clinical development.

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Introduction

The optimal cell based lineage for cardiac-regeneration still remains a mystery. Here we have developed a novel cell based assay to screen novel synthetic compounds for cardiovascular safety and regenerative profiles for clinical development. The human cardiomyocytes were isolated from adult ventricular and atrial appendages, characterized, and electrically profiled for functional physiological cardiac evaluations. The electrophysiology of adult human cardiomyocyte consists of 4X4 array of platinum microelectrodes and electronic measurement system based on a 1mV rms noise level amplifier and 16 channel 16bit data acquisition card. This High throughput ElectroPhysiological Assay (HEPA) cardiac chip technology was developed by Biopico Systems Inc., for characterization of the human cardiomyocytes electrophysiology functional index for cardiovascular safety and regenerative profiles. Prior to utilizing the human cardiomyocytes they were tested positive with Real time PCR, IHC and Western Blot Analysis for the following biomarkers: ckit, Actinin, ANP, Connexin 43, Desmin, KDR, Nkx2.5, GATA-binding protein 4 (GATA4) and SERCA2. The in-vitro assay procedure was established to determine cardiovascular safety and regenerative index of newly identified led molecules. This in-vitro cell based cardiac assay enabled us to determine the cardiac safety value help us determine the molecule to be a potential candidate for clinical development.

Methods:

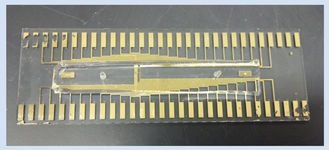
In-vitro Study:
The Human Cardiomyocytes ventricular derived (36044-15VT) was cultured at temperature and humidity controlled 37 ^{0}C incubator at 5% carbon dioxide. The cardiomyocytes were cultured in M36044-15VTS media and pre-coated ECM [E36044-15-T25] T25 flasks, and sucultured once in the same media and ECM. At the first passage the cells were transferred to the cardiac chip, with approximately 10,000 cells in a total volume of 100ul, the cells were cultured for 24 hours, and the drugs to be tested were incubated for 1hour, 2 hours and 3 hours. The same experiment was conducted with patch clamp studies and the results were compared to the data obtained from Biopico System.
Prior to utilizing the cells for the experiment the human cardiomyocytes were characterized by Flow, IHC, Real Time PCR, and western blot analysis for the following markers: cKit, Actinin, Connexin 43, Desmin, KDR, Nkx2.5, GATA-4 and SERCA2.

In-vivo study: We tested an anti-tumor drug CEP1430 for pancreatic cancer at the following doses: 10, 15 and 20 mg/kg once daily oral administration in SCID mice pancreatic cancer model established at Celprogen. There were 11 groups of 6 mice per group, two mice per dose, one female and one male mouse. The control group only received 1X PBS carrier vehicle, whereas, the experimental groups received once daily oral gavage [10,15& 20 mg/kg]. After 20 days post gavage administration, the study was terminated and the mice were sacrificed and their major organs were surgically removed for histological examination. At the end of the study the mice were sacrificed and the organ[heart, Lungs, Kidneys, Brain, Liver, Pancreas] tissues were sectioned into three compartment; 1. One section was fixed and H&E stained, 2. One section was cultured into monolayer and IHC studies and flow studies with various Biomarkers was performed, 3. One section was stored liquid nitrogen for genomic DNA and total RNA analysis for Real-time PCR.

Results:

The results are indicated in the figures and graphs below:

A



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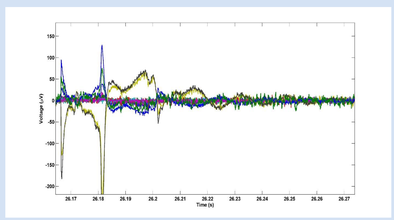

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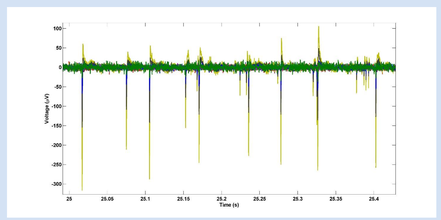


Figure 1: A. Cardiac Chip. B. Electrophysiology recording with drug incubation. C. Electrophysiological Recording with drug incubation.

Figure 2. A. Human cardiomyocyte –cardiac sphere, B. H&E staining of 3D Cardiac culture. C. Image of Cardiac cell in the cardiac chip prior to recording

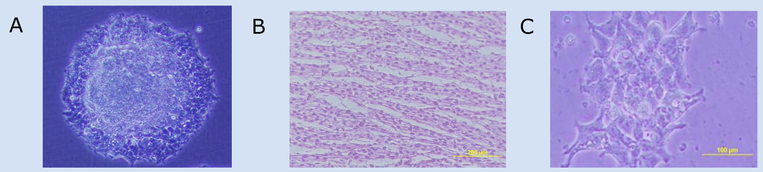
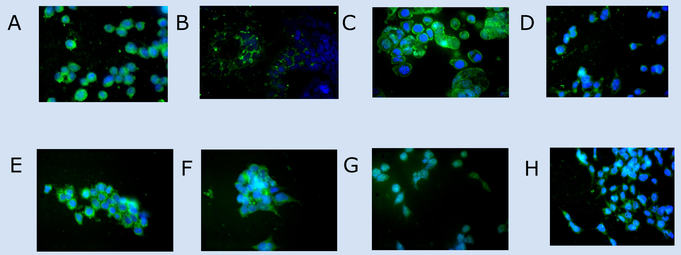


Figure 3 A. H. Cardiomyocyte IHC profile with FITC and BrdU. A. Actin FITC green, BrdU nuclear Blue. B. SERCA2 FITC green, BrdU nuclear blue. C. Connexin 43 FITC green, BrdU nuclear Blue. D. Troponin I FITC green, BrdU nuclear Blue. E. Tropomyosin FITC green, BrdU nuclear Blue. F. Desmin FITC green, BrdU nuclear Blue. G. cKit FITC green, BrdU nuclear blue. H. NKX2.5 FITC green, BrdU nuclear Blue



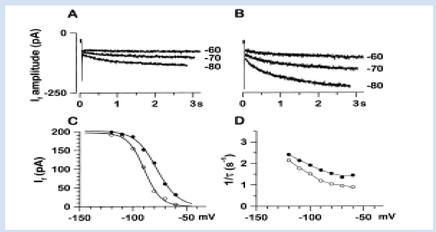


Figure 4. Human Cardiomyocyte Calcium ion Channel 36044-15VT cells, M36044-15S media and E36044-15-PD15mm

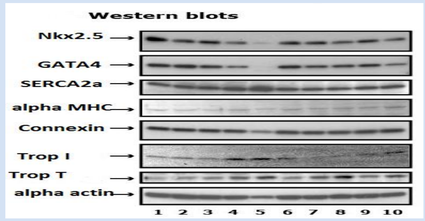


Figure 7. Western blot analysis of cardiac markers for cardiac cells 36044-15VT For 10 passages.

Figure 3: Comparison of treated and untreated human cardiac 3D culture for evaluation of lead compound CEP1430. A. Control, B. 24hours Incubation with CEP1430, C. 48 hours incubation with CEP1430, at concentration 1 uM. The cell density of Cardiac cells increases with incubation time, as indicated in the images when compared to the controls. D. Proliferation index.

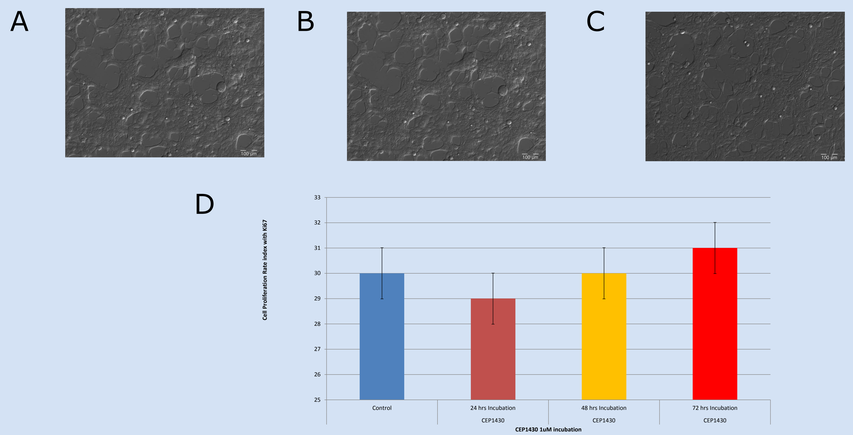
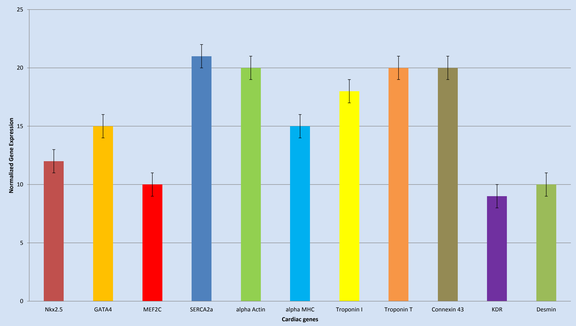


Figure 6: Gene expression profile of Cardiac Cells with CEP1430 treatment, The gene expression profile of Cardiac cells revealed high gene expression of Nkx2.5, GATA4, MEF2C, SERCA2a, alpha MHC, alpha Actin, Troponin I, Troponin T, Connexin 43, KDR and Desmin.



Conclusions:

In this study we tested CEP1430, a novel anti-tumor agent targeting pancreatic cancer stem cells for cardiovascular safety; our lead molecule had no adverse cardiovascular liability. In-vivo daily administration of CEP1430 for 20 days had no cardiovascular adverse effect. From this study we were able to conclude that we have identified a lead compound for targeting pancreatic cancer stem cells that has no adverse cardiovascular effect when monitored for 20 days with once daily administration of the compound by intraperitoneal injections. This in-vitro cell based cardiac assay enabled us to determine the cardiac safety value help us determine the molecule to be a potential candidate for clinical development. The electrophysiological data along with the gene expression data indicated that our compound had no adverse cardiac event.

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