Evaluating the utility of Human iPS Cell Derived Cardiomyocytes for Drug Toxicity Testing

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Background: During the recent years, pharmaceutical industry has been struggling with the costly withdrawals of a number of drugs from the market due to toxicity issues, often due to cardio toxicity. Present cell culture models based on cancer cell lines, can not predict toxicity response in specific normal tissue type. Animal models have an important role for detecting adverse effects of compounds, but these models are costly and associated with ethical issues.

Need: Failure to detect cardio toxic side effects of new drug before clinical launch can put patient on high health risks. As a result, there is an increasing demand for more relevant and predictive *in vitro* screening models for cardio toxicity testing.

Methodology/Principal Findings: Functional cardiomyocytes derived from pluripotent stem cells presents new opportunity to detect cardiotoxicity *in vitro*. Here, we use impendence measurement tool to show that cardiomyocytes grown *in vitro* maintain a uniform physiological state for up to 2-3 weeks in culture giving a window for drug testing. Additionally, the cardioactive drug (-Doxorubicin) affects the beating frequency and contractility of cardiomyocytes in a dose dependant way, and the results were compatible with empirical results in the clinic. The dose of 150nM of doxorubicin demonstrated prolonged arrhythmias with no significant cellular toxicity.

Conclusion: This system of interrogating human cardiac function *in vitro* opens new opportunity for predicting cardiac toxicity.

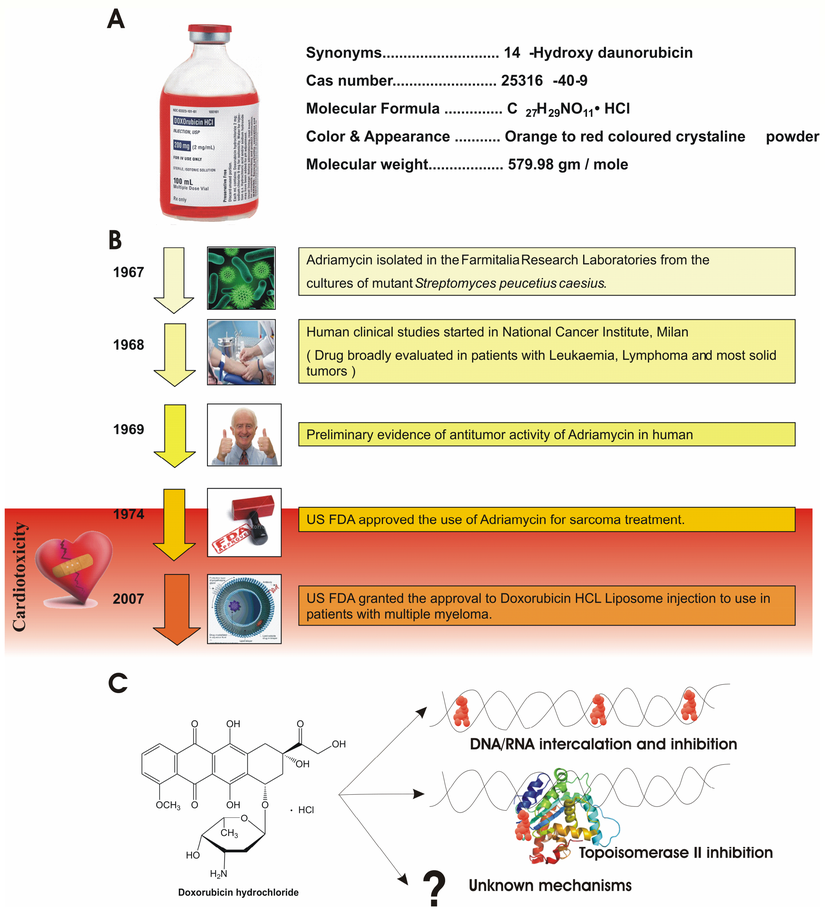

Figure 1: Drug selection strategy. Doxorubicin is a chemotherapeutic drug of choice for treatment of several solid tumors and disseminates cancers. The drug is used from past 3 decades and has resulted in increased survival in several human cancers. Doxorubicin is also a drug of choice for combination therapies. The most serious side effect of doxorubicin is life-threatening heart damage. The acute exposure of doxorubicin is shown to be associated with arrhythmias, and in severe cases cardiomyopathy. Doxorubicin cardio toxicity is characterized by a dose-dependant decline in mitochondrial oxidative phosphorylation. The 'red-devil' drug, although known as toxicant is still in use for chemotherapeutic use. The usual regimen in human use to be a dose of 60mg/m^2, and past few years the dose is reduced to 20-30mg/m^2. The wide use of doxorubicin and the year long history of cardio-toxicity make it a right model for testing it in a *in-vitro* model of cardio-toxicity. A) Basic of doxorubicin. B) Brief history of the drug and C) the mechanism of action of doxorubicin. The drug is known for its DNA intercalation and thus inhibiting the DNA synthesis, additionally, it is known to inhibit Topoisomerase-II.

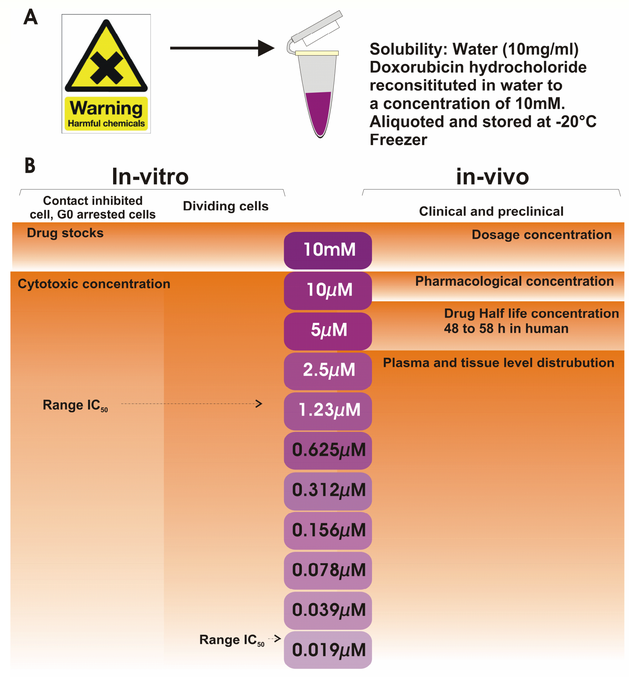

Figure 2: Preparation of drug. A) Doxorubicin is available as doxorubicin hydrochloride and is highly soluble in water. Doxorubicin stocks are prepared in water to achieve a final concentration of 10mmol/litre, and stored in deep freezers for a maximum period of 1year. B) Serial dilutions are prepared in culture medium just before the compound addition. Rational for selecting the dose is presented. A wide range of 2fold dilution was selected for testing. The drug has a half life of 48 to 58 hours and upto 2μM levels of drug concentration are reached in plasma, and lower concentration in tissues. *In-vitro*, doxorubicin is highly cytotoxic to dividing cells such as cancer cells and embryonic stem cells. However, non dividing cells are nearly one log value resistant. C) the drug is highly cytotoxic and should be handled with care. The liquid waste/spills are treated as hazarduos waste and disposed appropriately.

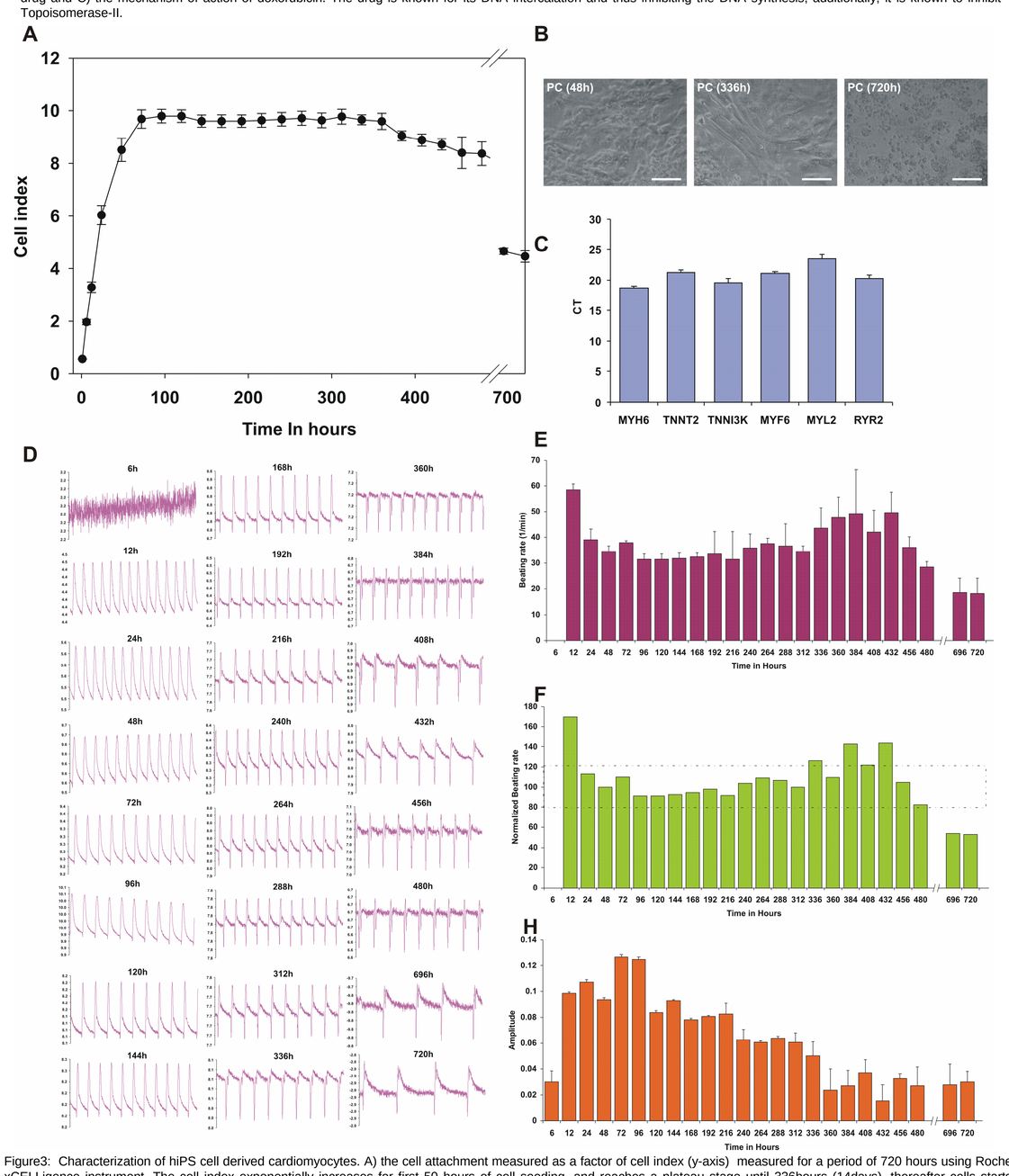

Figure3: Characterization of hiPS cell derived cardiomyocytes. A) the cell attachment measured as a factor of cell index (y-axis) measured for a period of 720 hours using Roche xCELLigence instrument. The cell index exponentially increases for first 50 hours of cell seeding, and reaches a plateau stage until 336hours (14days), thereafter cells starts detaching and eventually the cell-index drops significantly by 720hours (30day). This data suggests a testing-window for pharmacological substances for *in-vitro* tox-testing platform. An estimated time of 14 days can be used as a period of repeated dose testing strategy that can avoid noise-signal ratio of the test system. B) phase-contrast cell pictures taken on 48h, 336h and 720h. Cells are observed to slog off the surface during later time points, Scale bar corresponds to 100μ. C) Cardiac specific markers tested using real-time PCR. D, E, F, G) Data showing the beating rate/frequencies/amplitude measured using xCELLigence. The data is presented for every 24hours after plating of cells on e-plates. Note the initial (6h) cells do not show the characterized beating pattern and are in acclimatization phase. By 12h beating is normalized, but higher in beating rate. Beating rate and frequency is regular on and after 48h up to 384h(15day) and is in fidelity limits (20% plus and minus, shown as bracket in panel F) thereafter the irregular beating is observed, that also suggest a definitive testing period for cardio toxicity testing platform. The numerical data presented are mean+-standard deviation.

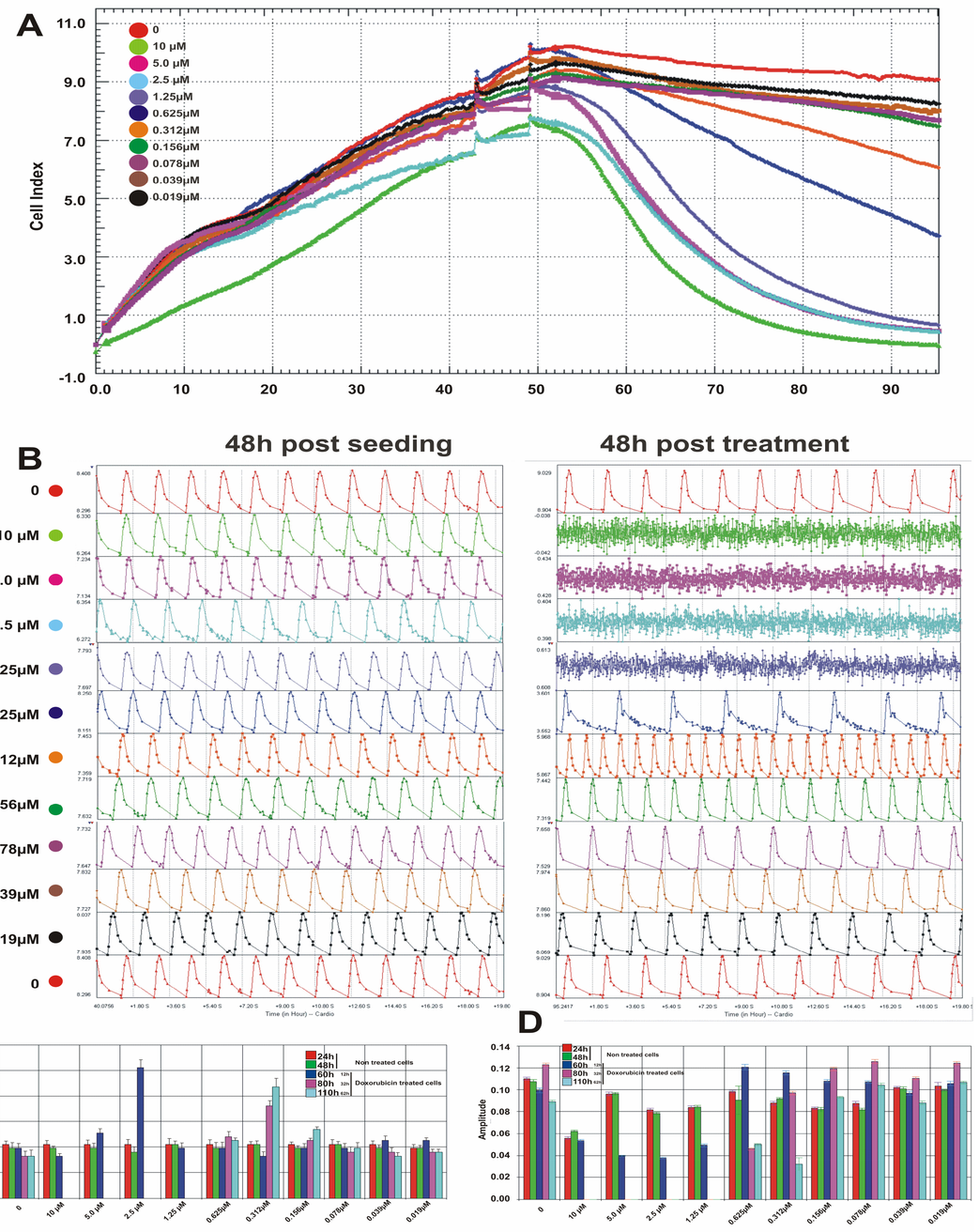

Figure4: Dose selection for repeated dose study. A) the cell index measurement on xCELLignece of cardiomyocytes. The scoring was performed for upto 100h post cell seeding. Doxorubicin at various concentration was introduced after 48h. Cell index is a measurement of cell attachment/proliferation/death. Higher concentration of drug (10, 5, and 2.5 μM) are observed to be cytotoxic and instantly kill cells within hours of introduction (<12h). Middle range concentration 1.25 and 0.625 μM) shows delayed cytotoxicty (>40h). However the lower concentrations (<0.3μM and lower) cells resists and shows not significant cell death. For a long term repeated dose study such concentrations should be suitable. Additionally, the beating rate is seen to be significantly (B,C, D) increased by a dose of 0.3 and 0.156μM in time-dependant manner. The beating rate is highest after 48hours of treatment at this concentration, and this time is also the half life of drug in human patient.