Cardiotoxicity Evaluation of Chronic BMS-986094 Exposure in Human iPSC-Derived Cardiomyocytes Using a Microelectrode Array (MEA) Assay.

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Abstract
BMS-986094 (INX-08189) was developed as a prodrug of a guanosine nucleotide analogue developed to treat Hepatitis C Virus (HCV). It was discontinued in Phase 3 clinical trials due to cardiotoxicity with 1 death and 8 patients hospitalized with significantly reduced left ventricular ejection fraction (LVEF). Further analysis has shown cardiotoxic effects in 14 of 34 patients where evaluation of the ECGs of patients with LV dysfunction showed depression, T-wave inversions, or loss of T-wave amplitude. Mitochondrial effects through inhibition of the mitochondrial RNA polymerase have been reported to be the mechanism for the toxicity. Retrospective animal studies with high doses of compound have shown recapitulation of some of the (2EF and EG) effects. Here, we explored HPSIC derived cardiomyocytes to BMS-986094 for 14 days and assessed their electrophysiological function using the MEA assay. In addition, we compared the results to those obtained from a cardiac mitochondrial protein assay and a cardiac cytotoxicity assay. We showed that although mitochondrial biogenesis and cytotoxicity can be detected with standard assays, the MEA assay is more sensitive to electrophysiological changes at 80nM and significant electrocardiographic functional effects at 80nM. At the 80nM concentration, BMS-986094 caused an increase in Nhr peak amplitude and a almost doubling of the beat rate. There was also a decrease in the T-wave amplitude and a reproducible depression in what would correspond to the ST region of the ECG in all of the electrodes of the MEA traces at this concentration. Notably, the MEA trace mimics the unique ST depression and T-wave amplitude effects consistent with observations in patients who had cardiotoxicity in the clinical trial. These results suggest that the cardiotoxicity from BMS-986094 is not related to the mitochondrial toxicity and likely related to a yet undetermined cumulative mechanism. This study shows that the use of stem cell derived cardiomyocytes in long term physiological based assays can improve the prediction of cardiac liabilities.

Introduction
Late stage failures of drugs in clinical trials have significant costs as well as significant safety risks to patients.
Identification of liabilities early will save money and allow for prioritization of better compounds.
BMS-986094 (INX-08189) was developed as a prodrug of a guanosine nucleotide analogue developed to treat Hepatitis C Virus (HCV). It was discontinued in Phase 3 clinical trials due to cardiotoxicity with 1 death and 8 patients hospitalized with significantly reduced left ventricular ejection fraction (LVEF). These effects were observed after taking the 300mg dose. Overall there was a 40% residence of cardiotoxicity. Can we identify this risk using IPSC derived human cardiomyocytes? What timepoints should be tested? Do the compound have no immediate effects but the toxicity was observed after extended use? IPSC cardiomyocytes can remain healthy for at least two weeks plated on an MEA-plate. Due to the fact that it measures electrical activity without addition of any reagents, extended multi-timepoint assays can be run. A Multi-timepoint 14 day assay was then run to determine the timescourse of the toxicity observed with BMS-986094

The Instruments
All recordings were acquired on the Asion-Maxinos platform using 48 well configured MEA plates. The Asion Ecentri was used to deliver pre-mixed CO₂ through the recordings. A Constant temperature of 37°C was maintained through the software controller.

Methods
- 48 wells MEA plates were pre-coated with 5μl of fibrinogen directly over the electrode grid and incubated at 37°C one hour before plating cells. Alternatively the cells were also seeded at a 96 well and 384 well fibrinogen coated plate.
- CxR cardiomyocytes were then reseeded in CxR cardiomyocyte plating medium and plated in 5μl at a density of 50,000 viable cells per well. They were seeded at a density of 40,000 cells per well for a 384 well plate and 100,000 cells per well for a 24 well plate.
- The cells were incubated, humidiﬁed at 37°C in 5% CO₂ for 6 days.
- 100% of the medium was changed every 2 days.
- After 6 days, medium was removed and medium spiked with compound was added to the 96 well and 384 well plates.
- Compounds were serially diluted in DMEM at 5000 the concentrations to be tested. Compound is diluted 50 fold in an intermediate plate followed by addition of compound in medium from the intermediate plate at a 10 fold dilution into the MEA plates. (See Graph)
- MEA recordings were acquired before compound treatment (baseline) and after dosing (1 hour). Readings were also taken after 2 days, 5 days, 10 days, 12 days, and 14 days. Medium was changed every two to three days.
- The cellular mtogenesis assay was run on day 8 after treatment with compound. Medium was changed every two to three days.
- The calcium flux assay was run after 14 days of treatment with compound. Medium was changed every two to three days. Cells were loaded with the Codex calcium dye for 45 minutes and then read in the Hamamatsu FDS55Cell.

Results: MEA Results

Results: Calcium Effects

Results: Mitochondrial Biogenesis Staining

Conclusions
The use of MEA and CxR IPSC derived cardiomyocytes identified a chronic liability that exists for INX-08189.
The compound caused a loss of beating at 10, 2, and 0.4μM concentrations by 14 days. This was a chronic issue as there was no effect from compound at lower concentrations.
There was an effect observed even at 80nM. The cells beat rate increased substantially and the Na amplitude increased significantly. This occurred on two separate plates.
The compound caused cytotoxicity as well as electrophysiological effects as demonstrated by ATP levels after completion of the 14 day MEA assay.
The calcium flux measurements basically confirmed what was found in the MEA assay with loss at the top two concentrations and very low calcium flux at the 0.4μM level. The beat rate for the 80nM concentration was recapitulated in this assay.
There have been reports of effects on mitochondrial biogenesis for INX-08189. Our data show an upregulation of mitochondrial coiled protein at 1μM concentration and below. Based on timing we observe and the expression of the mitochondrial proteins, it is likely this is unrelated to the toxicity observed.
The data here suggest the value of a chronic in vitro assay to determine cardiac safety. The use of multiple assay platforms and multiple timepoints would result in a significantly improved safety profile for compounds brought forward into animal and human clinical trials. This would also result in a significant savings for companies.

References