Use of a chronic multiplatform assay to evaluate cardiotoxicity of BMS-986094 in iPSC derived human cardiomyocytes

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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 cardiac toxicity 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 ST depressions, 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 been shown to recapitulate some of the LVEF and ECG effects. Here, we exposed hiPSC derived cardiomyocytes to BMS-986094 for 14-days and assessed their electrophysiologic function using a MEA assay. In addition, we compared these results to those obtained from a cardiac mitochondrial protein biogenesis assay, calcium flux assay, and a cardiac cytotoxicity assay. We show that the MEA assay is more sensitive at lower concentrations, with a complete loss of electrical activity at > 80nM and significant electrophysiological functional effects at 80nM. These effects were time dependent with many of the progressive effects taking place after 10 days of treatment. At the 80nM concentration, BMS-986094 caused a 20% increase in Na+ peak amplitude and a doubling of the beat rate. There was also a decrease in the T-wave amplitude at earlier timepoints before a loss in beating was observed. Notably, the MEA trace mimics the T-wave amplitude effects consistent with observations in patients who had cardiotoxicity in the clinical trial. We also show that the calcium flux effects mimic the effects seen in the MEA assay. Interestingly, although it has been suggested that mitochondrial biogenesis was blocked with this compound, we observe an increase in mitochondrial coded protein using a High content assay with immunofluorescent staining . 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 risk 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).
- BMS-986094 was discontinued in Phase 3 clinical trials due to cardiac toxicity with 1 death and 8 patients hospitalized with significantly reduced left ventricular ejection fraction (LVEF). These effects were observed after taking the 200mg dose. Overall there was a 40% incidence of cardiotoxicity
- Can we identify this risk using iPSC derived human cardiomyocytes?
- What timepoints should be tested?
- The compound did not have 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 timecourse of the toxicity observed with BMS-986094

Methods

- 48-well MEA plates were pre-coated with 5µl of fibronectin directly over the electrode grid and incubated at 37°C one hour before plating cells. Alternatively the cells were also seeded in a 96 well and 384 well fibronectin coated plate.
- iCell2 cardiomyocytes were then resuspended in CDI Cardiomyocyte plating medium and dot 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 96 well plate and 10,000 cells per well for a 384 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 DMSO at 500X the concentrations to be tested. Compound is diluted 50 fold in an intermediate plate followed by dilution of compound in medium from the intermediate plate at a 10 fold dilution into the MEA plate (500 fold dilution).
- 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 mitobiogenesis assay was run on day 8 after treatment with compound. Medium was changed every two to three days. Images were acquired on an Arrayscan VTI.
- 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 FDSS/µCell.

Results: MEA Results

A

Test Article	Time (hr)	Test Conc. (µM)	% of Vehicle: Average Beat Period	Average Na+ Slope	Average Na+ Amplitude	Average FPD
INX-08189	1	10	106 ± 5.4%	78 ± 10.8%	80 ± 11.7%	105 ± 6.2%
		2	106 ± 2.7%	112 ± 9.6%	111 ± 7.3%	107 ± 5.5%
		0.4	105 ± 1.8%	91 ± 23.1%	93 ± 26.2%	99 ± 8.5%
		0.08	106 ± 3.4%	122 ± 18.9%	129 ± 16.1%	105 ± 5.3%
		0.016	106 ± 4.6%	115 ± 16.7%	115 ± 16.3%	107 ± 5.9%
		MEC (µM)	NA	10	10	NA
	120	10	ND	ND	ND	ND
		2	100 ± 4.8%	57 ± 26.0%	41 ± 24.3%	102 ± 8.0%
		0.4	85 ± 2.0%	93 ± 35.1%	96 ± 36.0%	82 ± 10.6%
		0.08	88 ± 3.2%	185 ± 47.5%	203 ± 60.5%	86 ± 8.3%
		0.016	96 ± 3.3%	121 ± 50.7%	125 ± 53.1%	92 ± 4.7%
		MEC (µM)	10	0.08	0.08	10
	288	10	ND	ND	ND	ND
		2	ND	ND	ND	ND
		0.4	70 ± 5.1%	33 ± 13.1%	7 ± 4.6%	ND
		0.08	66 ± 3.0%	130 ± 19.5%	138 ± 24.2%	72 ± 6.5%
		0.016	91 ± 6.4%	107 ± 9.9%	107 ± 12.2%	89 ± 6.9%
		MEC (µM)	0.08	0.08	0.08	0.08
	336	10	ND	ND	ND	ND
		2	ND	ND	ND	ND
		0.4	ND	ND	ND	ND
		0.08	62 ± 5.5%	128 ± 11.7%	136 ± 19.1%	68 ± 7.0%
		0.016	93 ± 6.9%	108 ± 2.9%	110 ± 3.1%	90 ± 5.5%
		MEC (µM)	0.08	0.08	0.08	0.08

B

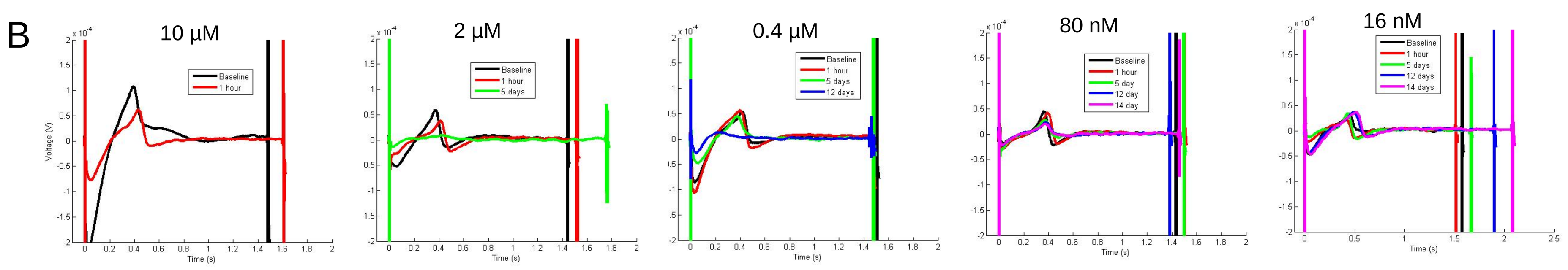
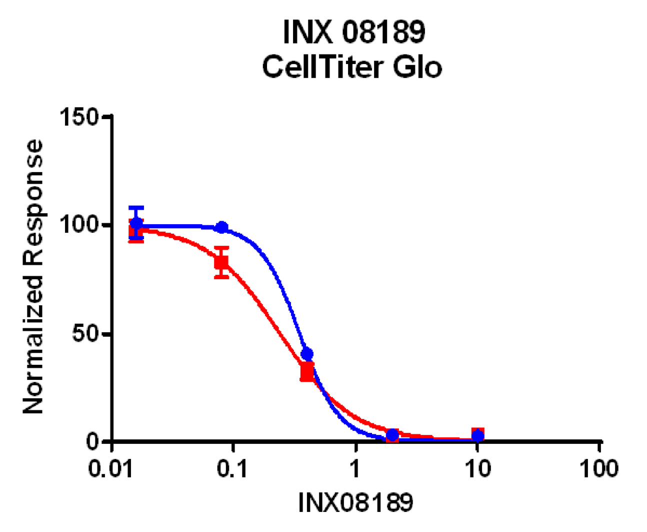


Figure 3. MEA data for INX-08189. A. Cells were treated with compound for 14 days with fresh medium and drug added every 3 days. The experiment consisted of a 5 point dose curve with 4 replicates at each dose. The 4 replicates consisted of 2 replicates on 2 separate plates. MEA measurements were taken at multiple timepoints during the 14 day experiment. All data is reported as a percent of the baseline corrected to the vehicle controls. The correction to the vehicle controls allows the experiment to correct for any maturation changes or condition differences that may exist over the 2 week long experiment. The table shows the changes that occur over the dose curve over the time course of the experiment. As the experiment progresses to the later time points, the lower concentrations continue to become more effected and stop beating. At 14 days, even the 80nM concentration has a significant change in the beat rate as well a reproducible and elevated increase in the Na amplitude. B. Traces of the different drug concentrations over time show the changes in the beat length as well as the overall change in the amplitude of the T-wave at the different doses and later time points.

Results:



	Experiment 1	Experiment 2
EC50	0.3469	0.2394

Figure 1. Cytotoxicity of MEA experiment. After completion of the 14 days assay, some medium was removed and CellTiter Glo was added to test for ATP levels. The compound was tested in two separate plates in 5 point dose curve (duplicate in each). The results show that the compound behaved comparably in the two plates with an IC50 of 0.35 and 0.24µM.

Results: Calcium Effects

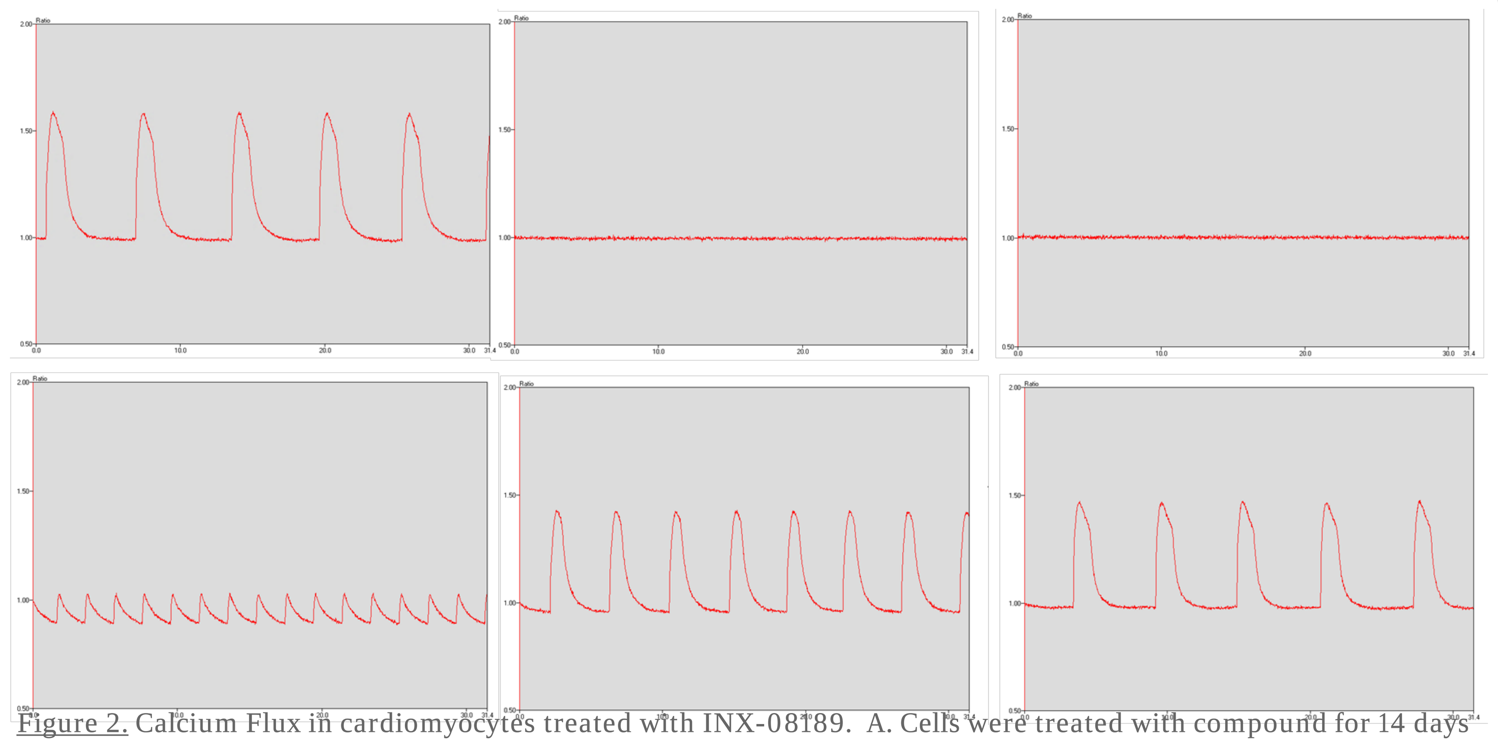


Figure 2. Calcium Flux in cardiomyocytes treated with INX-08189. A. Cells were treated with compound for 14 days with fresh medium and drug added every 3 days. The experiment consisted of a 5 point dose curve with 3 replicates at each dose. Calcium measurements were taken on a Hamamatsu FDSS/µCell. Images show the calcium flux measurements over time. The DMSO shows the normal cells with 0.2% DMSO. AT he highest concentrations, there is no beating and therefore no calcium flux. At 0.4 µM, the calcium levels are very low and the beating is very rapid. At 80nM, the beats are still rapid and at 16nM, they appear to return to normal levels as compared to DMSO.

Results: Mitochondrial Biogenesis Staining

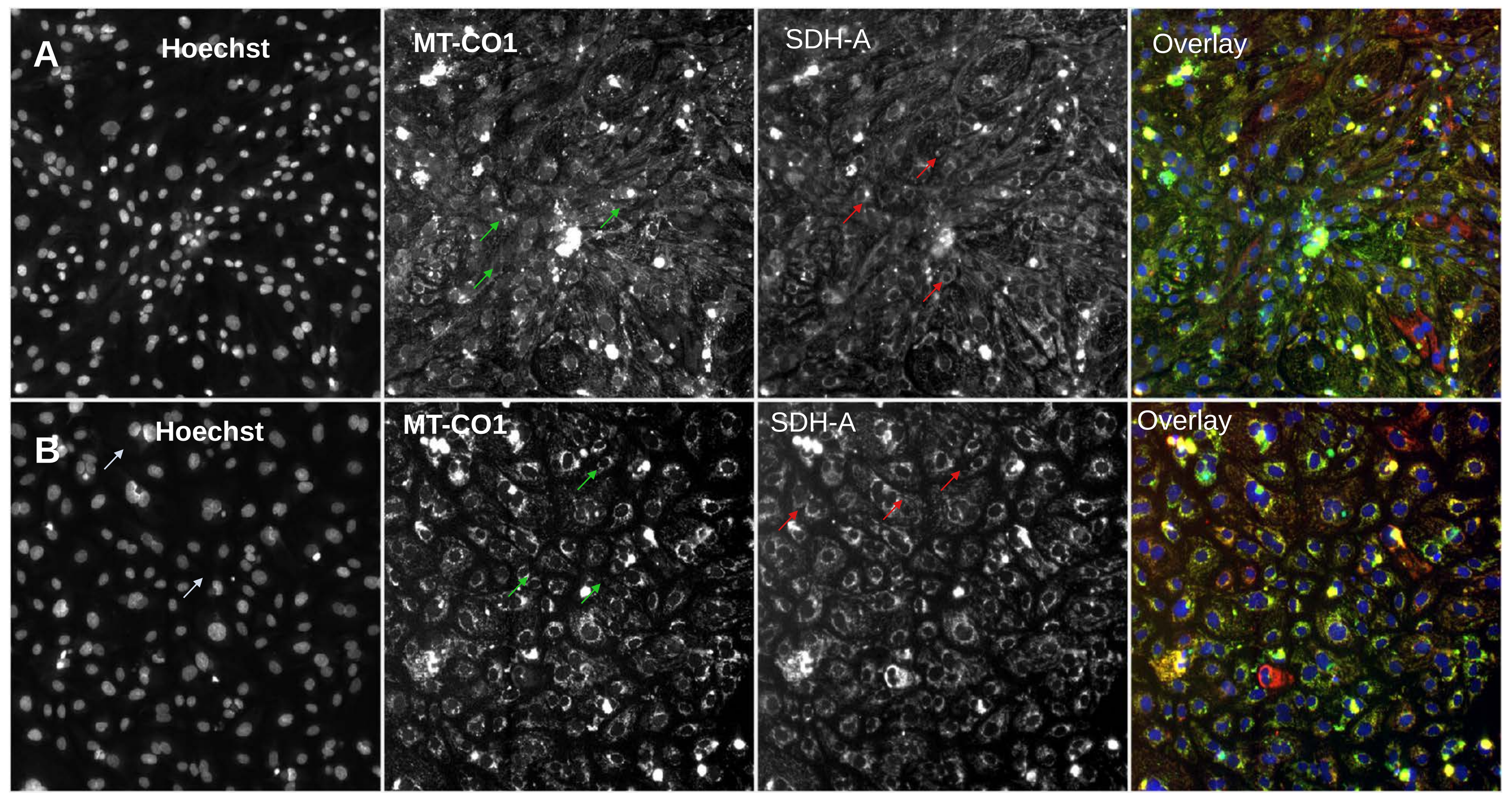


Figure 4. Mitochondrial Biogenesis in IPSC cartdiomyocytes treated with INX-08189. Cells were treated with drug or vehicle (0.2% DMSO) for 8 days. Cell were then fixed and stained with Hoechst, the mitochondrial coded protein subunit I of Complex IV (COX-I) (MT-CO1) and the nuclear coded mitochondrial protein SDH-A. Cells were then read in a Arrayscan VTI. A. Individual images of DMSO vehicle treated cells immunofluorescently stained with antibody listed. The first panel is Hoechst stained cardiomyocytes. The MT-CO1 stained image has arrows pointing to the mitochondrial localized staining which is the mitochondrial coded protein. The SDH-A stained image shows blue arrows pointing to the mitochondrial localized staining of the nuclear coded protein. B. Individual images of 1.25µM INX-08189 treated IPSC cardiomyocytes for 8 days stained with the antibody listed. The first panel is Hoechst stained nuclei. Green arrows point to the large abnormal nuclei that are present in these cells. The MT-CO1 stained image has arrows pointing to the mitochondrial localized staining which is the mitochondrial coded protein. It is obvious that this protein is actually expressed at higher levels than in the DMSO treated wells. The SDH-A stained image shows blue arrows pointing to the mitochondrial localized staining of the nuclear coded protein. This also looks upregulated. The overlay shows the colocalization of each. This data suggests that the compound does not block expression of the mitochondrial coded proteins and actually may cuase the cell to respond buy upregulating mitochondrial protein.

Conclusions

- Use of the MEA and CDI 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 this compound at 1 hour.
- 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 level calcium flux at the 0.4 µM 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 coded protein at 1 µM concentration and below. Based on timing we observe and the expression of the mitochondrial proteins, it is likely that 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

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