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Prediction of Liabilities with Chronic Drug Treatment in iPSC Derived Cardiomyocytes using an MEA Platform

Christopher Strock, PhD

In vitro Toxicology & Safety Assessment

Causes of drug failure in development and in the clinic

- **Toxicity remains a leading source of attrition in Drug Dx both pre-clinically but also in clinical development**
- **The industry has responded to this failure by developing early *in vitro* screen to minimize liabilities.**
- **Cyprotex has been working over the last 7 years developing *in vitro* models to address tissue specific liabilities**

Phase	Preclinical	Preclinical	Phase I-III	Phase I-III	Post Approval
Information	Causes of attrition	Causes of attrition	Causes of attrition	Causes of attrition	Withdrawl
Source	ABPI (2008)	Car (2006)	ABPI (2008)	Olson et al. (2000)	Stevens & Baker (2008)
Sample size	155 CD	88 CD	63 CD	82 CD	47 drugs
Cardiovascular	25%	27%	35%	21%	45%
Hepatotoxicity	15%	8%	29%	21%	32%
CNS	12%	14%	2%	21%	2%
Immunotox	7%	7%	10%	11%	2%
GI	5%	3%	2%	5%	2%
Reprotox	9%	13%	5%	1%	2%
Renal	6%	2%	5%	9%	0%
Carnogenicity	0%	3%	3%	0%	0%

Adapted fro, Redfern WS et al. SOT Poster 1081 and provided by Tim Hammond

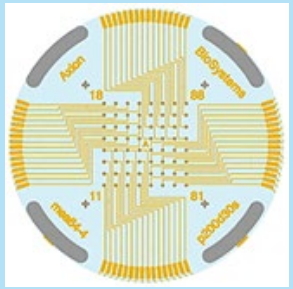
Introduction

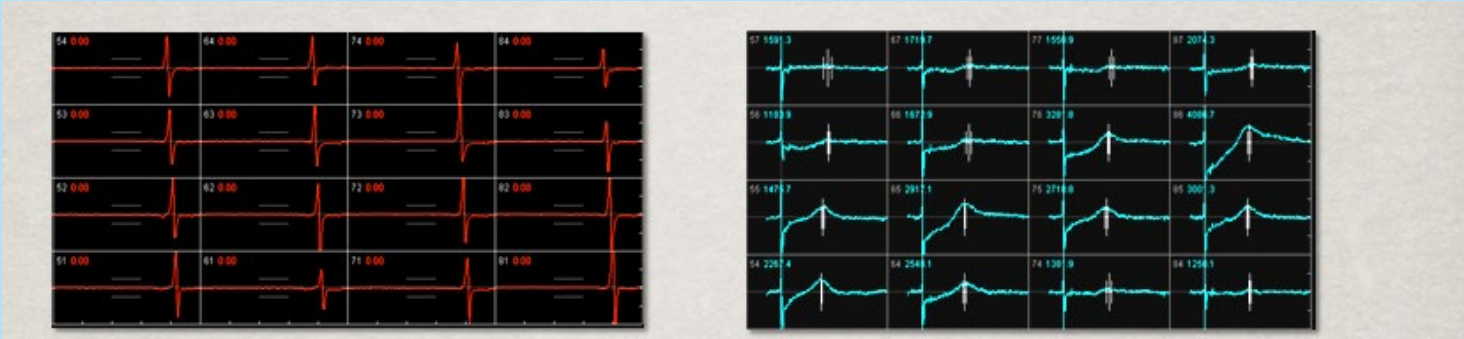
- Late stage failures of drugs in clinical trials have significant costs as well as significant safety risk to patients.
- Identification of liabilities early will save money and allow for prioritization of better compounds.
- Identification of ion channel liabilities will only identify specific acute liabilities for cardiac risk
- Telemerized dog studies are expensive and limit the amount of compounds against a target that can be screened. In vitro testing allows for multiple compounds and scaffolds to be tested to identify a safe compound at affordable prices.
- Other than animal studies, there is no effective way to identify chronic dosing effects
- iPSC cardiomyocytes can remain healthy for more than 2 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.

MEA Based Cardiotoxicity Assay

- MEA measure the electrical activity of entire cells and networks of cells
- Allows measurements of overall cardiac function as well as beat rate, sodium amplitude and QT conduction velocity

Instrument	Maestro 48-well MEA system (Axion BioSystems)
Cell Type	Human iPS cell-derived iCell® cardiomyocytes (Cellular Dynamics International) plated and allowed to fully mature and beat synchronously
Assay Details	Five concentrations in duplicate (dependent on customer requirements) Single time point Additional time points and washout (optional)
Data Delivery	Beat rate and number Field potential duration Amplitude Conduction velocity (optional)





MEA Based Cardiotoxicity

Physiological based toxicity

- MEA can be used to predict toxicity through sub-cytotoxic mechanisms which are the main causes of cardiac liability
  - Electrophysiologic effects driven by ion channel and calcium handling mechanisms
    - Identify hERG liabilities, both direct and trafficking
    - Identify other cardiac ion channel effects
    - Identify liabilities caused by calcium handling. e.g. sofosbuvir and amiodarone causing bradycardia
    - Identify arrhythmias and other liabilities from unknown causes
    - Identify responses to cardiac GPCRs
    - All of these are acute 1 hour responses
- Can run long term experiments
    - Assays can be run over days to weeks because no additional dyes or reagents need to be added
    - Identify delayed responses such as delayed arrhythmias or decreases in Na amplitude over time

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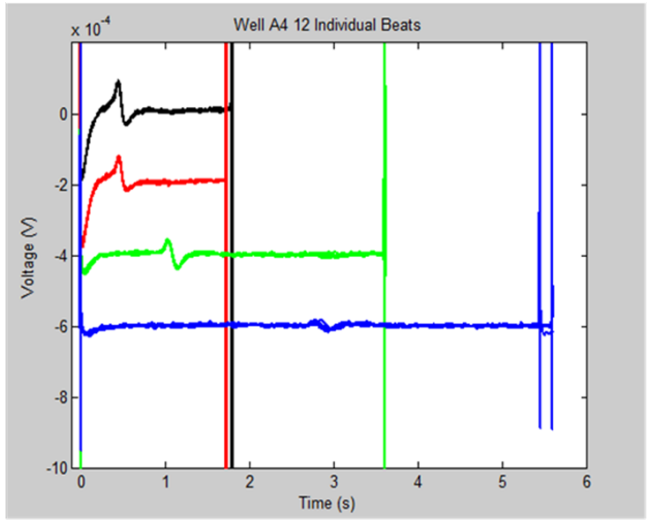
Compounds Tested

- Pentamidine – Known hERG trafficker
- Vanoxerine – Multichannel blocker failed for Torsades in clinical trial
- BMS-986094 (INX-08189) – Hepatitis C drug that caused death in clinical trial after multiple weeks
- Compound X – Caused loss of Na amplitude and slope after 24 hours with arrhythmias
- Compound Y – Caused delayed arrhythmias after 6 days with delay in repolarization but no hERG effect

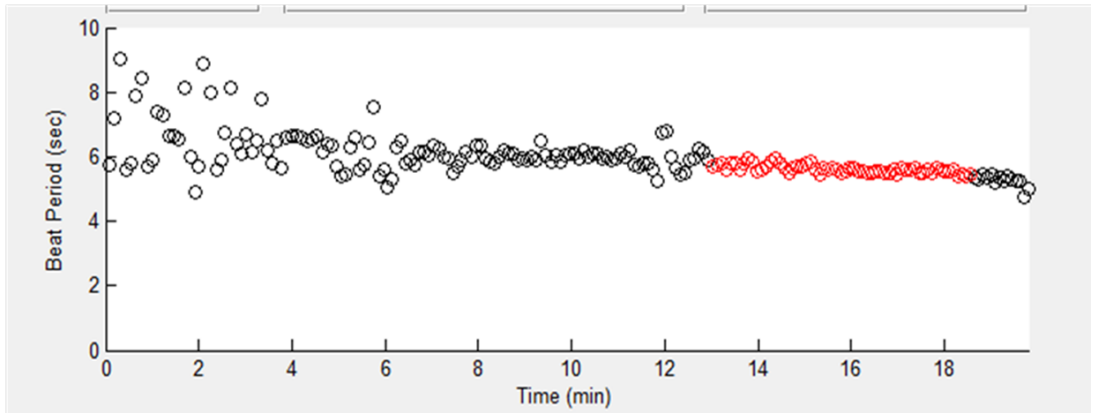
Pentamidine – hERG Trafficking

A



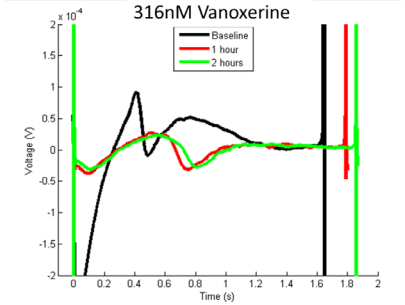
Black – Baseline
Red – 2 hours
Green – 24 hours
Dark Blue - 48 hours

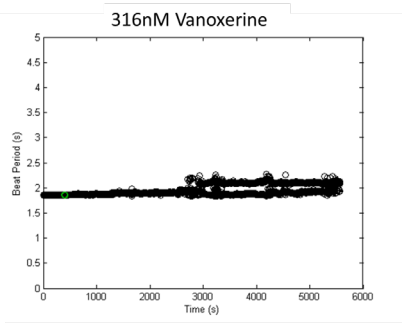
B

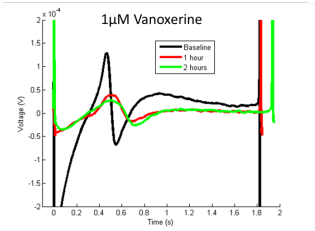


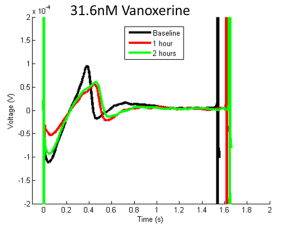
Vanoxerine Case Study – Multichannel Blocker

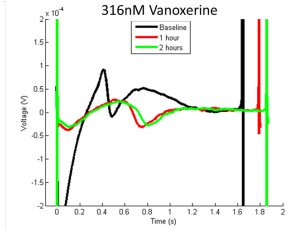
Treatment	Beat Period	Spike Slope	Spike Amplitude	Field Potential Duration (FPD)	Comments
1µM Vanoxerine	103 ± 0.7%	33 ± 5.0%	38 ± 5.0%	120 ± 4.4%	
316nM Vanoxerine	115 ± 2.9%	63 ± 7.8%	65 ± 9.3%	157 ± 7.3%	Arrhythmia
100nM Vanoxerine	110 ± 0.7%	87 ± 4.1%	88 ± 3.2%	145 ± 1.7%	
31.6nM Vanoxerine	106 ± 0.7%	95 ± 3.7%	95 ± 3.8%	114 ± 5.2%	
10nM Vanoxerine	102 ± 0.9%	98 ± 8.9%	97 ± 7.6%	107 ± 2.1%	

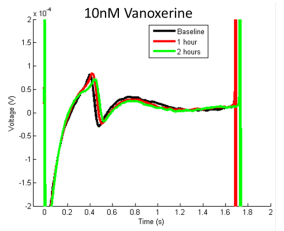


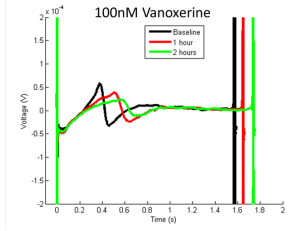












Multi channel blocker was in a clinical trial for atrial fibrillation. Dose was increased which caused torsades in two patients. Reached 0.85 µM.
Tested concentrations up to 1 µM and timepoints up to 4 hours

BMS-986094 caused Heart failure in patient

After Patient's Death, Study Shows HCV Drug Cardiotoxic in 14 of 34 Treated Patients

Michael O'Riordan
September 24, 2014

DURHAM, NC – The development of **BMS-986094** (Bristol-Myers Squibb), a nucleotide-polymerase inhibitor for the treatment of chronic hepatitis C (HCV) infection, was terminated back in August 2012, but a new retrospective review analyzing adverse events provides an in-depth look at the cardiotoxicity associated with the investigational agent.

The findings may have profound implications for other drugs in late-stage testing or others approved for use. Investigators say understanding the mechanisms of cardiac dysfunction and its relevance to other agents will require more study.

Development of BMS-986094 was stopped after a 25-year-old male treated with 200-mg dose experienced rapidly progressive heart failure and died. The patient had been hospitalized with shortness of breath and was found to have a left ventricular ejection fraction (LVEF) <10%.

Now, as part of a retrospective review of the phase 2 study that included the index death, researchers report that 14 of 34 patients treated with BMS-986094 had some evidence of cardiac dysfunction.

The review was published online September 24, 2014 in *Hepatology*.

In total, six patients had severe LV dysfunction (LVEF <30%) and eight had moderate LV dysfunction (LVEF 30%-50%) in one or more evaluations in the six months following drug discontinuation.

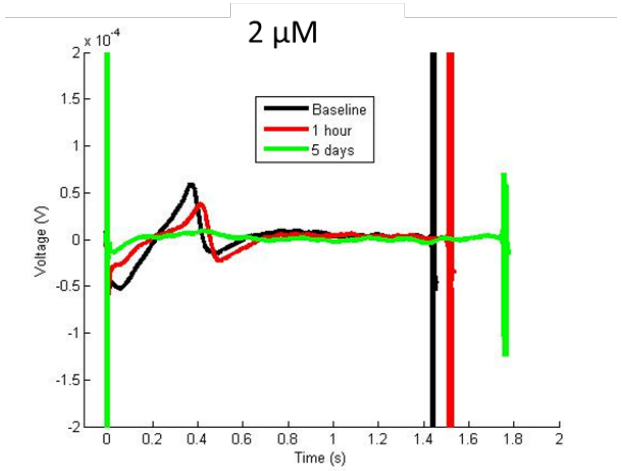
- In a phase 2 clinical trial, after 39 days of treatment a patient died from heart failure
- 14 of 34 patients treated at the same time also had some evidence of cardiac dysfunction (LVEF of less than 50%) in the 6 months following discontinuation.
- Chronic treatment with this drug caused unexpected liabilities not previously detected in animal studies
- Could we use human iPSC cardiomyocytes in *in vitro* assays to identify this liability?

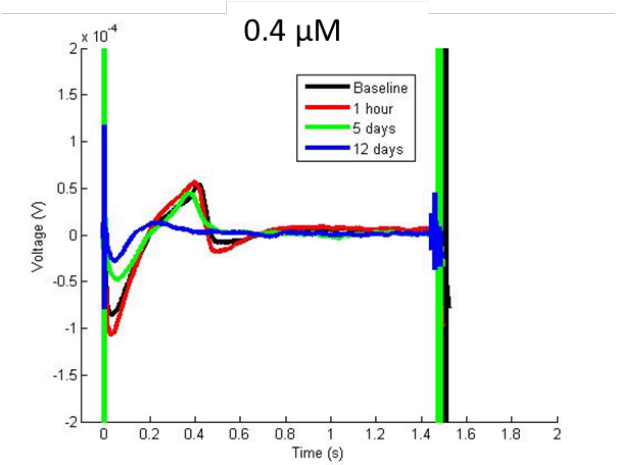
Chronic Dosing of BMS-986094 in MEA assay with Human iPSC Cardiomyocytes

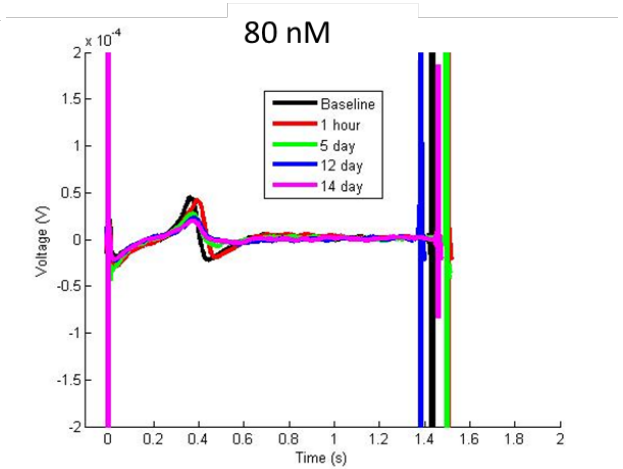
			% of Vehicle			
Test Article	Time (hr)	Test Conc. (µM)	Average Beat Period	Average Na+ Slope	Average Na+ Amplitude	Average FPD
INX-08189	1	10	106 ± 5.4%	78 ± 10.8%	80 ± 11.7%	105 ± 6.2%
		2	106 ± 2.7%	112 ± 9.6%	111 ± 7.3%	107 ± 5.5%
		0.4	105 ± 1.8%	91 ± 23.1%	93 ± 26.2%	99 ± 8.5%
		0.08	106 ± 3.4%	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

Compound causes a progressive loss of activity over time. By 14 days, there is no activity left at a concentration of 400nM and the 80nM concentration has a beat rate almost twice the vehicle treated cells suggesting significant activity at this concentration also.

BMS-986094 MEA Traces

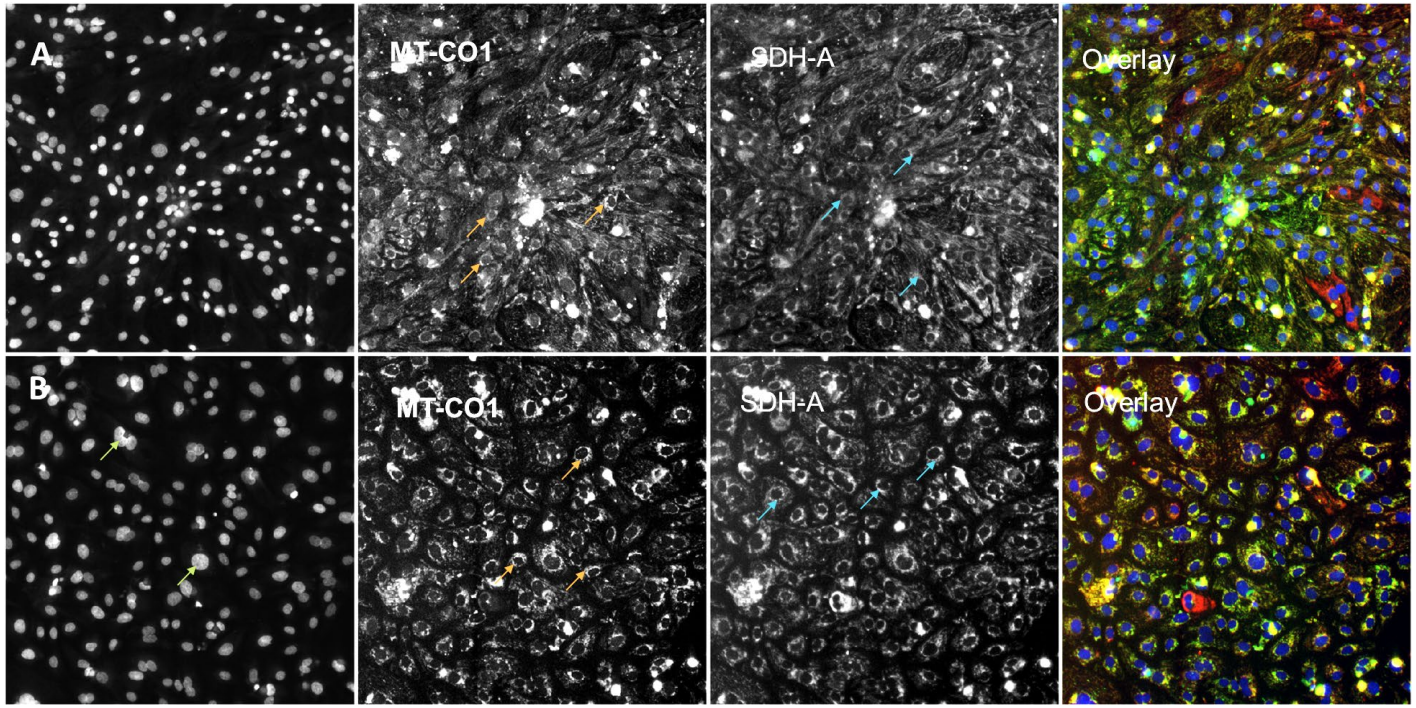






Mitochondrial Biogenesis

- Some publications have suggested an effect on mitochondrial biogenesis as the mechanism of toxicity



BMS-986094 Conclusions

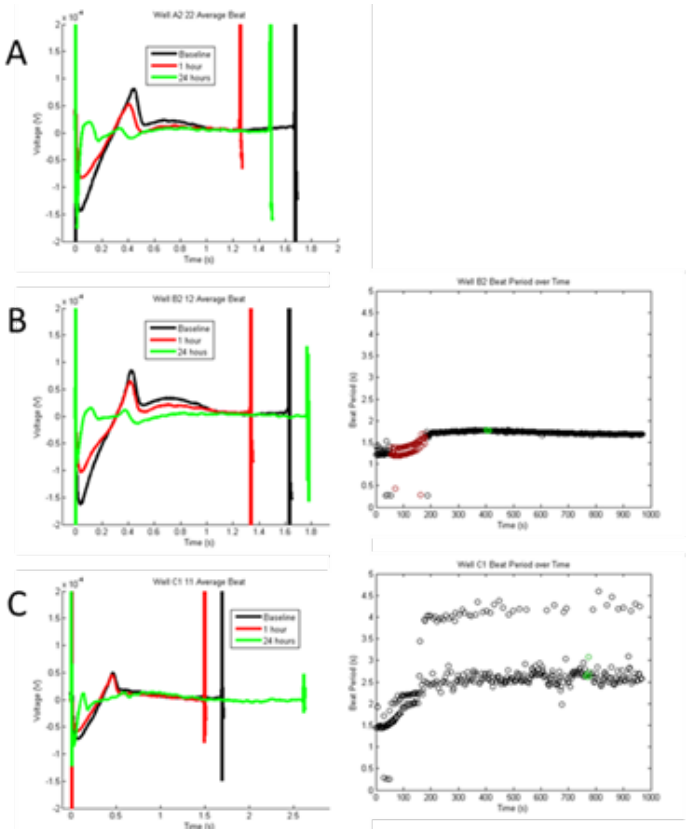
- Use of the MEA and CDI iPSC derived cardiomyocytes identified a chronic liability that exists for BMS-986094
- The compound caused a loss of beating at 10, 2, and 0.4μM concentrations by 14 days. This was a chronic issue as there was no effect from this compound at 1 hour.
- There was an effect observed even at 80nM. The cells beat rate increased substantially and the Na amplitude increased significantly. This occurred on two separate plates.
- These chronic effects were observed for this compound while other HepC drugs tested at the same time had no accumulating chronic effects
- 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 2.5 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 time points 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.

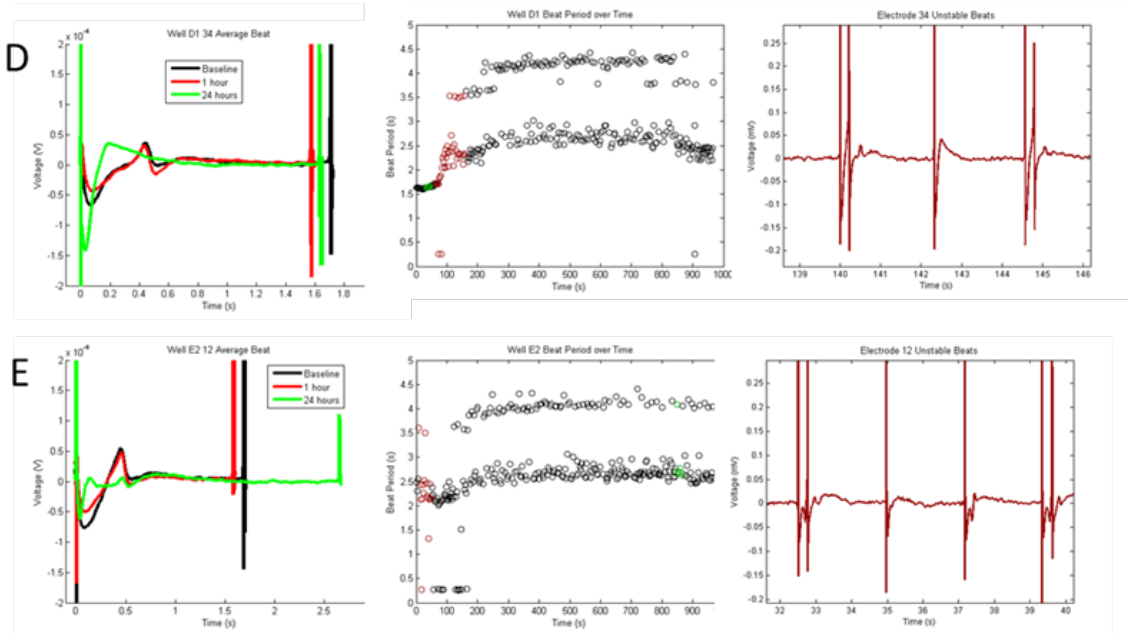
Compound X – Decrease in Na Amplitude at 24 hours

			% of Vehicle				
Test Article	Time Point	Test Conc. (μM)	Average Beat Period	Average Na+ Slope	Average Na+ Amplitude	Average FPD	Comment
Compound X	1 hour	150	76 ± 3.1%	62 ± 1.8%	65 ± 1.4%	93 ± 1.3%	
		75	85 ± 0.2%	72 ± 10.0%	76 ± 5.9%	97 ± 1.0%	
		30	92 ± 0.2%	82 ± 12.3%	83 ± 9.9%	99 ± 0.3%	
		15	96 ± 0.1%	95 ± 6.2%	95 ± 4.6%	100 ± 1.4%	
		7.5	98 ± 0.1%	85 ± 15.4%	92 ± 5.4%	101 ± 0.5%	
		MEC (μM)	30	75	75	NA	
	24 hours	150	86 ± 0.5%	12 ± 3.4%	22 ± 2.9%	72 ± 1.0%	
		75	94 ± 0.2%	23 ± 0.0%	30 ± 0.9%	83 ± 0.2%	A,C
		30	132 ± 17.1%	17 ± 0.4%	25 ± 0.9%	TND	A,C
		15	144 ± 21.4%	20 ± 2.3%	29 ± 2.7%	TND	A,C
		7.5	157 ± 1.3%	21 ± 1.8%	29 ± 0.9%	92 ± 6.9%	A,C
		MEC (μM)	150	7.5	7.5	15	

Compound X shows limited activity at 1 hour with increased beat rate at top two or three concentrations and decreased Na amplitude at top 2 concentrations. At 24 hours, all concentrations have greatly decreased Na amplitude and slope and at lower concentrations arrhythmias are occurring. There seems to be some multichannel inhibition with unexplained mechanisms driving changes.

Compound X MEA Traces





144 hour dosing of Compound Y – Delayed unexplained arrhythmic activity

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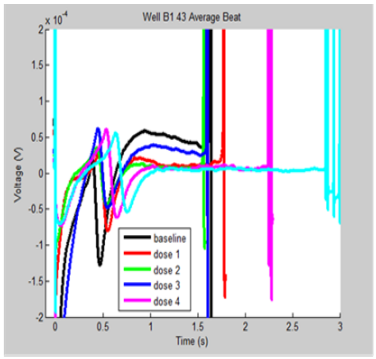
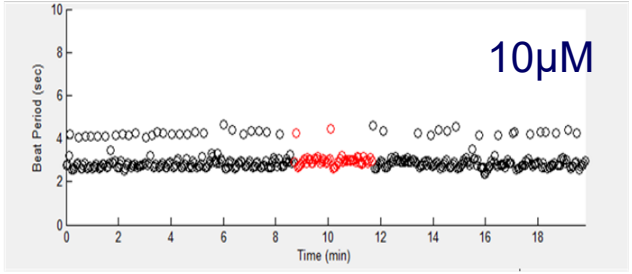


Black – Baseline
Red – 2 hours
Green – 24 hours
Dark Blue - 48 hours
Pink – 72 hours
Light Blue – 144 hours

B



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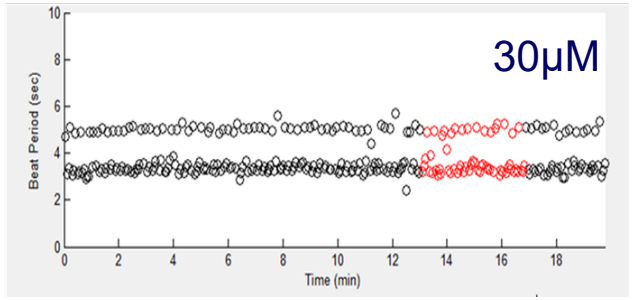

- Traces show the time course of the compound Y. The traces show that the repolarization becomes more delayed over time with the largest increase occurring at 6 days.
- It does not appear to be a hERG effect as we see no decrease in the repolarization peak amplitude. You can see multiple depolarization peaks showing arrhythmias.
- At 10 and 30µM, arrhythmias are observed at 6 days as can be observed in the beat plot.

Conclusions

- Use of an MEA cardiomyocyte assay is an effective way of identifying chronic liabilities.
- Pentamidine, a known hERG trafficking inhibitor, was identified using this assay
- Identified vanoxerine liabilities in extended exposure assay
- The chronic MEA assay was effective at identifying compound X which decreased Na amplitude at 24 hours.
  - May be an effect on cell health that is limiting cellular interactions
- The assay identified a compound which caused delayed arrhythmias (5-6 days) of unknown mechanism in telemerized dogs.
- The assay also identified INX-08189, a compound which caused a death in clinical trial, as a significant liability over the course of 14 days.