Ion Channel Retreat

Exploitation of Barracuda Automated Electrophysiology Platform to Improve Assessment of Cardiac Liability

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Diversity of drug-induced cardiovascular toxicity

- Major cause of drug attrition in pre-clinical and clinical development
- Cause of serious adverse drug reactions (ADRs) and withdrawal from the market.

Heart

- **Functional**
  - Arrhythmia
  - Contractility

QT / QRS/ PR prolongation

Cisapride

Blood Vessels

- **Functional**
  - Blood pressure changes
  - Orthostatic hypotension
  - Oedema
- **Structural**
  - Remodelling
  - Vasculopathies

Cardiomyopathy
Myocardial infarction
Heart failure

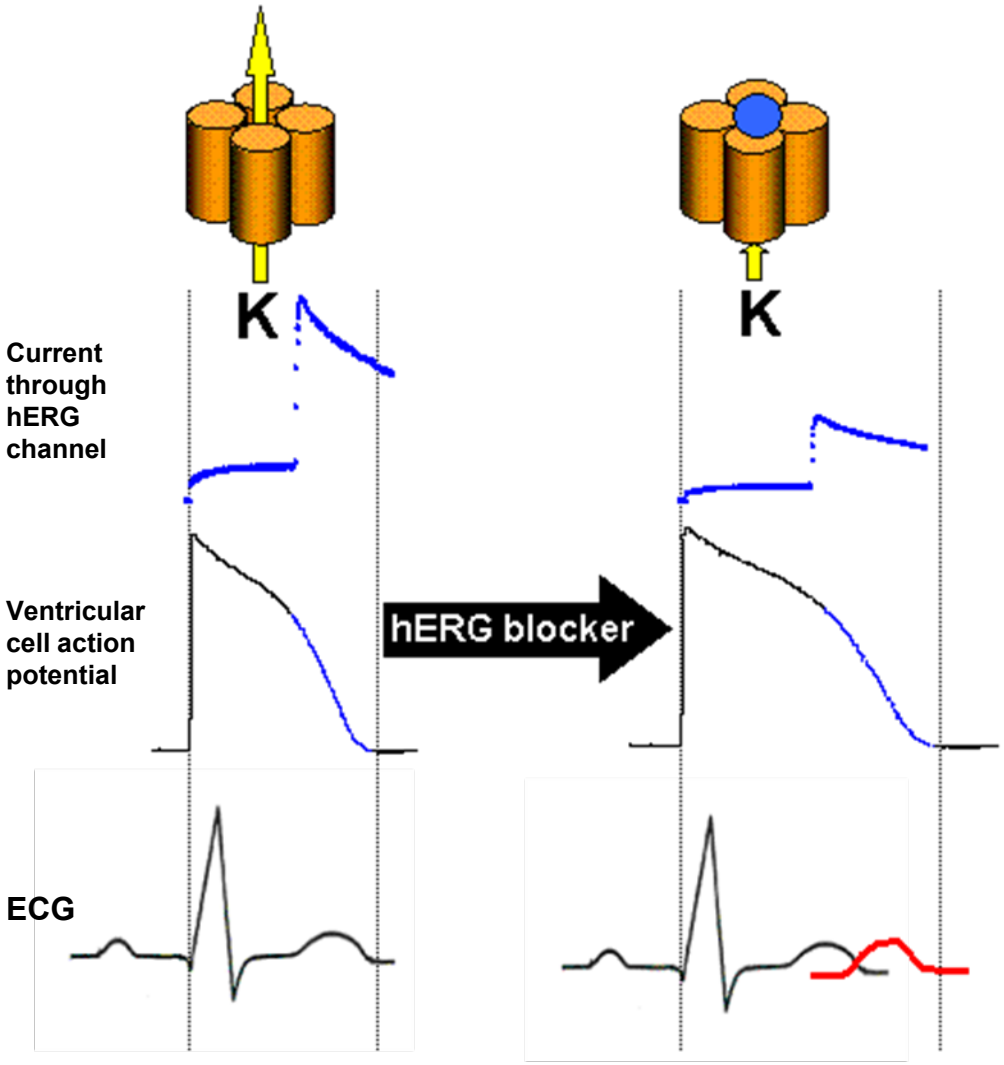
Heart

- **Structural**
  - Necrosis
  - Fibrosis
  - Hypertrophy
  - Valvulopathy

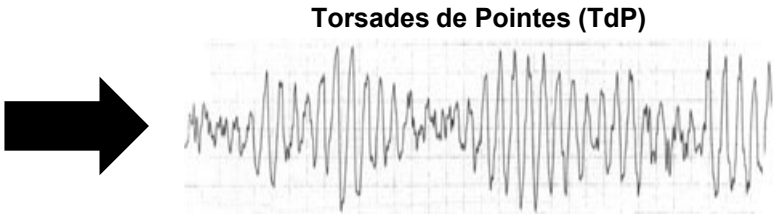
Blood

- Thrombosis
- Platelet function
- Anaemia/ RBC haemolysis
- Immune system

Drug-induced QT interval prolongation
Biological background



- hERG channel block prolongs ventricular cell action potentials
- Seen as QT interval prolongation on ECG
- QT interval prolongation is linked to proarrhythmic risk (Torsades de Pointes)
- hERG block is most common mechanism
- QT interval has to be corrected for heart rate changes (QTc)
- If drug significantly increases heart rate, this can lead to an apparent QTc increase

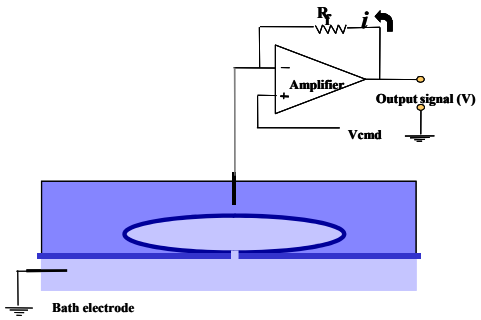


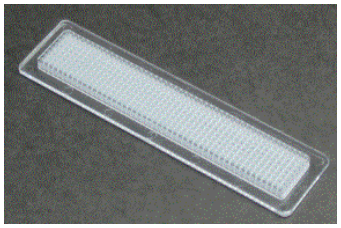
IonWorks versus GLP hERG

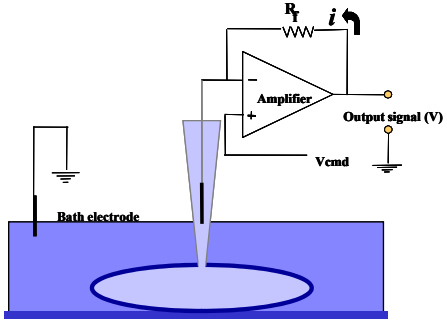
Technical differences

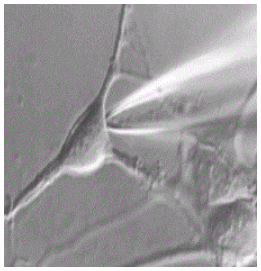
- IonWorks
  - 384-well, plate-based
  - Plastic surface area:buffer volume ratio, high
  - Intermittent voltage clamp
  - Rate of onset of block cannot be measured*
  - No measurement of drug concentration
  - Therefore, potency based on nominal concentrations

- Good Lab Practice (GLP)
  - Microelectrode-based conventional patch clamp
  - Plastic surface area:buffer volume ratio, low
  - Continual voltage clamp
  - Rate of onset of block can be measured
  - Drug concentration measured
  - Therefore potency based on measured concentrations





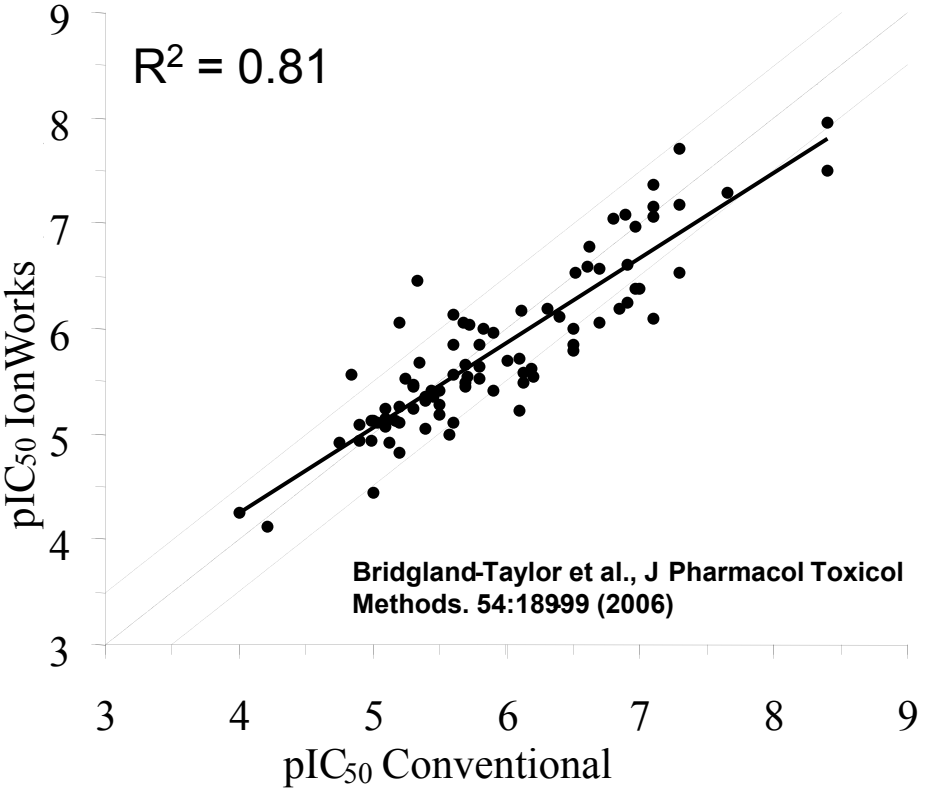




GLP versus IonWorks hERG

Data correlations

- Initial IonWorks validation prior to screen launch in 2003
  - For 89 compounds comparing IonWorks to microelectrode method, correlation was generally good with a few exceptions (for example AZ1)



Potential explanations for a difference in potency between IonWorks and GLP hERG are:

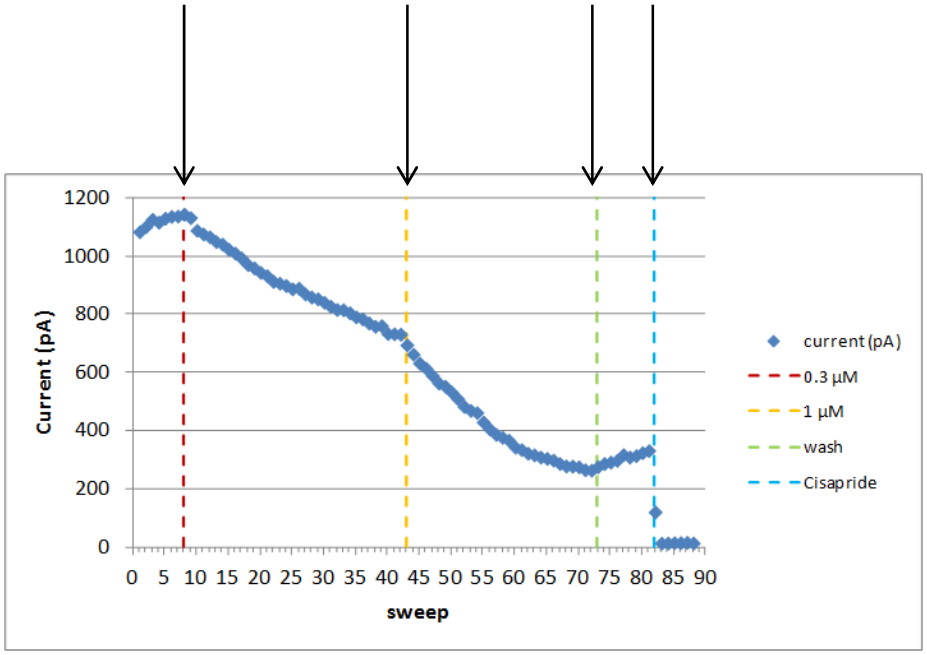
- The actual concentration in IonWorks is less than the nominal value
- The compound is a very slow on-set blocker such that its effect is under-estimated in IonWorks
- The lack of continuous voltage clamp in IonWorks

AZ1 IonWorks:GLP hERG data

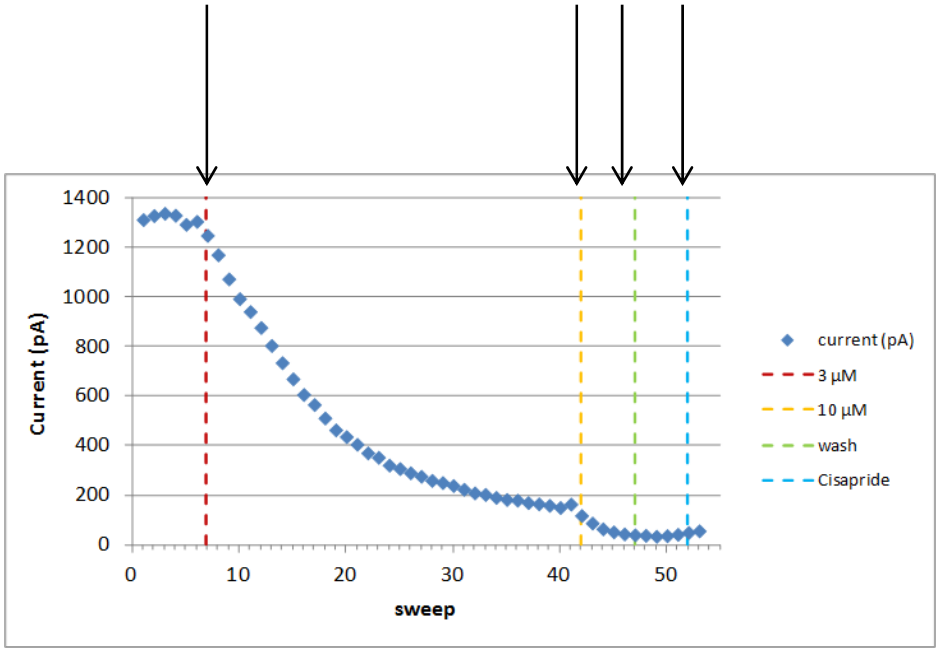
IC_{50} of 22.1 μM (n=6) for standard IonWorks-based assay (200 sec incubation)
Compared to IC_{50} of 0.25 μM in GLP-hERG (n=4) – is it a slow onset compound?

Trace profile characteristic of slow onset compound

Compound additions



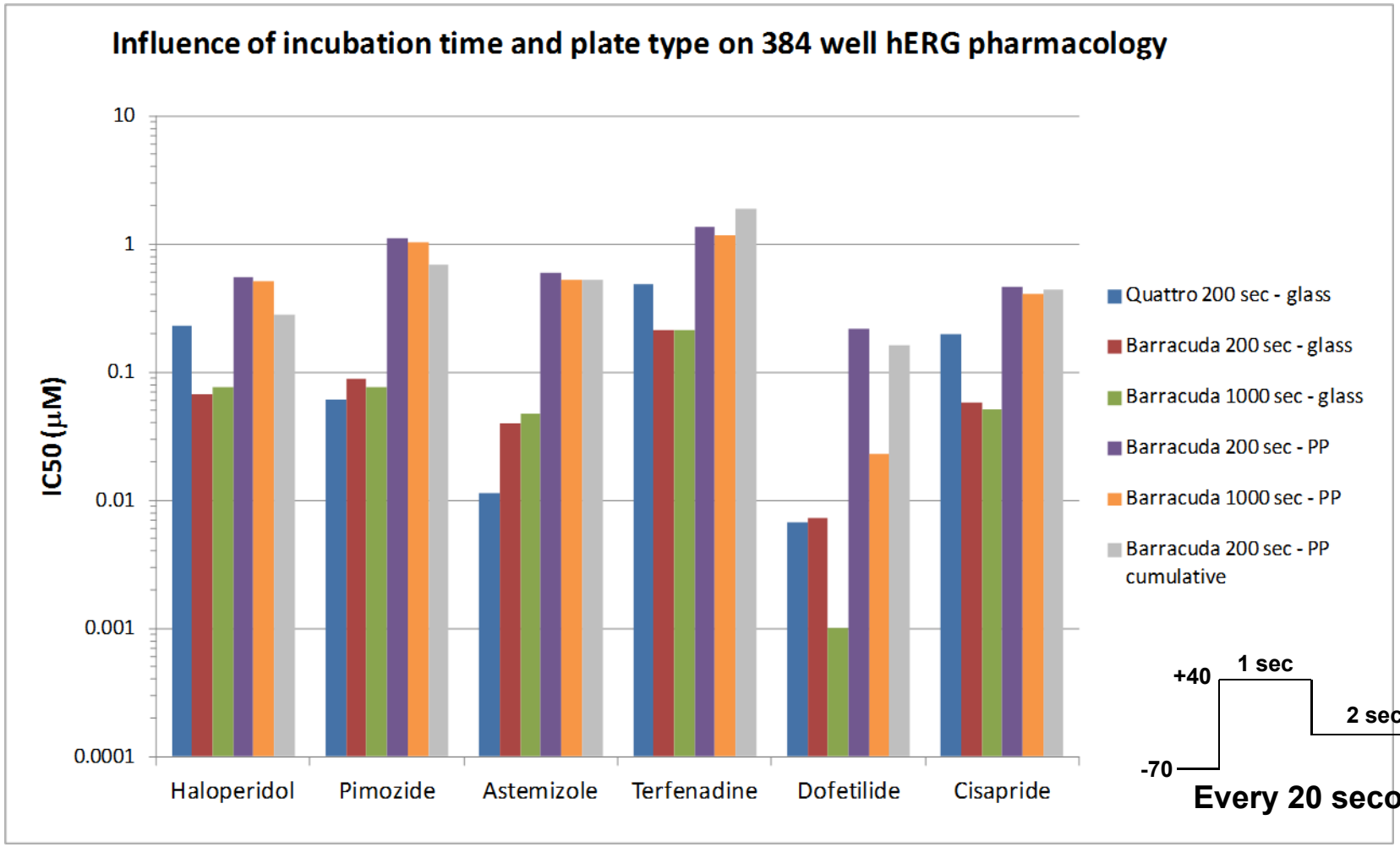
Compound additions



The next generation IonWorks device (Barracuda), like the GLP hERG assay, enables continuous voltage clamp and measurement of on-set of block

Barracuda validation - comparing incubation time

Assessing compound plate type and incubation time of hERG standards



Is AZ1 similar to dofetilide (slow onset blocker)?

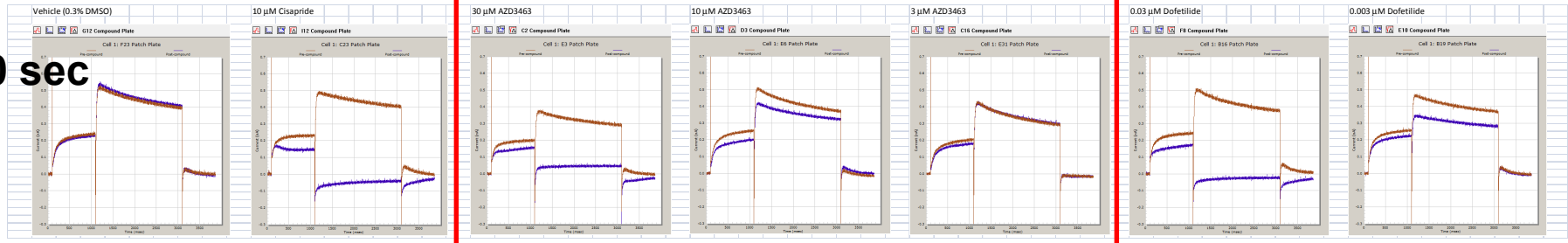
Side by side IonWorks vs Barracuda traces

IonWorks

AZ1

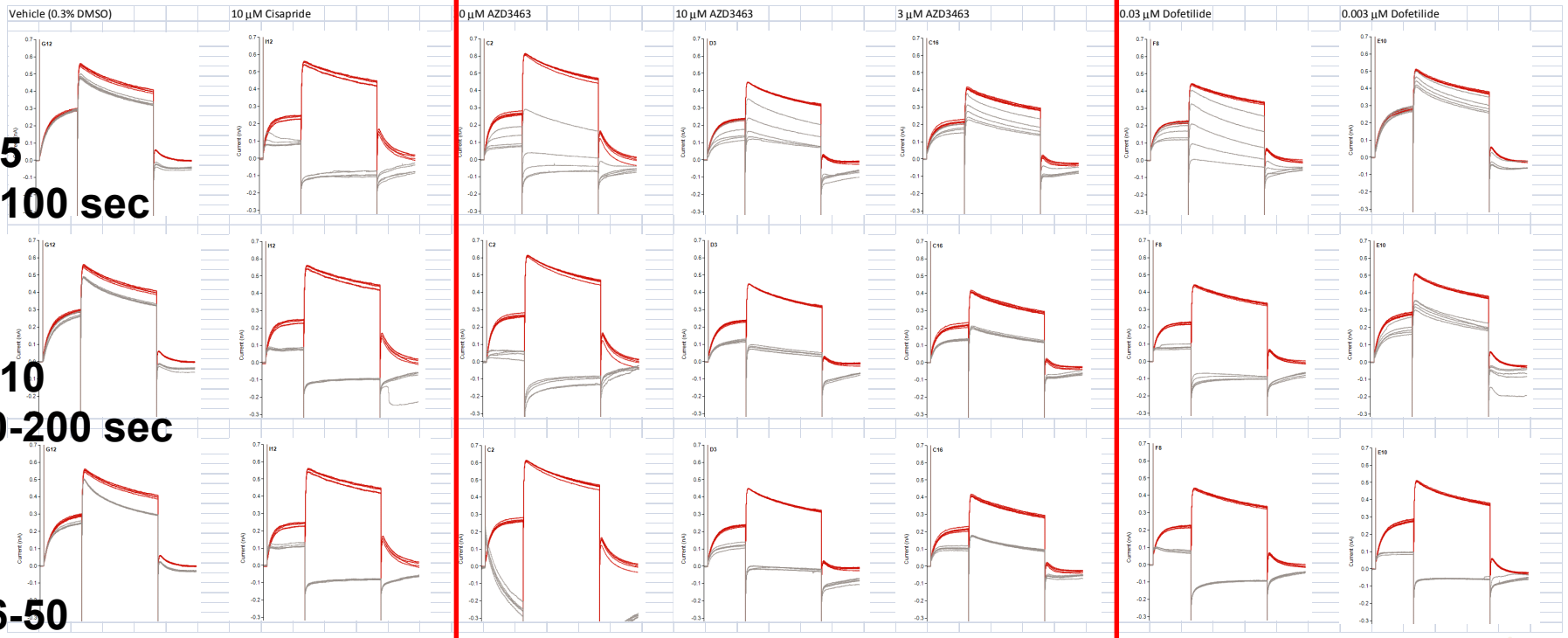
Dofetilide (slow)

200 sec



MAX **MIN** **30 µM** **10 µM** **3 µM** **0.03 µM** **0.003 µM**

Barracuda

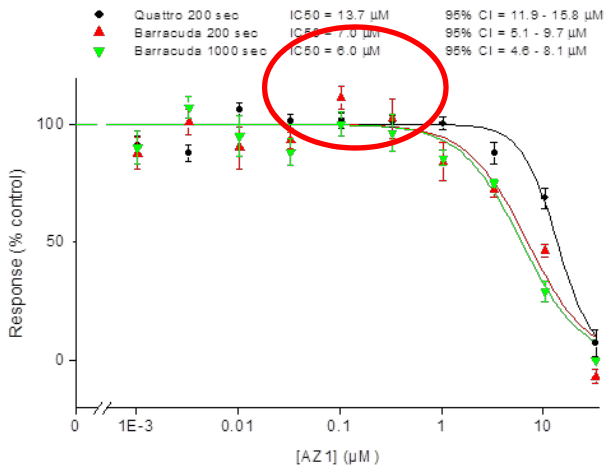


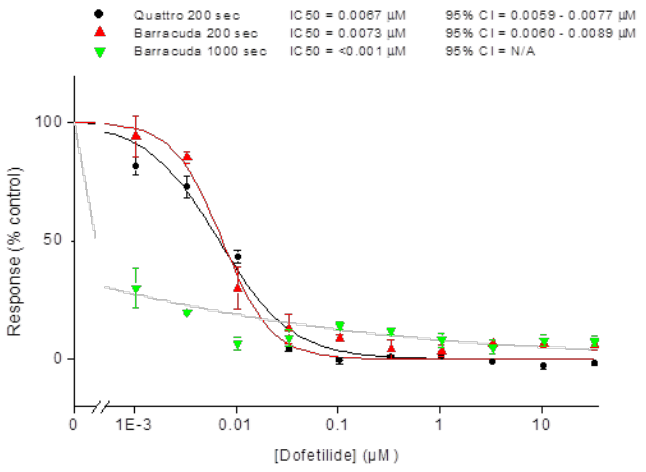
s1-5
20-100 sec

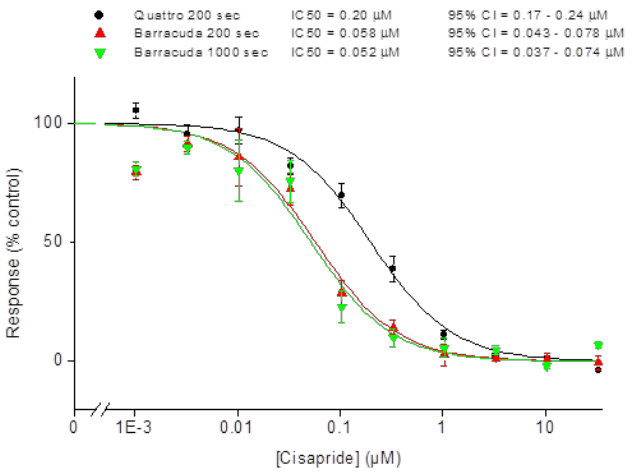
s6-10
120-200 sec

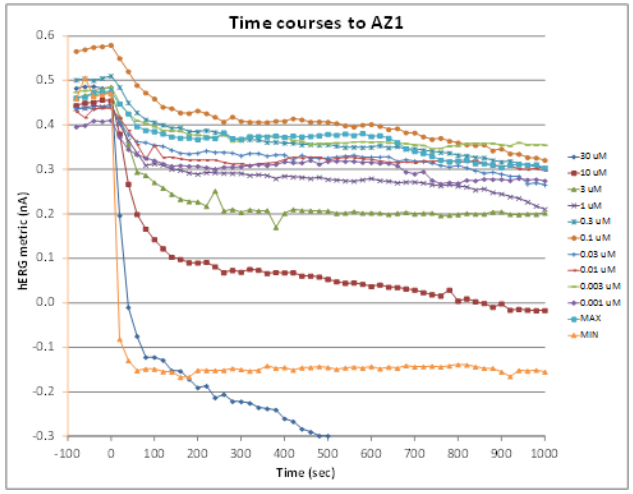
s46-50
920-100 sec

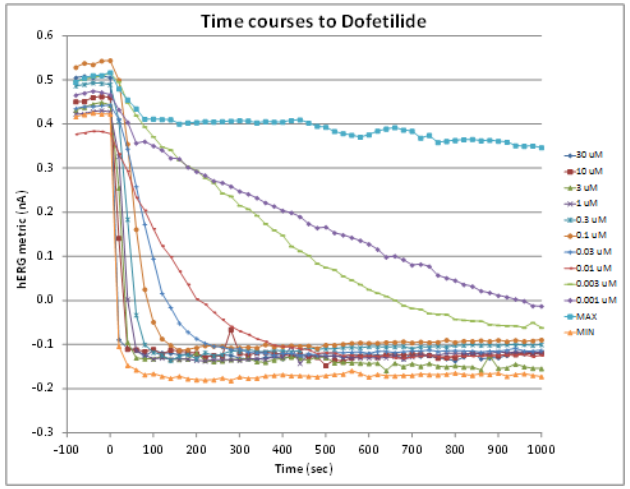
Data using glass-coated compound plates (n=4)

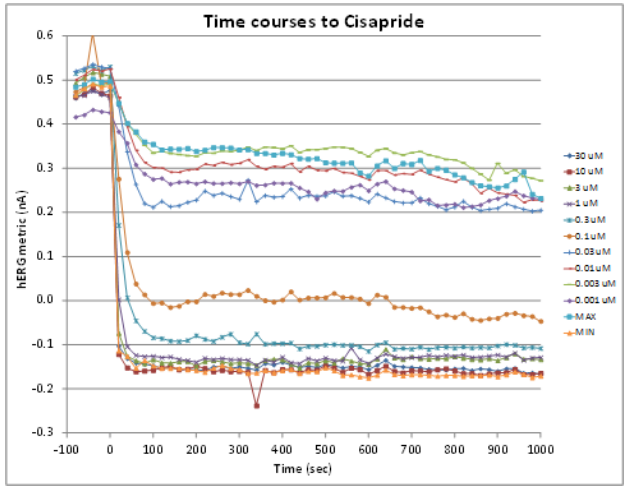












Comparing GLP, IonWorks & Barracuda

Summary of AZ1 hERG IC_{50} values using different methods

	IonWorks in HT mode: 96 well polyproylene (200 sec)	IonWorks in PPC mode: 384 well glass coated compound plate (200 sec)	GLP-hERG: minimal amount of plastic	Barracuda in PPC mode: 384 well glass coated compound plate (1000 sec)
Based on nominal concentration	22.1 μM	13.7 μM	0.42 μM	6.0 μM (7.0 μM at 200 sec plus detection of slow on-set of block)
Based on measured concentration	5.3 μM (extraction of contents from PatchPlate for DMPK analysis)	Not Determined	0.25 μM	**Not Determined

**** = if assume similar cell concentration & plastic surface area between IonWorks & Barracuda, then IC_{50} should drop to ~1.4 μM (5.6 fold from GLP-measured).**

Summary of work with AZ1

- IonWorks Barracuda™ was able to detect the very slow on-set of hERG block when testing AZ1. Some of the remaining difference in potency when compared to the GLP-hERG data is due to the compound sticking to the PatchPlate substrate or the difference in cell concentration.

- If we assume a similar drop in potency seen on IWQ when using the IWB PatchPlate, the potency of AZ1 is only 5.6 fold greater than the GLP-hERG IC_{50} value (~1.4 versus 0.25 μM).

- Gillie et al (2013) have reported the effects of longer exposure to Dofetilide in the IonWorks Barracuda™ system and here we have also demonstrated it is a very slow on-set blocker of the hERG channel. We have also seen that Dofetilide potency is influenced by increasing the incubation time with the IonWorks Quattro system (data not shown).

- Using IonWorks Barracuda™ for routine hERG screening is delivering a better assessment of compound block at this important ion channel.

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