Institution: University of Bristol		
Unit of Assessment: 1) Clinical Medicine		
Title of case study: Increasing the reliability of protocols for cardiac drug safety tests for international regulatory bodies and the pharmaceutical industry		
Period when the underpinning research was undertaken: 2004 - 2014		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Jules Hancox	Professor of Cardiac Electrophysiology	1991 - present
James Milnes	Postdoctoral Researcher	2001 - 2005
Harry Witchel	Research Fellow/Senior Fellow	1991 - 2007
John Ridley	Postdoctoral Researcher	2004 - 2007
Period when the claimed impact occurred: 2014 - 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

All new drugs have to undergo preclinical cardiac safety tests. However, previous ICH-S7B screening guidelines (in use since 2005) resulted in rejection of some potentially useful drugs. Professor Hancox has contributed to the multinational effort to produce better screening approaches that both improve cardiac safety and help reduce rejection of potentially useful drugs. This initiative is called the “Comprehensive in vitro Proarrhythmia Assay” (CiPA) and a protocol developed by the Hancox team is now integrated into the CiPA validation test suite. This test suite is designed for use by pharmaceutical companies, the high-throughput screen industry and guiding regulatory agencies, including the US FDA.

2. Underpinning research

One of the key ionic currents controlling termination of the cardiac action potential is carried by the hERG potassium channel. Professor Hancox has focussed on uncovering the biophysics of hERG channels as well as how mutations and drugs affect hERG activity which, by altering the cardiac action potential, may cause lethal arrhythmias (>60 journal papers).

The contributions of Professor Hancox's group to identifying undesired drug effects on hERG was described in REF2014. That work forms the backdrop to the new impact claimed here: namely the contributions to developing a suitable bioassay that has been incorporated in a framework to guide industrial drug safety evaluation of novel therapeutics as well as prevent premature abandonment of potentially useful drugs.

The University of Bristol (UoB) team characterized the (unintended) block of hERG by the anti-anginal drug lidoflazine, which was withdrawn due to its risk for lethal arrhythmia development [1]. Subsequent collaborative studies were carried out with Pfizer on moxifloxacin and the antidepressant doxepin to evaluate their hERG/safety profile. Importantly for this impact case study, the antiarrhythmic dofetilide and gastric prokinetic cisapride were examined by Professor Hancox's research team in a 2010 publication [2]. This study demonstrated clear differences in the behaviour of a drug that is ‘trapped’ in the hERG channel from one that is released between episodes of hERG channel activation. The ability to detect this important drug/receptor interaction mode depends on proper electrical pulse form and sequencing. The UoB team showed this could be achieved with a “long pulse” type protocol (herein referred to as the ‘Milnes

protocol', named after the first author of the study supervised by Professor Hancox) [2]. The UoB team and Pfizer subsequently used the same Milnes protocol in further hERG studies on a new SSRI (Selective Serotonin Reuptake Inhibitor) antidepressant drug [3].

The need for the pharmaceutical industry and academic community to adopt better electrophysiological measures to assess arrhythmia risk prompted Professor Hancox to co-author the "Lambeth conventions II" guidelines for the study of arrhythmias in animals and humans [4]. Professor Hancox's knowledge of the strengths and limitations of preclinical approaches to evaluating drug-induced arrhythmia liability led to authorship of guides in key literature intended to inform drug development and safety testing (e.g. [5]). The importance of the need for new safety testing paradigms, as well as new integrative approaches to safety testing in industry, are described in the eBook "Safety Pharmacology – Risk Assessment QT Interval Prolongation and Beyond" (see evidence source [E]).

Work by both the US FDA [6] and CiPA linked investigators (which includes Prof Hancox), has successfully demonstrated the benefit of the Milnes protocol for evaluation of hERG blocking kinetics. The Milnes-based protocol, as recommended by the FDA for assessing kinetics of hERG blockers, is detailed in evidence source [C].

3. References to the research

[1] **Ridley JM**, Dooley PC, **Milnes JT**, **Witchel HJ**, **Hancox JC.** (2004). Lidoflazine is a high affinity blocker of the hERG K^+ channel. *Journal of Molecular & Cellular Cardiology*, 36(5), 701-705. DOI:10.1016/j.yjmcc.2004.02.009

[2] Milnes JT, Witchel HJ, Leaney JL, Leishman DJ**, Hancox JC**. (2010). Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37°C: Cisapride versus dofetilide. *Journal of Pharmacological & Toxicological Methods*, 61(2): 178-191. DOI:10.1016/j.vascn.2010.02.007

[3] Alexandrou AJ, Milnes JT, Sun SZ, Fermini B, Kim SC, Jenkinson S, Leishman DJ, Witchel HJ, **Hancox JC**, Leaney JL. (2014). The human ether-a'-go-go related gene (hERG) K^+ channel blockade by the investigative selective-serotonin reuptake inhibitor CONA-437: limited dependence on S6 aromatic residues. *Journal of Physiology & Pharmacology,* 65(4): 511-523. PMID:25179083 www.jpp.krakow.pl/journal/archive/08_14/pdf/511_08_14_article.pdf

[4] Curtis MJ, **Hancox JC,** Farkas A, Wainwright CL, Stables CL, Saint DA, Clements-Jewery H, Lambiase PD, Billman GE, Janse MJ, Pugsley MK, Ng GA, Roden DM, Camm AJ, Walker MJA. (2013). The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. *Pharmacology and Therapeutics*, 139, 213-248. DOI:10.1016/j.pharmthera.2013.04.008

[5] **Hancox JC**, McPate MJ, El Harchi A, Zhang YH. (2008). The hERG potassium channel and hERG screening for drug-induced torsades de pointes. *Pharmacology & Therapeutics,* 119: 118–132. DOI:10.1016/j.pharmthera.2008.05.009

[6] Li Z, Dutta S, Sheng J, Tran PN, Wu W, Chang K, Mdluli T, Strauss DG, Colatsky T. (2017) Improving the In Silico Assessment of Proarrhythmia Risk by Combining hERG (Human Ether-à-go-go-Related Gene) Channel-Drug Binding Kinetics and Multichannel Pharmacology. *Circulation: Arrhythmia & Electrophysiology,* 10(2): e004628. DOI:10.1161/CIRCEP.116.004628

4. Details of the impact

Impact follows the adoption of the Milnes protocol [2] approach to characterizing a type of ion channel block by drugs. The CiPA designated ion channel working group (which includes Professor Hancox) critically evaluated tests and recommended that the Milnes et al. protocol be adopted. As documented by the CiPA initiative steering team [Ai]: "I*.3.a The original hERG protocol proposed by the Ion Channel Working Group proved to be inadequate in determining kinetics of block for the training set of drugs. After careful review of the literature, and a series of preliminary experiments, the protocol from Milnes et al., (2010) was adopted*.".

Drug safety testing

Drug safety testing is a key component of drug development by the pharma industry and of great importance for the general public who should not be exposed to increased risk as a result of their medication(s). On the other hand, if the tests are not optimal, potentially useful drugs might be rejected with the loss of revenue for developers and to public detriment. The pharma industry spends approximately USD1 billion on developing each new drug, of which approximately 9% are subsequently rejected due to cardiovascular safety concerns.

The CiPA initiative [A] began following a workshop in July 2013 at the US FDA with the objective of engineering a reliable assay system for assessment of the proarrhythmic potential of new drugs. The idea was the newly designed assays should have improved specificity compared to the existing ICH S7a/b screening "hERG assay plus thorough QT study" approach. Thus the multinational CiPA initiative has two goals: (1) to increase the efficiency of new drug development by reducing drug rejection from false positives in preclinical tests; and (2) to increase patient safety by rejecting drugs that do indeed carry increased risk for arrhythmias. This is underscored on the HESI CiPA website [Aii]: "*The objective of the CIPA initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation. The new CIPA paradigm will be driven by a suite of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes*.". The importance and influence of the CiPA initiative (and by extension of the utility of the Milnes hERG protocol which originated in the Hancox laboratory [2]) is summarized in a number of review articles (e.g. [B]).

The approaches developed through CiPA are already being exploited by industry. The UoB team protocol [1-3] that revealed undesired drug-hERG interactions (to minimise risk for arrhythmogenesis) was then adapted for drug screening purposes [C] and this provides pharmaceutical companies with a valuable tool for late stage validation work prior to regulatory approval.

Development and application of regulatory guidelines

The CiPA steering group and the FDA released details of specific protocols and measurement conditions to be used in independent validation studies, including the use of the modified 'Milnes et al' hERG protocol [B, C]) This forms a key part of a continuously evolving cardiac drug safety testing framework for international regulatory bodies, such as the FDA, as well as the pharma and drug testing industries [D].

Additional evidence for the impact of the Milnes et al [2] long pulse protocol for safety evaluation has been description of its use for "uncertainty characterization" in drug-hERG binding studies in a pharmacology safety reference collection written to guide drug development and industry testing [E, F] as well as for obtaining parameters for the protocol-dependence of drug hERG blocking potency [G] for examination by in-silico drug interaction models (which form another limb of the CiPA initiative).

Adoption by industry

Thus the impact of work from the UoB is: (1) the CiPA acknowledgement of the utility of Professor Hancox's approach for evaluating blocking kinetics of the hERG channel (the results of which are required to paramaterize subsequent *in silico* work estimating the likely QT_c/proarrhythmia risk); and (2) the independent work conducted by multiple companies that have shown that their high-throughput screening platforms can apply the Milnes et al. protocol. Specific examples of the latter include Metrion Biosciences [Hi], Sophion Bioscience [Hii] and Ncardia [Hiii]. The ability of such platforms to exploit the Milnes et al. protocol has also been used for marketing purposes [Hi-iii]. In connection with this, the level of detail provided by the Milnes et al. protocol [2] requires more time to conduct than may be suitable for some high-speed high-throughput automated drug screening systems. This makes it better targeted to the final stages of detailed drug validation [I] but does not diminish the impact of the basic science carried out at UoB on drug development/testing. In terms of reach, it should also be noted that high-throughput screening is a multinational growing USD14 billion+ industry [e.g. J] so that adoption of protocols that improve performance in drug screening by automated platforms also has value for investors.

Beneficiaries of this impact are:

(i) Regulatory bodies, such as the FDA, who must find and approve optimal methods for drug safety.
(ii) Drug industry companies intending to produce drugs for US, UK and EU markets.
(iii) Manufacturers of electrophysiological screening platforms (who are already marketing based on CiPA objectives) [H, I].
(iv) Users of pharmaceuticals who will be better treated with new drugs that have passed the new stringent CiPA tests for cardiac safety.

5. Sources to corroborate the impact

[A] i) CiPA (2017). Question & Answer with the CiPA Steering Team
ii) CiPA (2020). About CiPA

[B] Fermini B, **Hancox JC**, Abi-Gerges N, Bridgland-Taylor M, Chaudhary KW, Colatsky T. (2016). A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive In Vitro Proarrhythmia Assay Paradigm. *J. Biomolecular Screening*. 21(1): 1–11. DOI:10.1177/2168479017720249

[C] FDA (2018). Recommended voltage protocols to study drug-cardiac ion channel interactions using recombinant cell lines

[D] Rouse *et al.* (2018). Translating New Science Into the Drug Review Process: The US FDA's Division of Applied Regulatory Science. *Therapeutic Innovation and Regulatory Science*. 52(2): 244–55. DOI:10.1177/2168479017720249

[E] Grandi *et al.* (2018). Editorial: Safety Pharmacology - Risk Assessment QT Interval Prolongation and Beyond. *Frontiers in Physiology*, 9, 678 DOI:10.3389/fphys.2018.00678

[F] Lee *et al.* (2017). In Vitro and In Silico Risk Assessment in Acquired Long QT Syndrome: The Devil Is in the Details. *Frontiers in Physiology* 8: 934. DOI:10.3389/fphys.2017.00934

[G] Ridder *et al.* (2020). A systematic strategy for estimating hERG block potency and its implications in a new cardiac safety paradigm. *Toxicology and Applied Pharmacology* 394: 114961. DOI:10.1016/j.taap.2020.114961

[H] i) Metrion Biosciences Application Report CiPA hERG Milnes kinetic assay on QPatch
ii) Sophion (2018). CiPA recommended Milnes kinetic hERG assay on QPatch
iii) Nanion Technologies (2017). HTS automated patch clamp takes cardiac safety testing to the next level

[I] Kramer *et al.* (2020). Cross-site and cross-platform variability of automated patch clamp assessments of drug effects on human cardiac currents in recombinant cells. *Scientific Reports,* 10(1): 5627–15. DOI:10.1038/s41598-020-62344-w

[J] Report Linker (2020). Global High Throughput Screening (HTS) Industry