Institution: University of Bristol

Unit of Assessment: 1 – Clinical Medicine

Title of case study: Expressed hERG potassium channel bioassays in mammalian cell lines to evaluate safety and efficacy of new drugs.

1. Summary of the impact (indicative maximum 100 words)

All new drugs are required to undergo cardiac safety testing to avoid dangerous side effects on contractility and excitability. Of particular concern is the risk of developing a lethal arrhythmia from inhibition of hERG (human Ether-à-go-go-Related Gene) potassium channels. The Bristol laboratory of Professor Hancox and colleagues demonstrated the utility of hERG-transfected mammalian cell lines for investigation of hERG-related effects and risk. Now most drug discovery programmes utilise hERG screens as part of an integrated assessment of cardiac risk (as recommended by the FDA and MHRA). Second, their work linked hERG inhibition to cardiac risk for certain psychotropics (and other agents) that have been either withdrawn or now carry warnings as to their cardiac safety.

2. Underpinning research (indicative maximum 500 words)

The discovery that *human-Ether-a-go-go-Related Gene (hERG)* is responsible for channels mediating the cardiac 'rapid' delayed rectifier K^+ current, I_{Kr} in 1995 underpins understanding of mechanisms that control the length of ventricular action potentials and the QT interval of the electrocardiogram. Although it was already known that some drugs produce unwanted prolongation of the QT interval of the ECG (acquired Long QT syndrome; LQTS) that could lead to a fatal arrhythmia called *torsades de pointes* (TdP), the mechanism was unclear.

In 1998, Professor Hancox's group showed that native I_{Kr} was reproduced by hERG protein expression during action potentials at body temperature in a mammalian cell line.[1] The close concordance between hERG and native I_{Kr} under physiological conditions provides an underpinning rationale for the use of mammalian cell lines expressing hERG channels for drug screening and toxicity tests which are now current (and ongoing) pharmaceutical company practice. Between 1998 and 2004, Professor Hancox's laboratory also demonstrated pharmacological inhibition of recombinant hERG in mammalian cell lines by a range of cardiac and non-cardiac drugs, including: tricyclic antidepressants (imipramine and amitriptyline), Class Ia and Ic antiarrhythmics (disopyramide, procainamide, flecainide, propafenone), selective serotonin reuptake inhibitors (SSRIs) (citalopram and fluvoxamine) and antianginals (lidoflazine).

In 2002, they produced an 'Appraisal state of the art' article on troubleshooting issues associated with screening drugs for QT interval prolongation using hERG channels in cell lines and *Xenopus* oocytes. In addition to discussing a number of issues pertaining to the use of mammalian cell lines for this kind of screening, this article also pointed out that *Xenopus* oocytes (then widely used for ion channel structure function work) are not appropriate for hERG drug potency screening. This article has been cited in subsequent work from a range of drug companies (including Pfizer, Abbott, Millipore, AstraZeneca, Lundbeck, Johnson and Johnson) and by the UK Medical and Healthcare Products Regulatory Agency (MHRA).

From 2004, Professor Hancox and colleagues continued to publish work on pharmacological inhibitors of hERG in cell lines, including antiarrhythmic drugs (amiodarone and dronedarone) antimicrobials (erythromycin, ketaconazole, moxifloxacin), antihistamines (clemastine) and psychotropic drugs (TCA – doxepin; antipsychotic – thioridazine) and (recently) collaborative work with Pfizer comparing drug potencies obtained with different hERG screening protocols. In 2000 and 2008 Professor Hancox and colleagues produced substantial reviews/position papers linking I_{Kr}/hERG to acquired LQTS and evaluating hERG based screening assays. To underscore the value of this work, some drugs (cisapride, terfenadine) unexpectedly

caused arrhythmias and the mechanism of that action was subsequently traced to hERG (side) effects in cell systems. Also, many other drugs now have QT-associated warnings as a result of indicative hERG effects (see: http://www.azcert.org/index.cfm). This is particularly important for the SSRIs that are widely used to treat depression, anxiety and eating disorders, as well as other drugs (for example, see Doggrell and Hancox, Expert Opinion on Drug Safety 2013;12(3):421-431). Furthermore, even after careful drug design to minimize depressant cardiac effects, approximately 1-2% of patients still exhibit dangerous arrhythmias after overdose (Kerr, Emergency Medicine Journal 2001;18(4):236-241) and 8% of psychiatric patients develop ECG abnormalities associated with their drug therapy (Reilly et al, Lancet 2000;355(9209):1048-1052), recapitulating the importance of cardiac electrophysiological screening for the development of better and safer drugs.[b]

Jules Hancox was Reader from 1991 to 2002 and Professor of Cardiac Electrophysiology from 2002 to date, in the School of Physiology and Pharmacology at the University of Bristol.

3. References to the research (indicative maximum of six references)

[1] Hancox, J.C. Levi, A.J. and Witchel, H.J. (1998) Time course and voltage dependence of expressed HERG current compared with native "rapid" delayed rectifier K current during the cardiac ventricular action potential. *Pflugers Archiv*, 436(6), 843-853. PMID: 9799397

[2] Witchel, H.J., Milnes, J.T. Mitcheson, J.S. Hancox, J.C. (2002) Troubleshooting problems with *in vitro* screening of drugs for QT interval prolongation using HERG K^+ channels expressed in mammalian cell lines and *Xenopus* oocytes. *J. Pharm. Toxicol. Methods,* 48, 65-80. PMID: 14565563

[3] Ridley J.M., Dooley, P.C., Milnes, J.T. Witchel, H.J. Hancox, J.C. (2004) Lidoflazine is a high affinity blocker of the HERG K^+ channel. *J. Mol. Cell. Cardiol.,* 36(5), 701-705. PMID: 15135665

[4] Alexandrou AJ Duncan RS, Sullivan, A, Hancox JC Leishman DJ, Witchel HJ, Leaney JL (2006) Mechanism of hERG K channel blockade by the fluoroquinolone antibiotic moxifloxacin. *Br. J. Pharmacol.*, 147(8), 905-916. PMID: 16474415

[5] Hancox JC, McPate MJ, El Harchi A, Zhang Yh (2008) The hERG potassium channel and hERG screening for drug-induced torsades de pointes. *Pharm. Ther.*, 119, 118-132. PMID: 18616963

[6] Milnes JT, Witchel HJ, Leaney JL, Leishman DJ, Hancox JC (2010) Investigating dynamic protocol dependence of hERG potassium channel inhibition at 37^{o}C: cisapride versus dofetilide. *J. Pharm. Toxicol. Methods,* 61, 178-191. PMID: 20172036

4. Details of the impact (indicative maximum 750 words)

Impact on clinical guidelines

By establishing the reliability of mammalian cell systems for drug screening and potential cardiac toxicity, the research conducted by Professor Hancox and colleagues at the University of Bristol forms a cornerstone for the validity of mammalian cell line screens for drug certification by pharmaceutical companies (as well as for basic science researchers to better understand how hERG gene products work). Their research not only showed that mammalian cell lines can be used to identify cardiac risk but also revealed some unexpected and clinically important interactions of disparate drugs on cardiac repolarization mechanisms (see above). For example, the US Food and Drug Administration (FDA) had warned about the use of high doses of SSRI antidepressants because of concerns over its association with prolonged QT intervals. This prompted the UK's regulatory body, the Medicines and Healthcare Products Regulatory Agency (MHRA) to amend its dosage guidance so that it no longer recommends high doses of SSRIs because of cardiac risk. However, not all SSRIs have the same risk and in-vitro hERG bioassays can help identify the less dangerous SSRIs (that is, those with least effect on hERG) to allow continued use with less risk and avoid unwarranted concerns as to cardiac safety.[a, b, c]

Impact on pre-clinical screening

For an integrated assessment of cardiac risk in drug discovery programmes, both the current

European Medicines Agency (EMA) and FDA guidelines S7B (2005) recommend the use of mammalian cell line (for example, CHO) hERG screens (hERG expression in CHO in physiological conditions was pioneered by Professor Hancox and colleagues). These guidelines pertained throughout the REF period, and the EMA guidelines were updated in December 2009 in ICH guideline M3 (R2).[c] Virtually all companies now employ screens against hERG for their lead compounds. Such tests are also used to help define structure/activity relationships for lead compounds (these data are commercially sensitive for drug companies and thus cannot be documented here). A 2005 survey of 119 pharmaceutical companies found that 93% employed hERG assays [d] and approximately 71% of test substances have undesired effects on hERG.[e] The hERG screens allows companies to:

- Develop new antiarrhythmic agents directed toward selective hERG channel inhibition. This can be carried out more efficiently with modern automated patch clamp screens using stably transfected mammalian cell lines.
- Determine cardiac toxicity due to promoting long QT syndrome of all other drugs they develop. (It should be noted that there are real concerns as to the validity of small animal models for human conditions which is reduced by using the human cell line approach.)
- Achieve early rejection of prototype drugs. This creates considerable cost savings by preventing progression to expensive animal and clinical trials with subsequent rejection due to long QT effects, which represent about 70% of the estimated development cost of around $800M per drug entity (see http://content.healthaffairs.org/content/25/2/420.long).

Impact of new screening technology

The utility of using hERG assay screens has also led to new spin-offs and development of new hERG-based ion channel screens. Just a few examples of major pharmaceutical laboratories developing and using hERG-based ion channel screens are: Abbot Labs,[f] Pfizer,[g] Millipore,[h] AstraZeneca [i] and Johnson and Johnson.[j] The utility of this method has also led to the development of companies that carry out confidential screening of prototype drugs for the pharmaceutical industry, such as Cytoprotex (Macclesfield, UK http://www.cyprotex.com/toxicology/cardiotoxicity/hergsafety/), which recorded revenues of £8.33m in 2012.

The ability to use mammalian cell lines (such as CHO cells as demonstrated by Professor Hancox) for drug screening is not only cost-effective but has also led to the development of automated patch clamp machines as an important new industry. Some examples are:

- Multichannel Systems: http://www.multichannelsystems.com/products/automated-patch-clamp
- Fluxion: http://www.fluxionbio.com/
- Cytocentrics: http://www.cytocentrics.com/
- Molecular Devices: http://www.moleculardevices.com/Products/Instruments/Automated-Electrophysiology/IonWorks-Quattro.html

Such companies represent a growing $375m industry in the $60-100 billion drug screening industry (Global Industry Analysts Inc. “Top 10 Drug discovery technologies” Market Strategic Analysis and Global Forecasts (2010-2015); Ion Channel Trends 2011, HTS Tec Ltd.).

Impact on drug development legislation

The development of reproducible and documented drug screens also allows regulatory bodies produce guidelines as to how the pharmaceutical industry can document the safety of their compounds; this necessary documentation must be developed before human trials to minimize risk for patients enrolled in the trial.[c]

Societal impact

Societal impact arises from increased patient safety; by uncovering important interactions between non-cardiac drugs and arrhythmia risk, patient mortality is reduced. As one example, during treatment of heroin addicts methadone is usually prescribed but it is now known that methadone also carries a risk for TdP.[k] Finally, impact also arises from replacement of animal models by suitable cell systems that have been documented to reproduce native cell

behaviour. This not only reduces cost and increases speed for drug testing and screening but also reduces ethical concerns on animal use, which has important societal impact. It also fits with the Research Councils' desire to reduce, refine and replace animal use where possible (see also http://www.nc3rs.org.uk/ and http://www.iacuc.org/alternate.htm).

5. Sources to corroborate the impact (indicative maximum of 10 references)

[a] **Evidence for linkage of TdP with serotonin uptake inhibitors and role of hERG**: Kogut, C., Crouse, E.B., Vieweg, W.V.R., Hasnain, M., Baranchuk, A., Digby, G.C., Koneru, J.N., Fernandez, A., Deshmukh, A., Hancox, J.C., Pandurangi, A.K. (2013). Selective serotonin reuptake inhibitors and torsade de pointes: new concepts and new directions derived from a systematic review of case reports. *Therapeutic Advances in Drug Safety*, 4(5), 189–198. DOI: 10.1177/2042098613492366

[b] **Evidence for hERG in TdP and linkage of TdP with serotonin uptake inhibitors**: Witchel, H.J., Hancox, J.C. & Nutt, D.J. (2003). Psychotropic drugs, cardiac arrhythmia, and sudden death. *Journal of Clinical Psychopharmacology*, 23(1), 58–77. PMID: 12544377

[c] **Current regulatory guidelines documenting use of bioassays for hERG effects for pre-clinical safety studies**: EMA ICH M3 (R2) guidelines, "Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals" (2009). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf

[d] **Evidence that pharmaceutical company use of hERG bioassays is widespread**: Friedrichs G.S., Patmore L., and Bass A. (2005) Non-clinical evaluation of ventricular repolarization (ICH S7B): results of an interim survey of international pharmaceutical companies. *Journal of Pharmacological and Toxicological Methods*, 52, 6-11. PMID: 15975833

[e] **MHRA view on the utility of cell-based hERG bioassays**: Shah, R.R. (2005) Drug-induced QT interval prolongation-regulatory guidance and perspectives on hERG channel studies. In hERG Cardiac Potassium Channel: Structure, Function and Long QT Syndrome Book Series: Novartis Foundation Symposium Volume: 266, 251-285. DOI: 10.1002/047002142X

[f] **Evidence that Abbot labs uses hERG bioassays**: Gintant, G (2011) An evaluation of hERG current assay performance: Translating preclinical safety studies to clinical QT prolongation. Pharm. Thera. 129(2): 109-119 DOI: 10.1016/j.pharmthera.2010.08.008.

[g] **Evidence that Pfizer uses hERG bioassays**: Mo, Z-L, Faxel, T., Yang, Y-S, Gallavan, R., Messing, D., Bahinski, A.B. (2009) Effect of compound plate composition on measurement of hERG current IC50 using PatchXpress. *Journal of Pharmacological and Toxicological Methods* 60(1): 39-44 DOI: 10.1016/j.vascn.2009.04.198

[h] **Evidence that Millipore uses hERG bioassays**: Helliwell, Ray M (2008) Recording hERG Potassium Currents and Assessing the Effects of Compounds Using the Whole-Cell Patch-Clamp Technique. Methods in Molecular Biology Book Series: Methods in Molecular Biology. Ed. Lippiat, JD Vol 491:279-295. DOI: 10.1007/978-1-59745-526-8_22

[i] **Evidence that Astra Zeneca uses hERG bioassays**: Bridgland-Taylor, M. H., Hargreaves, A. C., Easter, A., Orme, A., Henthorn, D. C., Ding, M., Davis, A. M., Small, B. G., Heapy, C. G., Abi-Gerges, N., Persson, F., Jacobson, I., Sullivan, M., Albertson, N., Hammond, T. G., Sullivan, E., Valentin, J. -P., Pollard, C. E. (2006) Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks(TM) *Journal of Pharmacological and Toxicological Methods* 54(2):189-199. PMID: 16563806

[j] **Evidence that Johnson & Johnson uses hERG bioassays**: Dubin, AE ; Nasser, N ; Rohrbacher, J ; Hermans, AN ; Marrannes, R ; Grantham, C ; Van Rossem, K ; Cik, M ; Chaplan, SR; Gallacher, D ; Xu, J ; Guia, A; Byrne, NG ; Mathes, C (2005) Identifying modulators of hERG channel activity using the PatchXpress((R)) planar patch clamp. *Journal of Biomolecular Screening* 10(2): 168-181 DOI: 10.1177/1087057104272295

[k] **Evidence for the need to employ hERG drug screening to identify TdP risk in existing drugs:** Methadone-associated Q-T Interval Prolongation and Torsades de Pointes. Stringer, J., Welsh, C., Tommaselli, A (2009). *American Journal of Health-System Pharmacy* 66:825-833. PMID: 19386945