Institution: University of Dundee
Unit of Assessment: UoA1 Clinical Medicine
Title of case study: New Approaches to Drug and Chemical Safety Assessment

1. Summary of the impact

The development of new paradigms for toxicity testing has benefitted the Scottish economy and population in Tayside through two biotechnology companies which, between them, employ up to 40 staff and have had a combined turnover of some £15M over the last five years. The benefits extend to the international pharmaceutical, cosmetic, chemical and consumer product industries, which have gained access to innovative new methods for safety testing at a time of acute need for more predictive methods to evaluate drug safety and better *in vitro* tests for consumer products. Patients and consumers in Europe and worldwide have benefitted indirectly from improved risk assessment of drugs, consumer products and environmental contaminants.

2. Underpinning research

The research underpinning this impact was led by Prof. C. Roland **Wolf** (at the time Director, Biomedical Research Centre; now Director, Medical Research Institute; Ninewells Hospital and Medical School Dundee) and used biotechnology approaches to develop better models for the study of xenobiotic disposition and toxicity. Its aim was to address differences between humans and commonly-used rodent models in terms of responses to xenobiotics and to create *in vitro* systems which can be used to accelerate drug development and reduce candidate attrition in clinical trials.

Research published in 1997 developed new technology for the heterologous expression of functional human cytochrome P450s (P450s) in bacteria, yeast and mammalian cells [i]. The proteins thus generated are used to predict pathways of drug disposition and toxicity, making the safety assessment of drug candidates more predictive of likely outcomes in humans. Cell lines expressing P450s can be used in bioreactors to generate human-specific metabolites, with significant cost savings over what are often challenging chemical syntheses. This work resulted in patents granted worldwide and to the establishment in 1998 of the spinout company Cypex.

The aim of the second phase of this research was to develop novel *in vivo* approaches for the assessment of drug and chemical safety by generating transgenic reporter models in which processes such as oxidative stress can be visualised by histochemical staining [ii] and/or the synthesis of excretable reporter molecules. The results and know-how from this programme were central to the creation in 2002 of the spinout company, CXR Biosciences (CXR).

One of the primary aims of the ongoing collaboration between Dundee Medical School and CXR has been to create genetically-engineered models which can be used to improve the predictivity of human risk assessments. Among the first of these was the Hepatic Reductase Null™ mouse [iii] in which the entire hepatic P450 system is ablated in adult mice allowing the dependence of metabolic and toxic processes on P450-dependent hepatic metabolism to be evaluated.

Of particular note has been the collaboration between the Medical School, CXR, ITI Life Sciences and TaconicArtemis, Cologne which led to the generation of a unique panel of transgenic mouse models humanised for pathways of drug metabolism [iv,v]. By replicating entire pathways of Phase One human drug metabolism in mice, the profound species differences in these pathways were circumvented, allowing more informed predictions of human drug responses to be obtained, accelerating the process of drug development and benefitting the pharmaceutical industry by reducing the proportion of drug candidates which undergo costly failure late in development.

Work in the Medical School has also generated cell-line based reporter systems for the detection of oxidative stress and associated modes of toxicity. These include the human-derived reporter cell line AREc32 [vi], which contains a luciferase gene construct controlled by the antioxidant response element and responds robustly to compounds such as the redox-cycling agent *tert*-

butylhydroquinone which disrupt cellular oxidation status. Such cell lines offer the possibility that the safety of substances such as cosmetic ingredients can, in future, be evaluated without animal testing (http://ec.europa.eu/enterprise/epaa/3_events/3_3_workshops/18-llna-taalman.pdf).

3. References to the research

Key Participants

- Prof John Hayes, Dr Colin Henderson and Dr Michael Pritchard, Biomedical Research Centre, Ninewells Hospital & Medical School, Dundee.
- Prof Bruce Whitelaw, Division of Developmental Biology, The Roslin Institute, University of Edinburgh.
- Dr Nico Scheer, TaconicArtemis GmbH, Cologne, Germany.

References

i. Pritchard MP, Ossetian R, Li DN, Henderson CJ, Burchell B, **Wolf** CR and Friedberg T (1997) A general strategy for the expression of recombinant human cytochrome P450s in Escherichia coli using bacterial signal peptides: expression of CYP3A4, CYP2A6 and CYP2E1. *Arch. Biochem. Biophys.* **345**, 342-354 (DOI: 10.1006/abbi.1997.0265).

ii. Young R, **Wolf** CR, Brown K, Hayes JD and Whitelaw CB (2010) Spatial monitoring of toxicity in HMOX-LacZ transgenic mice. *Transgenic Res*. **19**, 897-902 (DOI: 10.1007/s11248-010-9363-z).

iii. Henderson CJ, Otto DME, Carrie D, Magnuson MA, McLaren AW, Rosewell I and **Wolf** CR (2003) Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J. Biol. Chem.* **278**, 13480-13486 (DOI: 10.1074/jbc.M212087200).

iv. Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N and **Wolf** CR (2008) A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *J. Clin. Invest.* **118**, 3228-3239 (DOI: 10.1172/JCI35483).

v. Scheer N, Kapelyukh Y, McEwan J, Beuger V, Stanley LA, Rode A and **Wolf** CR (2011) Modelling human cytochrome P450 2D6 metabolism and drug-drug interaction by a novel panel of knockout and humanized mouse lines. *Mol. Pharm.* **81**, 63-72 (DOI: 10.1124/mol.111.075192).

vi. Wang XJ, Hayes JD and **Wolf** CR (2006) Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of nrf2 by cancer chemotherapeutic agents. *Cancer Res.* **66**, 10983-10994 (DOI: 10.1158/0008-5472.CAN-06-2298).

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- **Wolf** CR *et al*: MRC/BBSRC-funded LINK P450 Programme; Industry Sponsors: Astra, Glaxo, Janssen Pharmaceutica, Lilly, Novo Nordisk, Parke-Davis, Pfizer, Roche Products, Sanofi-Winthrop, Servier, SmithKline Beecham, Wellcome, Wyeth (1993-1998) £3.5M.
- **Wolf** CR *et al:* The Toxicology Consortium; Industry Sponsors: AstraZeneca, Eli Lilly, GlaxoSmithKline, Novartis, Pfizer, Roche, Wyeth (1998-2005) £6.0M.
- CXR Biosciences and TaconicArtemis: ITI Life Sciences Transgenic Screening and Safety Models (TSM/TSE); Scottish Enterprise (2005-2009) £8.0M.
- **Wolf** CR *et al:* MARCAR; EU Innovative Medicines Initiative Award (2010-2015) Total budget €25M; €12M in kind from industrial sponsors; Dundee component £1.1M.
- **Wolf** CR: REDOX; European Research Council Senior Investigator Award (2011-2016) £2.1M.

Patents

- **Wolf** CR, Friedberg TH and Pritchard MP: Cytochrome P450 expression in enterobacteria; Patent Numbers EP0914446, US6566108, CA22559619; assigned to British Technology Group (BTG).
- **Wolf** CR and Henderson CJ: Transgenic animals for assessing drug metabolism and toxicity in man; Patent Numbers EP1711051, US2009013417. Patents owned by Imperial Cancer Research Technology; licensed to CXR and five pharmaceutical companies which participated in the Toxicology Consortium.
- **Wolf** CR and Henderson CJ: Modulation of cytochrome P450 reductase activity; Patent Numbers EP1521827, EP2065464, US7700822, CA2403235, JP005532807, GB2391231. Patents owned by Imperial Cancer Research Technology.
- **Wolf** CR and Clark AJ: Methods and kits for drug screening and toxicity testing using promoter-reporter cells derived from embryonic stem cells; Patent Number EP1893749, US2006292694, US2006292695, US2008152632, CA2613529,JP2008546417. Patents owned by CXR Biosciences/Geron Corporation.

4. Details of the impact

This research has benefitted the Scottish economy and local population through the establishment of two biotechnology companies, Cypex and CXR which, between them, employ up to 40 staff and generate an average annual turnover of £2.4M. Professor **Wolf**, a Director of CXR from its formation until August 2012 and a member of the Scientific Advisory Group of the Translational Medicine Research Initiative, received the OBE in 2010 for his contribution to life sciences in Scotland.

Cypex (www.cypex.co.uk) was formed in 1998 and has, since releasing its first recombinant P450 in 2000, developed a portfolio of >100 products including 19 human P450s, eight sulphotransferases, murine and canine P450s, fine chemicals and antibodies. These are marketed in Europe via Tebu-Bio [1], in the US via XenoTech LLC [2] and in Asia and Africa. Their use helps pharmaceutical companies to eliminate drug candidates which are likely to fail during development or cause drug-drug interactions in patients. Cypex also offers contract services in custom protein expression and the production of drug metabolites. Its products (in the form of cell lines which express human P450s) are used in bioreactors to generate drug metabolites in sufficient quantities for toxicological evaluation, as is now required by the FDA when human-specific metabolites represent more than 10% of the administered dose [3]. This application has been adopted by pharmaceutical companies including Novartis and Hoffmann-La Roche [4]; the intellectual property has been licensed to BTG for further commercialisation and Novartis has licensed the technology (£400K) for further marketing and use.

CXR (www.cxrbiosciences.com) was formed in 2002 to provide services in preclinical drug development and chemical safety. It currently has >60 clients worldwide and has pioneered the application of humanised mouse models in drug development. It opened its first international sales office in the USA in May 2012 [5]. It also markets a number of cell lines and other reagents derived from University-based research [ii,iii,vi]; these include the Hepatic Reductase Null™ mice and a range of reporter mice, licensed for marketing by CXR via Taconic in the USA and Europe. AstraZeneca, Pfizer and Nestlé have also taken out licenses to these models.

The second aspect of the impact of this research has had international reach, benefitting the pharmaceutical industry and regulators. This aspect arose from an ITI Life Sciences-sponsored research and development programme (2005-2009) in which CXR and TaconicArtemis GmbH (Cologne) collaborated in the generation of humanised mouse models to determine the human-specific systemic effects of drugs and chemicals [iv,v]. The generation of these exciting new research tools, which are now marketed via Taconic [6,7], has been welcomed by pharmaceutical companies and regulators because of their applicability in drug regulatory submissions [7].

These models have important applications in, for example, allowing the species specificity of non-genotoxic carcinogenesis to be established [8]. This is central to the predictive risk assessment of

drugs and consumer products; to that end the use of these humanised and reporter models is embedded in the EU Innovative Medicines Initiative programmes MARCAR (http://www.imi-marcar.eu/partners.html) and DILI (http://www.imi.europa.eu/content/mip-dili), whose aim is to identify new biomarkers to predict the effects of non-genotoxic carcinogens and predict the potential for drug-induced liver injury. CXR is a key small/medium enterprise participant in this €25M collaboration, led by Dundee, between academia, the pharmaceutical industry and drug regulators, and the extent of industrial involvement (>€5M) is a direct result of the Dundee team's track record in developing commercially-applicable novel models for assessing chemical safety.

In a third aspect of impact, this research has benefitted industry, patients and consumers in Europe and beyond as a result of the development of innovative methods for *in vitro* toxicity testing. Better *in vitro* testing methods are urgently needed as a result of the enactment in 2007 of the REACH regulations, which demand toxicity testing of some 30,000 substances using non-animal methods wherever possible, and the staged implementation of the 7th Amendment to the EU Cosmetics Directive, which has prohibited the use of animals for skin irritation, corrosion, and genotoxicity testing since 2009 and for characterising the disposition of cosmetic ingredients since March 2013. The AREc32 cell line [iv], licensed via CXR to Givaudan, has been shown to be useful as part of an integrated testing strategy for the prediction of chemical-induced skin hypersensitivity reactions without the need for animal testing [9,10] and has been adopted for water quality assessment and the evaluation of disinfection by-products by institutions in Australia and Germany [11]. The use of this cell line provides the pharmaceutical, cosmetic, consumer product and chemicals industries with a cost-effective, legally-permissible method for assessing potential skin sensitisers and benefits patients and consumers by improving safety assessments.

5. Sources to corroborate the impact

1. Example press release dated 24-Oct-08 at http://www.prlog.org/10132610-tebu-bio-and-cypex-ltd-introduce-seven-new-dog-bactosomes.html.
2. Cypex-Xenotech product brochure at http://204.12.1.74/uploaded/documents/Cypex.pdf.
3. FDA Guidance for Industry Safety Testing of Drug Metabolites (February 2008) http://www.fda.gov/OHRMS/DOCKETS/98fr/FDA-2008-D-0065-GDL.pdf.
4. Letter of corroboration from PTDCA Biocatalysis Department, F. Hoffmann-La Roche Ltd.
5. Press release dated 17-May-12; http://www.cxrbiosciences.com/news_article.php?news_id=43.
6. tADMET™: A unique and evolving portfolio of **t**ranslational (humanized & knockout) mouse models, *in vitro* tools and services for an improved prediction of the **A**bsorption, **D**istribution, **M**etabolism, **E**xcretion and **T**oxicity characteristics of new compounds in humans. http://www.taconic.com/wmspage.cfm?parm1=1792.
7. Letter of corroboration from TaconicArtemis GmbH, Cologne, Germany.
8. Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, Henderson CJ, **Wolf** CR and Elcombe CR (2010) Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. *Toxicol. Sci.* **116**, 452-66 (DOI: 10.1093/toxsci/kfq118).
9. Natsch A, Emter R and Ellis G (2009) Filling the Concept with Data: Integrating Data from Different *In Vitro* and *In Silico* Assays on Skin Sensitizers to Explore the Battery Approach for Animal-Free Skin Sensitization Testing *Toxicol. Sci.* **107**, 106–121 (DOI:10.1093/toxsci/kfn204).
10. Presentation by Joanna Matheson, US Consumer Product Safety Commission, "OECD Dermal Sensitization AOP: Regulatory Perspective" on the US Environmental Protection Agency website; http://www.epa.gov/oppfead1/cb/ppdc/testing/2013/july/workshop/session2-oecd.pdf.
11. Escher BI, van Daele C, Dutt M, Tang JYM and Altenburger R (2013) Most Oxidative Stress Response In Water Samples Comes From Unknown Chemicals: The Need For Effect-Based Water Quality Trigger Values. *Environ. Sci. Technol.* **47**, 7002−7011 (DOI: dx.doi.org/10.1021/es304793h).