Institution: University of Manchester

Unit of Assessment: UoA5

Title of case study: Accurate *in vitro* prediction of *in vivo* genotoxicity and cancer hazard; reducing costs to industry and the use of animals in research

1. Summary of the impact

Development of the human cell GADD45a assay enabled accurate identification of carcinogens *in vitro*, with a low rate of misleading positives. Through the spin-out company Gentronix, this research is reducing costs to industry and decreasing the use of animals in research. Industrial collaboration has enabled commercial adoption of the technology in many sectors. With a 10-fold increase in orders in 2012 versus 2008, Gentronix is a profitable business employing 17 people and with an annual turnover of £1.88m. During 2008-12, Gentronix released a series of new products, established testing services, and signed a product license agreement with GlaxoSmithKline. More than 100 companies worldwide are using Gentronix kits, including pharmaceutical, agricultural and health and beauty companies, along with manufacturers of food flavourings and household goods. The Gentronix assay is currently being reviewed by the European Centre for the Validation of Alternative Methods.

2. Underpinning research

The impact is based on research initiated in 1996, and continuing. The key researchers at the University of Manchester (UoM) were:

Professor Richard Walmsley (1996 to date, Gentronix Founder & Scientific Director, 1999 to date)
Dr Nick Billinton (PhD student, 1996-1999; Gentronix Scientist, 1999 to date)
Mr Paul Cahill (Research Assistant, 1996-1999; Gentronix Lab Manager, 1999 to date)
Dr Andrew Knight (Post-Doctoral Research Associate, 1997-2000; Gentronix Scientist, 2000-2012)
Dr Christopher Jagger (Post-Doctoral Research Associate, 2006-2008; Gentronix Scientist, 2008 to date)
Dr Mathew Tate (Post-Doctoral Research Associate, 2006-2008; Gentronix Senior Scientist, 2006 to date)
Christopher Hughes (Research Assistant, 2007-2008; Gentronix Technician, 2008 to date)

The initial aim of this research was to develop a screening assay for genes involved in the DNA damage response. A reporter assay was developed, linking Green Fluorescent Protein (GFP) expression to expression of the yeast RAD54 DNA repair gene [1] (*Yeast cell genotoxicity* patents granted, PCT/GB98/00786). Cells exposed to the potent genotoxic carcinogen methane methyl sulfonate became increasingly fluorescent, making it apparent that the assay might have an additional and valuable use in the detection of genotoxic carcinogens. The assay was validated against diverse mechanistic classes of genotoxic carcinogen [1]. The field of genetic toxicology was discovering that the then internationally required battery of regulatory genotoxicity tests was very effective in producing positive results for carcinogens, but also produced positive results for most non-carcinogens (i.e. 'misleading positives', sometimes called 'false positives'). The time was right for new more accurate assays, and the yeast test produced fewer misleading positives. The group went on to investigate the feasibility of using human cell reporters:

- An improved assay using human cells and linked to GFP expression ("GreenScreen HC") was developed from 2003 and patented in 2005 (*Human cell genotoxicity* patents, PCT/GB2005/001913, granted in EC, Canada, USA, Japan and China). The human GADD45a gene in the new reporter is unrelated to the yeast RAD54 gene, but was known to be induced by DNA damage. A key finding, exploited in the new reporter, was that DNA sequences in intron 3 were required for the proper biological response to DNA damage. These included a p53 response element, which Walmsley's research group subsequently implicated in regulation of GADD45a through its promoter WT1 element. Initial validation and subsequent studies [2, 3] showed the GADD45a assay to have far superior specificity to commonly used regulatory tests.
- In 2008, a modified assay was developed to detect compounds that become genotoxic following metabolism in animals [4].

- In 2008-10 the lab led two international 'ring trials', through collaboration with three global pharmaceutical and household products companies. These trials validated the transferability and reproducibility of GreenScreen HC with and without [5] S9 metabolic activation.
- In 2010 a new version of the GADD45a assay was developed, "BlueScreen HC", with and without metabolism. Replacement of GFP with the Gaussia 'flash' luciferase gene in this assay (Genotoxicity luciferase patents pending, PCT/GB2010/000581) allowed the testing of coloured and fluorescent compounds that interfered with GFP fluorescence. It also allowed higher throughput deployment [6]. BlueScreen HC was further developed for 384-well deployment in collaboration with GlaxoSmithKline.

3. References to the research

Genetic Toxicology is a specialist area encompassing academic, regulatory and industrial interest groups. In order to make impact in this field, it is necessary to publish in the appropriate specialist journals. Birrell et al [2] was featured in the 25 Hottest Articles from Mutation Research/Genetic Toxicology and Environmental Mutagenesis in 2010.

1. **Walmsley, R.M.**, **Billinton, N.**, Heyer, W.D. (1997) Green fluorescent protein as a reporter for the DNA damage-induced gene RAD54 in *Saccharomyces cerevisiae*. *Yeast*. 13 (16). p. 1535-1545. DOI:10.1002/(SICI)1097-0061(199712)13:16<1535::AID-YEA221>3.0.CO;2-2
2. Birrell, L., **Cahill, P., Hughes, C.**, **Tate, M., Walmsley, R.M.** (2010) GADD45a-GFP GreenScreen HC assay results for the ECVAM recommended lists of genotoxic and non-genotoxic chemicals for assessment of new genotoxicity tests. *Mutation Research.* 695. p. 87-95. DOI:10.1016/j.mrgentox.2009.12.008
3. **Jagger, C., Tate, M.**, **Cahill, P.A., Hughes, C., Knight, A.W., Billinton, N., Walmsley, R.M.** (2009) Assessment of the genotoxicity of S9-generated metabolites using the GreenScreen HC GADD45a–GFP assay. *Mutagenesis.* 24 (1). p. 35-50. DOI:10.1093/mutage/gen050
4. **Billinton, N.,** Hastwell, P.W., Beerens, D., Birrell, L., Ellis, P., Maskell, S., Webster, T.W., Windebank, S., Woestenborghs, F., Lynch, A.M., Scott, A.D., Tweats, D.J., van Gompel, J., Rees, R.W., **Walmsley, R.M.** (2008) Inter-laboratory assessment of the GreenScreen HC GADD45a-GFP genotoxicity screening assay: an enabling study for independent validation as an alternative method. *Mutation Research.* 654. p. 23-33. DOI:10.1016/j.mrgentox.2008.02.011
5. Topham, C.H., **Billinton, N., Walmsley, R.M.** (2012) Non-genotoxic apoptosis inducers do not produce misleading positive results in the TK6 cell-based GADD45a-GFP genotoxicity assay. *Toxicological Sciences.* 128 (1). p. 79-91. DOI:10.1093/toxsci/kfs132
6. **Hughes, C.,** Rabinowitz, A., **Tate, M.,** Birrell, L., Allsup, J., **Billinton, N., Walmsley, R.M.** (2012) Development of a high-throughput Gaussia luciferase reporter assay for the activation of the GADD45a gene by mutagens, promutagens, clastogens, and aneugens. *Journal of Biomolecular Screening*. 17 (10). p. 1302-1315. DOI:10.1177/1087057112453312

4. Details of the impact

Context

Internationally required regulatory genotoxicity tests are very effective in producing positive results for carcinogens. However, they can also produce positive results for most non-carcinogens which are termed 'misleading positives'. Walmsley and his team realised that the development of a more accurate assay would reduce the number of misleading positives and ultimately save money and animals.

Pathways to impact

Walmsley founded the spin out company Gentronix in 1999. The direct beneficiaries of the company's technology cover diverse sectors, including pharmaceuticals, biotechnology, agrochemicals, foodstuffs, biomaterials, flavours and fragrances. Accurate early *in vitro* screening, as provided by the Gentronix GADD45a assays, reduces the need for animal testing and can rescue compounds misclassified as genotoxins by other tests, which that might otherwise be

discarded, thereby saving these industries considerable sums of money.

Reach and significance of the impact

Business growth and performance:

Gentronix is a successful and growing business. It relied on private and university investments of £3m from 1999 to 2010, and became profitable in 2011. In the year ending August 2012, there were 286 revenue generating orders, representing a 32% increase on the same period in 2011. A significant development was customers taking advantage of the rapid testing service, which accounted for 78% of revenue. Despite flat or declining revenues in the broader economy the total revenue from orders during the 2012 financial year was £1.88m [A], representing an increase of 50% over 2011 and 330% over the financial year ending 2008. Cumulative revenues since 2008 are £5.54m. Gentronix employs 17 full-time staff. Key to the success of the company has been the retention of a core team of four scientists from the time when the company was founded.

Reducing the numbers of animals used in research:

In 2007, the European Centre for the Validation of Alternative Methods (ECVAM) recognised the critical need to reduce the misleading positives in *in vitro* genotoxicity assays to avoid unnecessary animal testing [B]. Whilst only 30-60 animals are used for a regulatory *in vivo* genotoxicity study when *in vitro* genotoxicity results are negative, a 'positive' *in vitro* genotoxicity assay can trigger the use of up to 200 animals in follow-up mechanistic studies. Typically, a two year rodent study on a novel compound destined for use as pharmaceuticals, household products or cosmetics can use 400-500 rats and mice per compound. Historically, many of those animal studies were needless because the *in vitro* result was misleading.

The high specificity of the GreenScreen HC assay produces fewer, but reliable positive results, and hence triggers fewer needless animal studies [text removed for publication]. Also in 2007, the UK National Centre for the Replacement, Refinement and Reduction of animals in research (NC3Rs) awarded the 'Reduction' prize to Gentronix, following the successful completion of research they sponsored in the Walmsley lab [C]. This supported the development of a modified GreenScreen assay that included the addition of minute quantities of preserved rodent liver extracts. This allowed the detection of genotoxic metabolites, a key requirement for *in vitro* assays.

Reducing drug development costs for the pharmaceutical industry:

In the pharmaceutical industry it can cost $6m to get a compound to first time in human. Most compounds do not get that far so it is important to identify liabilities early on. Regulatory preclinical genotoxicity safety assessment tests require gram quantities of product and cost $60k to complete. The Gentronix test costs $250 to $2,500, and requires only sub-milligram quantities of product, which is all that is available in early screening. Over 70 companies, predominantly in the EC, US and Japan now use the test, either using kits, or by sending compounds to the Manchester labs for service testing. For example [text removed for publication] flavour and fragrance molecules are currently being tested for the Research Institute for Fragrance Molecules.

Positive results in regulatory tests can be misleading, but a positive result during preclinical safety assessment can trigger additional mechanistic studies delaying pre-booked clinical trials, costing millions of dollars. Early screening with Gentronix tests reduces late stage failure. Independent users of the GADD45a assay in the global pharmaceutical industry – Galderma (GreenScreen HC) [D] and GlaxoSmithKline (BlueScreen HC) [E] have described the utility of the assay in peer-reviewed manuscripts. *"BlueScreen-384 was found to reduce the need for costly and time-consuming analogue testing using traditional genotoxicity tests, such as the Ames test"* [E].

Collaborations with industry:

More than 100 pharmaceutical and fine chemical companies in 17 countries have used the Gentronix assays and services as part of their genotoxicity profiling strategy.

In the development of GreenScreen HC, Gentronix performed international ring trials to demonstrate its transferability and reproducibility to industry sectors [6]. This saw the company working with Unilever (UK), GSK (UK), Johnson & Johnson (Belgium), BioReliance Corp. (USA), Leo Pharma (Denmark).

New products, services, partnerships and license agreements:

The GADD45a assay has attracted new customers every year across the US, UK and Europe and it is continuing to expand further. The assay has been developed in GreenScreen HC and BlueScreen HC forms, and both are available with and without 'S9' metabolic activation. Customers gain access to the assay by buying test kit products, undertaking Gentronix in-house service testing, undertaking service testing through third-party contract research organisations (CROs) or by signing a license agreement:

- In 2010 GlaxoSmithKline, which had supported early development of the GADD45a assay for high throughput screening (384-well format), signed a 3-year license agreement for use of GreenScreen and BlueScreen HC in genotoxicity screening [F].
- Gentronix has formed partnerships with international CROs including BioReliance, Charles River, and LGC Standards.
- GreenScreen HC with S9 was introduced in 2008, leading to a dramatic increase in sales (80%) and BlueScreen HC with and without S9 was introduced in 2010. The latter opened up new markets excluded by patents on GFP. For example in the flavours and fragrance industry, 500 compounds were tested in 2012.
- In-house service testing began in 2008 with GreenScreen HC (+/-S9), and has expanded through the formation of a strategic partnership with Apredica in 2010 to include BlueScreen HC (2010), the Comet and micronucleus assays and in 2011 the Ames test. In 2012, Gentronix announced its first screening contract in Japan, with the pharmaceutical manufacturer Kowa.

Recognition by policy-making and validation/regulatory bodies:

Walmsley has given invited presentations to national and international policy makers such as the Department of Health Committee on Mutagenicity and the International Life Sciences Institute. Both presentations led to published guidance in 2011 that highlight the assay [G, H].

In 2009 Walmsley gave an invited presentation on the GADD45a assay to the New Chemical Entity review group of the US Food and Drugs Administration (FDA). A key step towards the assay being approved by a regulatory body such as the FDA is recognition and assessment by an international validation authority. At the request of one such validation authority, ECVAM, Walmsley undertook a "Step 1, pre-submission", which was accepted on 29/06/12. As a result the GreenScreen HC protocols are now published in the INVITTOX database (available for over 2500 registered users in 75 countries). A "Step 2, Complete submission of project" to ECVAM was made in April 2013.

5. Sources to corroborate the impact

A. Gentronix audited accounts VAT ref GB727056828; Company registration 03810162. Available on request.

B. Kirkland et al. "How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop". Mutation Research. 2007 Mar 30;628(1):31-55. doi:10.1016/j.mrgentox.2006.11.008

C. UK NC3Rs Award: http://www.nc3rs.org.uk/news.asp?id=414

D. Galderma: Luzy et al. "Evaluation of the GADD45a-GFP GreenScreen HC assay for rapid and reliable in vitro early genotoxicity screening". J Appl. Toxicol. 2012; DOI:10.1002/jat.2793

E. GlaxoSmithKline: Simpson et al. "The BlueScreen-384 assay as an indicator of genotoxic hazard potential in early stage drug discovery". J Biomol Screen. 2013 Apr;18(4):441-52 DOI: 10.1177/1087057112470858

F. GlaxoSmithKline license agreement: http://www.pharmpro.com/news/2010/03/outsourcing-news-Gentronix-Signs-Three-Year-Agreement-with-GlaxoSmithKline-for-GreenScreen-HC-and-BlueScreen-HC-Genotoxicity-Assays/

G. Strategy for testing chemicals for mutagenesis, September 2011 http://iacom.org.uk/guidstate/documents/COMGuidanceFINAL.pdf

H. Lynch A.M., et al. New and Emerging Technologies for Genetic Toxicity Testing. Environmental and Molecular Mutagenesis 2011; 52(3): 205-23. DOI:10.1002/em.20614