Institution: Queen's University Belfast

Unit of Assessment: 1

Title of case study: Development and Commercialization of a Technology Platform to Enable Biomarker Discovery and Validation

1. Summary of the impact

Research at Queen's University Belfast has led to the successful development and commercialization of a DNA chip technology platform that facilitates the rapid discovery and validation of new diagnostic tests in cancer. A spin out company has been established called Almac Diagnostics that currently employs 85 staff, thereby significantly contributing to the knowledge based economy in Northern Ireland. Almac has used this technology to develop and validate a number of genomic tests that have been successfully licensed to established US based diagnostic companies, thereby securing long term revenue streams. Almac is now recognised internationally as a worldwide industry leader in this area.

2. Underpinning research

A key issue holding back successful gene expression based biomarker discovery and validation projects in oncology is access to well annotated tissue banks with linked clinical information. The majority of high throughput gene expression technologies require high quality fresh frozen tumour samples to obtain good quality RNA for reproducible expression studies. This has created a major bottleneck since very few such tumour banks exist. Professors Harkin (Professor of Molecular Oncology since 2004 at Queen's) and Johnston (Professor of Oncology at Queen's since 1996) developed two new array based technology platforms termed DSA and Xcel capable of generating robust gene expression data from partially degraded RNA derived from formalin-fixed, paraffin-embedded (FFPE) material[1,2] and as a result it is now possible to exploit the many high quality banks of FFPE tumours to both develop and validate new gene expression based tests to improve the management of cancer patients.

Two major innovations based on the research by Harkin and Johnston underpin the generation of the DSA and Xcel arrays. The first concerns the content of the arrays. Although it has been established that the human genome encodes approximately 23,000 protein coding genes, it is now clear that many non-coding RNA species that are not well characterized, but have high functional significance are also generated. Harkin and Johnston initiated a high throughput sequencing programme at Queen's to identify the complete transcriptome for the five major cancers: breast, colon, lung, prostate and ovarian cancer. All the sequence information was aligned to identify the unique transcripts from each individual disease type and that information was used to develop a panel of cancer disease specific arrays (DSA's).

The second major innovation relates to the design of the probe sets on the arrays to detect transcript expression from degraded RNA derived from FFPE material. Harkin and Johnston observed that the 3-'ends of transcripts extracted from FFPE tumours tended to be protected from degradation by the poly-A tail. As a consequence the arrays were designed to specifically probe the expression of the extreme 3' ends of each transcript resulting in a significant improvement in the ability to detect gene expression from FFPE derived RNA. Through a partnership agreement with Affymetrix, the world leader in microarray manufacture, arrays were generated for lung, colon, breast, prostate and ovarian cancer [1,2,3,4,].

Subsequently, the information from the individual DSA's was combined to generate the Xcel array which encodes approximately 92,000 unique transcripts representing the most comprehensive coverage of the cancer transcriptome. Proof of the clinical utility of the technology came from further development of two cancer diagnostic tests. The first of these termed Col-Dx identifies patients at high risk of recurrence following surgery for stage II colon cancer[5]. A second assay termed DDRD-Breast-Dx predicts the benefit from DNA damage based chemotherapy in node negative and node positive breast cancer and is now in press in the Journal of the National Cancer Institute[6].

3. References to the research

1. Farragher SM, Tanney A, Kennedy RD, **Paul Harkin D**. RNA expression analysis from formalin-fixed, paraffin-embedded tissues. Histochem Cell Biol. 2008 Sep;130(3):435-45. doi: 10.1007/s00418-008-0479-7. Epub 2008 Aug 5. Review

This manuscript highlights Almac's expertise in the analysis of degraded RNA derived from FFPE tissue (cited 99 times).

2. Tanney A, Oliver GR, Farztdinov V, Kennedy RD, Mulligan JM, Fulton CE, Farragher SM, Field JK, **Johnston PG, Harkin DP**, Proutski V, Mulligan KA. Generation of a non-small cell lung cancer transcriptome microarray. BMC Med Genomics. 2008 May 30;1:20. doi: 10.1186/1755-8794-1-20

This represents the first publication highlighting the approach taken to develop Almac's Disease Specific Array platforms (cited 14 times).

3. Hosey, A.M., Gorski, J.J., Murray, M.M., Quinn, J.E., Chung, W.Y., Stewart, G.E., James, C.R., Farragher, S.M., Mulligan, J.M., Scott, A.N., Dervan, P.A., **Johnston, P.G.**, Couch, F.J., Daly, P.A., Kay, E., McCann, A., Mullan, P.B. and **Harkin, D.P.** (2008) "Molecular Basis for Estrogen Receptor Alpha Deficiency in BRCA1-Linked Breast Cancer." Journal of the National Cancer Institute 99(22): 1683-1694. Doi: 10.1093/jnci/djm207.

This represents the first manuscript in which the breast cancer DSA was used to translate biology identified using *in vitro* models in a clinical setting.
Impact Factor: 14.3 paper cited 105 times.

4. Tejpar S, Bertagnolli M, Bosman F, Lenz HJ, Garraway L, Waldman F, Warren R, Bild A, Collins-Brennan D, Hahn H, **Harkin DP**, Kennedy R, Ilyas M, Morreau H, Proutski V, Swanton C, Tomlinson I, Delorenzi M, Fiocca R, Van Cutsem E, Roth A. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. The Oncologist. 2010;15(4):390-404. doi: 10.1634/theoncologist.2009-0233. Epub 2010 Mar 29. Review.

This review was the result of an international collaboration on how to best advance the integration of genomic technologies to aid prognosis and prediction in colorectal cancer (cited 55 times).

5. Kennedy RD, Bylesjo M, Kerr P, Davison T, Black JM, Kay EW, Holt RJ, Proutski V, Ahdesmaki M, Farztdinov V, Goffard N, Hey P, McDyer F, Mulligan K, Mussen J, O'Brien E, Oliver G, Walker SM, Mulligan JM, Wilson C, Winter A, O'Donoghue D, Mulcahy H, O'Sullivan J, Sheahan K, Hyland J, Dhir R, Bathe OF, Winqvist O, Manne U, Shanmugam C, Ramaswamy S, Leon EJ, Smith WI Jr, McDermott U, Wilson RH, Longley D, Marshall J, Cummins R, Sargent DJ, **Johnston PG, Harkin DP**. Development and independent validation of a prognostic assay for stage II colon cancer using formalin-fixed paraffin-embedded tissue. J Clin Oncol. 2011 Dec 10;29(35):4620-6. doi: 10.1200/JCO.2011.35.4498. Epub 2011 Nov 7

This manuscript represents the first successful discovery and validation of a colon cancer prognostic assay from historical FFPE samples using microarray technology. The work represented a major collaborative effort across 10 global Cancer Centres in order to ensure a representative patient population for the validation study.
Impact Factor: 18; paper cited 30 times.

6. Mulligan JM, Hill LA, Deharo S, Irwin GW, Boyle D, Keating KE, Raji OY, McDyre FA, O'Brien E, Bylesjo M, Quinn JE, Lindor NM, Mullan PB, James CR, Walker SM, Kerr P, Salto-Tellez M, James J, Davison TS, Proutski V, **Johnston PG**, Couch FJ, Harkin DP, Kennedy DK. Identification and validation of an anthracycline/cyclophosphamide based chemotherapy response assay in breast cancer. Journal of the National Cancer Institute (In Press).

This manuscript describes the identification and validation of a microarray based test to predict response to DNA damage based chemotherapy in early stage breast cancer (Journal Impact Factor is 14.3).

Grants Awarded:
Technology Strategy Board: Awarded a grant of £2.3 Million to Almac Diagnostics in July 2013 to fund the further development of its breast cancer DDRD test for launch into the UK market. This represents one of only four such awards across the UK and highlights the clinical utility of Almac's breast cancer test.

4. Details of the impact

The research highlighted above has had a significant economic impact on the local economy in N. Ireland by the development of a company called Almac Diagnostics in 2004. Without the research by Harkin and Johnston this company would not have been established. However, more importantly, it has allowed the development of new diagnostic products, which are likely to improve the management of colon and breast cancer patients. Almac's proprietary microarray technology has been the basis of the company's success both in terms of its internal research and development programme and its external contract research commercial activities. In October of 2012 the Xcel array was licensed by Affymetrix for global distribution based on the realization that this technology platform substantially accelerates the rate at which novel array-based biomarkers can be discovered, validated and commercialized[1]. This licensing deal involved an upfront technology access fee in addition to volume based milestone payments and royalty payments for the lifetime of the product. To date Almac has chosen to retain exclusive distribution rights to its DSA technology platforms. As stated above Almac has also used its DSA technology to discover and validate two genomic tests in colon and breast cancer.

The first of these tests termed, Col-Dx, identifies those patients who are at an increased risk of recurrence following surgery for stage II colon cancer. This information can help clinicians and patients make a more informed decision about the need for additional chemotherapy to optimally manage their disease. The Col-Dx assay was licensed to Precision Therapeutics, a US based diagnostic company for commercialization in the US market. The Col-Dx test (rebranded as GeneFx) has seen Clinical Laboratory Improvements Amendments (CLIA) validated for use in the US and has subsequently been launched [2]. Significantly, Almac has carried out a second successful independent validation of the GeneFx assay in collaboration with the Cancer and Leukemia Group B (CALGB) clinical trial group in the US. The results from this study are currently embargoed but will be presented at the American Society for Clinical Oncology meeting in January 2014.

Almac's second genomic test termed DDRD-Breast-Dx, identifies early stage node negative and node positive breast cancer patients who are likely to benefit from the addition of chemotherapy following surgery. Existing prognostic tools classify early stage patients into low, intermediate and high risk of recurrence. Low risk patients are not recommended for chemotherapy whilst high risk patients are. However, approximately 40-60% of patients are classified as intermediate risk where the benefit from chemotherapy is unclear. For these patients the DDRD-Breast-Dx test allows clinicians to make an objective decision on likely benefits of chemotherapy. Almac's DDRD-Breast-Dx assay has also been successfully licensed to a major US diagnostic company. The successful commercialization of Almac's core technology platform to Affymetrix and the subsequent licensing of two highly complex genomic cancer assays into the US market has established Almac Diagnostics as a recognised international leader in the cancer diagnostics industry[3,4,5,6].

In 2012 Almac announced the opening of its CLIA laboratory in Craigavon, N. Ireland[7]. This laboratory, which has been approved by the College of American Pathologists, was established to facilitate the delivery of novel diagnostic tests to help select patients for enrolment in pharma sponsored biomarker driven clinical trials. Currently Almac is supporting approximately 15 phase I and II global clinical trials from its CLIA laboratory, which further emphasises its prominence with the pharmaceutical industry.

The economic importance of Almac Diagnostics is evidenced by its continued growth and expansion of its operations in Europe and the US. Specifically over the last 5 years a total of 50 new positions have been created within Almac Diagnostics across areas such as molecular biology, bioinformatics, project management, business development and marketing. Furthermore approximately 90% of the staff employed are graduates and 50% have PhD qualifications emphasising the contribution research has made to the knowledge-based economy in N. Ireland. In addition, Almac Diagnostics opened an office in Manchester in 2009 as a hub for bioinformatics support and now employs 4 members of staff in that location as well as two business development managers. Finally, Almac Diagnostics have also expanded into the US market and now employ two business development managers there. Almac's reputation in the cancer diagnostics industry has led to substantial new opportunities for the business through strategic partnerships with many of the world's leading pharmaceutical companies. In 2009, Almac announced a strategic partnership with Pfizer, the world's largest pharmaceutical company. The agreement provided Pfizer access to Almac's Colon-DSA array to discover and validate new predictive markers of response to chemotherapy in colon cancer[5]. Similarly in 2009, Almac announced a partnership with Eli-Lilly and the Medical Research Council to help develop new predictive markers for drugs within their pipelines[6].

5. Sources to corroborate the impact

1 **Almac and Affymetrix Announce a Global Distribution Agreement for Almac's Xcel™ Array** 29 Mar 2012 http://www.almacgroup.com/2012/03/almac-and-affymetrix-announce-a-global-distribution-agreement-for-almac%E2%80%99s-xcel%E2%84%A2-array/

2 **Launch of GeneFx by Precision Therapeutics**

http://www.genefxcolon.com/

3 **Almac Collect Top Recognition for New Ovarian Cancer Research Product**, March 10, 2009 http://www.almacgroup.com/2009/03/almac-collect-top-recognition-for-new-ovarian-cancer-research-product/

4 **Almac wins top award at Tech Idol Showcase**, December 18, 2007 http://www.almacgroup.com/2007/12/almac-wins-top-award-at-tech-idol-showcase/

5 **Almac Announces Collaboration with Pfizer and the PETACC3 Translational Research Working Party** May 18, 2009 http://www.almacgroup.com/2009/05/almac-announces-collaboration-with-pfizer-and-the-petacc3-translational-research-working-party/

6 **Almac and Lilly Partner on Companion Diagnostic Development**, September 15, 2009 http://www.almacgroup.com/2009/09/almac-and-lilly-partner-on-companion-diagnostic-development/

7 **Almac open CLIA laboratory in Craigavon, N. Ireland to support biomarker driven clinical trials**. http://www.almacgroup.com/2011/03/almac-open-clia-lab-for-biomarker-clinical-trials/