Institution: University of Southampton

Unit of Assessment: 08 Chemistry

Title of case study: 08-02 Scorpion Primers and the Advance of Medical Diagnostics

1. Summary of the impact

Research from Professor Tom Brown's group in Chemistry at Southampton has made a major contribution towards tackling the issue of ineffective drug therapies using Scorpion Primer Technology. Originally commercialised through DxS Ltd., the major impacts derived since 2008 include:

- DxS launching, in 2008, its first companion diagnostic product to allow European sales of Amgen's colorectal cancer drug, Vectibix®;
- The acquisition of DxS by QIAGEN N.V. for US$120 million in 2009;
- FDA approval in 2012 of the KRAS kit for use with the colorectal cancer drug, Erbitux®;
- FDA approval in 2013 of the EFGR kit for use with the non-small cell lung cancer drug, Afatinib;
- A 230% increase in QIAGEN staff based at the Manchester site between 2009 and 2012;
- Current annual sales of Scorpion Primer diagnostics are around US$100 million.

2. Underpinning research

The prescription of ineffectual drug therapies is a recurring problem in global healthcare, putting patients' lives at risk and wasting vast sums of health service money. Around 90% of drugs are effective on only 30-50% of individuals and inappropriate drug treatments can lead to distressing side effects. A colossal US$350bn of the US$770bn global annual drugs sales in 2008 was spent on ineffective medicines.

Rapid and accurate analysis of specific DNA sequences can identify groups of patients that are most likely to respond positively to a particular drug, thereby enabling improved and cost-effective care. The need to invent a more effective method of genetic analysis drove a body of research, from 1998 to 2003, by a team in the University of Southampton Chemistry Department, led by Tom Brown **[3.1, 3.2 ,3.3]**. Brown was a Professor of Biological Chemistry in Southampton Chemistry (until October 2013) and was also the founder of three biotech companies: Oswel, ATDBio and PrimerDesign. Brown's group specialises in nucleic acids chemistry, in particular structure-function relationship, DNA sequence recognition and the application of oligonucleotide chemistry to genetic analysis, diagnostics and therapeutics.

The work was conducted in Southampton, mainly by three PhD students under Brown's supervision: Antonio Solinas, Lynda Brown and Jamie Nicol. In collaboration with Dr David Whitcombe and Dr Steve Little at AstraZeneca Diagnostics, the research programme produced an entirely original method of genetic analysis that was particularly suitable for real-time PCR-based diagnostics. AstraZeneca and Oswel Research Products, a company specialising in the synthesis of chemically modified and complex oligonucleotides and their analogues, DNA, RNA and PNA, funded the projects jointly. Oswel was founded by Brown and at the time was based in the University of Southampton.

Brown's primary contribution was to provide the unique chemical expertise necessary to construct the complex modified oligonucleotides that form the basis of the technology. Beginning with the analysis of genes involved in breast cancer susceptibility, the group developed bi-functional molecules in which a primer is covalently linked to the probe, which is held in a scorpion-shaped tail (see **Figure**). The molecules also contain a fluorophore and a quencher. During the Scorpion polymerase chain reaction (PCR), in the presence of the target, the fluorophore and the quencher separate, leading to an increase in the fluorescence emission, which can be detected and measured in the reaction tube.

Mechanistic studies followed to test the performance of these systems. Compared with dual-labelled probes, the uni-molecular mechanism acts around four times faster in solution, for

instantaneous fluorescence in real-time PCR. Its other key advantages, over standard PCR probes that existed at the time, are enhanced sensitivity, simplified assay design, high signal-to-noise ratio and greater specificity.

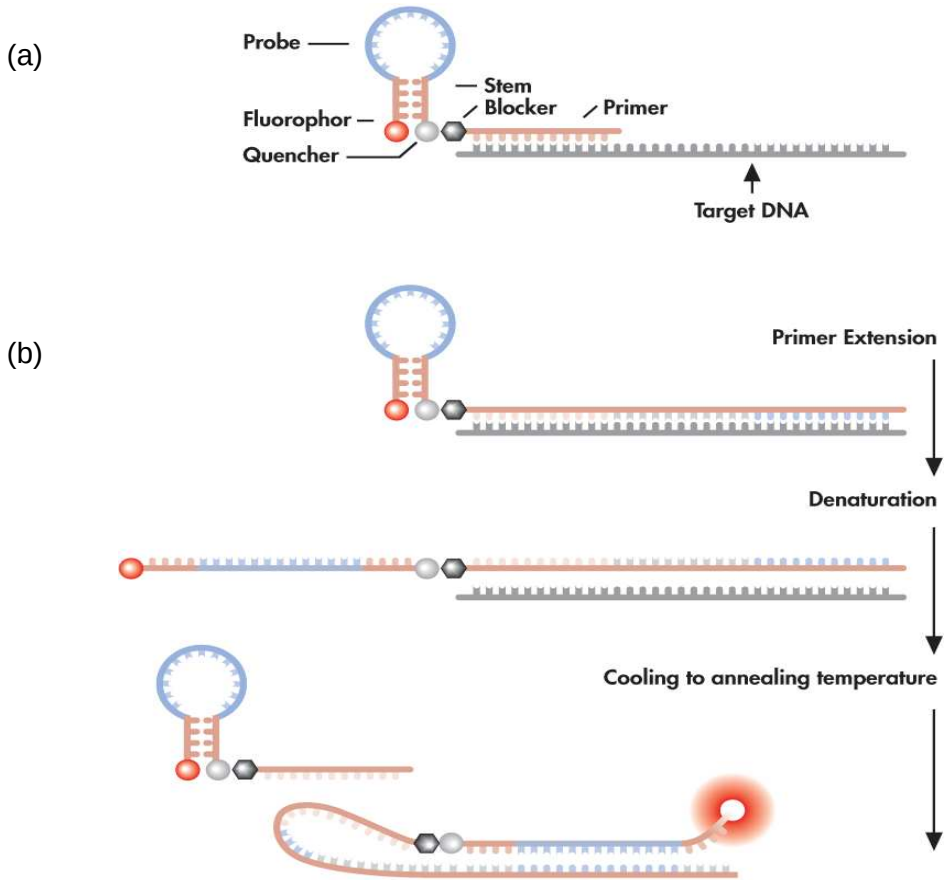

Figure: (a) illustrating the key elements of a Scorpion primer and (b) how it functions.

The research resulted in several highly cited publications, e.g. **[3.1]**-**[3.5]**. AstraZeneca filed a US patent for the **Scorpion Primers** technology in 1999; a UK patent for "Fluorophore/quencher labelled oligonucleotides" was filed in the same year.

3. References to the research (the best 3 illustrating the quality of work are starred)

Papers:

***[3.1]** Whitcombe D., Theaker J., Guy S.P., Brown T., Little S. 'Detection of PCR products using self-probing amplicons and fluorescence'. *Nature Biotechnol.* **1999**, *17*, 804-807

***[3.2]** Thelwell N., Millington S., Solinas A., Booth J., Brown T. 'Mode of action and application of Scorpion primers to mutation detection'. *Nucleic Acids Res.* **2000**, *28*, 3752-3761.

***[3.3]** Solinas A., Brown L.J., McKeen C., Mellor J.M., Nicol J.T.G., Thelwell N., et al. 'Duplex Scorpion primers in SNP analysis and FRET applications'. *Nucleic Acids Res.* **2001**, *29*, art. no.-e96.

[3.4] McKeen, C. M.; Brown, L. J.; Nicol, J. T. G.; Mellor, J. M., Brown, T. 'Synthesis of fluorophore and quencher monomers for use in Scorpion primers and nucleic acid structural probes'. *Org. Biomol. Chem.* **2003**, *1*, 2267-2275.

[3.5] May, J. P.; Brown, L. J.; van Delft, I.; Thelwell, N.; Harley, K, Brown. T. 'Synthesis and evaluation of a new non-fluorescent quencher in fluorogenic oligonucleotide probes for real-time PCR'. *Org. Biomol. Chem.* **2005**, *3*, 2534-2542.

Patents filed prior to DxS being founded:

'Methods for detecting target nucleic acid sequences', US patent number US6326145, Applicant: Zeneca Ltd.; Inventors: D. M. Whitcombe, J. Theaker, N. J. Gibson and S. Little (filed 1999, granted 2001); 'Fluorophore/quencher labelled oligonucleotides', Applicant: Zeneca Ltd.; Inventors: N. J. Gibson and T. Brown, UK patent number GB2337992B (filed 1999, granted 2001).

Funding for the underpinning research and studentships was provided through industrial contributions from the collaborating partner, AstraZeneca Diagnostics.

4. Details of the impact

After the development of the new Scorpion Primers technology in collaboration with Professor Brown (Southampton Chemistry), two scientists from AstraZeneca (Little and Whitcombe – key contacts) left the company to set up an SME; **DxS Ltd,** in Manchester in 2001 (and acquired by Qiagen in 2009) **[5.1, 5.2]**. DxS began developing mutation detection products including an assay for 29 epidermal growth factor receptor (EGFR) mutations. This led to a range of research products (EGFR, KRAS, BRAF, PI3K, T315I), a selection of which progressed into TheraScreen CE-marked diagnostic kits. **In January 2008 DxS launched its first companion diagnostic product to allow European sales of Amgen's colorectal cancer drug, Vectibix®.** Vectibix was initially rejected by the European Medicines Agency (EMA) on the basis of limited efficacy. Amgen then used the DxS kit to stratify the population on the basis of KRAS mutation status and Vectibix was approved by EMA for the KRAS wild-type population. Further diagnostic products followed, with the EGFR kit being used to establish the mutation status of non-small cell lung cancer tumours, to determine likely response to Iressa® or Tarceva®.

DxS was acquired by QIAGEN N.V. for US$120m in 2009 [5.1, 5.2]. QIAGEN, the world-leading provider of assay technologies, is listed on NASDAQ and the Frankfurt stock exchanges and operates more than 30 subsidiaries in 18 countries. The acquisition of DxS was described by QIAGEN's CEO as *"strategically a highly important transaction" and "a key element"* in its aim to *"lead in molecular diagnostic-based prevention, profiling and personalised healthcare"* **[5.2]**. The sale was recognised as the "Transaction of the Year" (2010) at the Mediscience Awards, London **[5.3]**. It also benefited the UK economy, with QIAGEN establishing a Centre of Excellence in Pharma Partnering at DxS's Manchester headquarters. **The workforce expanded from 67 (2009) to ~160 (2012) after a move to a purpose-fitted facility on the Manchester Science Park in 2010.** The success of the DxS assays has led to the growth of QIAGEN's personalized healthcare portfolio, which now makes US$100 million of sales annually (Peer Schatz, CEO, QIAGEN N.V.) **[5.4]**.

In the US, Food and Drug Administration (FDA) approval requires diagnostic assays be associated with a clinical trial. DxS had been approached by drug companies Amgen, BMS Lilly and Boehringer Ingelheim to provide companion diagnostic assays for use in their clinical trials. **This led to the approval (July 2012) of the KRAS kit in the US for use with the colorectal cancer drug, Erbitux® [5.5]**. The US market for this drug is expected to be in the region of US$20 million. The EGFR kit was submitted to the FDA in November 2012 for use with the Boehringer Ingelheim non-small cell lung cancer drug, Afatinib; **approval was received in July 2013.**

The EGFR and KRAS kits are now being used worldwide by hospital laboratories, central testing facilities and clinical research organisations. In the US the KRAS assay is being offered by Lab21, Inc, The Mayo Clinic, Applied Diagnostics, Inc, Boyce and Bynum Pathology Laboratories. **In January 2013 Clarient also began offering the assay [5.6]**. The CEO of Clarient, Carrie Eglinton Manner, stated: *"We believe precision medicine is the new direction in diagnosing and treating cancer and Clarient uses state-of-the-art diagnostic technologies like the therascreen KRAS test to bring clarity and precision to physicians to assist them in making better treatment decisions for their patients. Clarient's comprehensive offering and fast turnaround time coupled with our experience with the therascreen KRAS test permits us to provide a higher level of performance. Also, the fact that it is FDA approved provides Clarient with additional assurance of its quality and reliability."* **[5.6]**.

On-going development of the companion diagnostic portfolio is clear from the **agreement between QIAGEN and Eli Lilly, signed in February 2013**, to develop companion diagnostics for drugs for all therapeutic areas of interest **[5.7]**. A further expansion of the biomarker portfolio is evidenced by agreements and licences to develop assays for glioblastoma, lymphoma and rheumatoid arthritis, in deals with Insight Genetics, Drug Response Dx, InsermTransfert, Columbia University and the BC Cancer Agency, Canada **[5.4]**.

Since 2001 Professor Brown has continued to work on Scorpion design with DxS and later QIAGEN on a consultative basis. Since 2005 this has mainly been channelled through ATDBio (a spin-out company founded by Tom Brown) for the provision of specialist advice and custom oligonucleotides **[5.8]**.

David Whitcombe (co-founder of DxS) has stated *"Not only was Professor Brown instrumental in the initial development of Scorpions (suggesting and supplying appropriate blocking and quenching groups, and also creating monomer phosphoramidites), but as the technology matured, he was able to advise and assist on the high quality manufacture of Scorpion constructs. In addition, his group were highly engaged in the investigation of the properties and performance of these molecules. This enabled DxS to move into the manufacture of high quality and high value Diagnostic tests with significant clinical benefits for large numbers of cancer sufferers.* ***Ultimately it was the utility and quality of these tests that underpinned the sale of DxS for US$120m"*** **[5.9]**.

This is supported by Steve Little (co-founder of DxS), who states that *"The core technology used by DxS was Scorpions and, following the acquisition, the technology continues to be used in an expanded range of personalised medicine products provided by QIAGEN.* ***Scorpions technology now supports annual sales of around US$100m.*** *The input of Professor Tom Brown in providing chemistry expertise was central to the success of the Scorpions technology."* **[5.10]**.

5. Sources to corroborate the impact

[5.1] Qiagen company web-site: http://www.qiagen.com/ , main information under section on Molecular Diagnostics: http://www.qiagen.com/Products/Lab-Focus/Molecular-diagnostics

[5.2] Press releases announcing acquisition of DxS by Qiagen: http://www.qiagen.com/About-Us/Press-Releases/PressReleaseView/?PressReleaseID=268&lang=EN; http://www.genomeweb.com/dxpgx/qiagen-acquires-uks-dxs-deal-worth-130m

[5.3] QIAGEN's acquisition of DxS Ltd. in 2010 was recognized as the "Transaction of the Year" at the annual Mediscience Awards (London), the largest annual gathering of publicly quoted healthcare, biotech and life science companies in Europe: http://www.qiagen.com/About-Us/Who-We-Are/Awards-and-Partnerships/Awards-and-Recognitions/

[5.4] QIAGEN Sales and Expansion of biomarker portfolio: http://www.qiagen.com/About-Us/Press-Releases/PressReleaseView/?PressReleaseID=414&lang=EN

[5.5] Press release on the market size for Erbitux: http://www.genomeweb.com/mdx/fda-clears-qiagen-kras-test-cdx-erbitux

[5.6] Press release announcing Clarient to offer KRAS assay: http://www.qiagen.com/About-Us/Press-Releases/PressReleaseView/?PressReleaseID=404&lang=EN

[5.7] QIAGEN-Eli Lilly agreement: http://www.genomeweb.com/pcrsample-prep/qiagen-eli-lilly-enter-cdx-deal

[5.8] ATDBio web-site: http://www.atdbio.com/

[5.9] Corroborating contact, ex-AstraZeneca collaborator and co-founder of DxS.

[5.10] Corroborating contact**,** ex-AstraZeneca collaborator and co-founder of DxS and ex-Vice President QIAGEN.