Institution: University of Southampton

Unit of Assessment: 08 Chemistry

Title of case study: 08-02 Novel oligonucleotide technologies improving targeted cancer therapies and disease diagnostics.

Period when the underpinning research was undertaken: 2000 – 2017

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Tom Brown	Professor of Nucleic Acid Chemistry	1995 – 2013
Ali Tavassoli	Professor of Chemical Biology	2006 – present
Phil Bartlett	Professor of Electrochemistry	1992 – present
Eugen Stulz	Assoc Prof in Bioorganic Chemistry	2006 – present

Period when the claimed impact occurred: August 2013 – December 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

Research into nucleic acid structures within the University of Southampton's School of Chemistry resulted in the design of novel oligonucleotide technologies for therapeutic and diagnostic purposes that have developed along four pathways to commercialisation. The original research was responsible for the creation of technologies required for the launch and sale of targeted cancer diagnostics and therapies that have benefited hundreds of thousands of patients globally and generated revenues in excess of GBP10 billion between 2014 and 2020 through direct sales and licensing arrangements. A new technique for rapid DNA sequence analysis made a key contribution to the GBP200 million sale of a diagnostic company and led to new forensics tools for law enforcement agencies. The research also led directly to the formation of two UK companies, resulting in commercial impact valued at tens of millions of GBP, supporting dozens of high-skilled jobs and allowing the sale of products that rapidly diagnose infectious diseases such as Ebola, African Swine Fever and COVID-19.

2. Underpinning research

Fundamental and interdisciplinary research led by Professor Tom Brown has focused on the design and synthesis of unique, chemically modified oligonucleotides as potential therapeutic and diagnostic agents. This led to new methods of identifying mutations in the human genome and of pathogens. In collaboration with international biotechnology companies, Brown's group translated these underlying principles into the design of novel oligonucleotide technologies that have been applied to specific industry challenges.

A collaboration with LGC beginning in 2000 led to the development of a new technology for genetic analysis [**3.1**]. **HyBeacons** is a novel oligonucleotide probe technology that identifies mutations more rapidly, accurately and reliably than previously possible. Multiple subsequent papers demonstrated the practical application of HyBeacons for rapid DNA sequence analysis to determine whether an individual might respond positively to certain treatments or to quickly diagnose suspected bacterial infections. Brown and LGC further developed HyBeacons for forensic applications (STR profiling) to be used for human identification at crime scenes [**3.2**]; for the identification of sexually transmitted infections; for Multiplex Genetic Analysis; and for single nucleotide polymorphism detection in cancer. The technology was patented in 2009 (Brown as co-author) and licensed to biotech firm Evogen in the same year for all applications except forensics (LGC forensics, now Eurofins). Evogen further licensed the technology to Focus Diagnostics who developed the Simplexa molecular assay product line incorporating HyBeacons, which in turn was sold to DiaSorin in 2016 who developed HyBeacon Generation II.

Concurrently, Brown's group developed new methods of mutation analysis. Working with AstraZeneca, researchers led by Brown invented a novel real-time PCR method to identify mutations in the human genome, later marketed as **Scorpion Primers**. Mechanistic studies [**3.3, 3.4**] followed to test the performance of the technology; because probe and primer are combined into a single molecule, the new method provides enhanced sensitivity, shorter reaction times and greater specificity than conventional bi-molecular mechanisms. One of the first uses of the Scorpion technology was for the detection of mutations in genes that have the potential to cause cancer, guiding decisions by clinicians and pharmaceutical companies to select safe and effective cancer therapies that could be adapted as changes occurred in the genetics of the cancer.

Another related research strand emerged out of Brown's studies into the genetic and biophysical analysis of DNA. Brown founded a company called ATDBio in 2005 to develop **custom-made,**

chemically modified novel oligonucleotide analogues for a wide range of applications to meet the needs of customers**,** drawing closely on his research expertise in the underlying chemistry. ATDBio continues to support research at Southampton. Brown and Bartlett – in the field of DNA sequence analysis – produced a new method for identifying mutations in the human genome using a combination of Surface-Enhanced Raman Spectroscopy and electrochemical DNA melting [**3.5**]. Brown and Tavassoli designed the first unnatural DNA backbone linkage that can be formed in high yield and read through by polymerase enzymes [**3.6**], culminating in the entirely chemical synthesis of functional genes, thus strengthening ATDBio's offering in click-ligation of DNA. Stulz was supported by ATDBio to carry out research on supramolecular DNA structures and DNA-origami protein complexes using click-ligation [**3.7**], which has furthered ATDBio's knowledge in chromophore and protein modifications.

Around the same time that ATDBio started up, Brown co-founded (along with Dr Jim Wicks and Dr Rob Powell in the University of Southampton's Faculty of Medicine) another company called **Primerdesign** in 2005 applying his research into novel labelled oligonucleotides and fluorescent probes to **the development of diagnostic kits used in medical, genetic, and research applications**. Brown's research has continued to feed into the creation of new products.

3. References to the research

3.1 French, D. J., Archard, C. L., Brown, T. & McDowell, D. G. HyBeacon (TM) probes: a new tool for DNA sequence detection and allele discrimination. *Mol. Cell. Probes* **15**, 363-374 (2001). https://doi.org/10.1006/mcpr.2001.0384

3.2 Gale, N., French, D. J., Howard, R. L., McDowell, D. G., Debenham, P. G. & Brown, T. Rapid typing of STRs in the human genome by HyBeacon melting. *Org Biomol Chem* **6**, 4553-4559 (2008). https://doi.org/10.1039/B813431F

3.3 Solinas, A., Brown, L. J., McKeen, C., Mellor, J. M., Nicol, J. T. G., Thelwell, N. & Brown, T. Duplex Scorpion primers in SNP analysis and FRET applications. *Nucleic Acids Res.* **29**, p. e96 (2001). https://doi.org/10.1093/nar/29.20.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.* **1**, 2267-2275 (2003). https://doi.org/10.1039/B301859H

3.5 Mahajan, S., Richardson, J. A., Brown, T. & Bartlett, P. N. SERS-Melting: A New Method for Discriminating Mutations in DNA Sequences. *J. Am. Chem. Soc.* **130**, 15589-15601 (2008). https://doi.org/10.1021/ja805517q

3.6 El-Sagheer, A. H., Sanzone, A. P., Gao, R., Tavassoli, A. & Brown, T. Biocompatible artificial DNA linker that is read through by DNA polymerases and is functional in E.coli. *Proc. Natl. Acad. Sci. USA* **108**, 11338-11343 (2011). https://doi.org/10.1073/pnas.1101519108

3.7 Marth, G., Hartley, A. M., Reddington, S. C., Sargisson, L. L., Parcollet, M., Dunn, K. E., Jones, D. D., Stulz, E. Precision Templated Bottom-Up Multiprotein Nanoassembly through Defined Click Chemistry Linkage to DNA. *ACS Nano* **11**, 5003-5010 (2017). https://doi.org/10.1021/acsnano.7b01711

4. Details of the impact

4.1. HyBeacons: delivering commercial impact and a cheaper, more efficient forensic testing capability for law enforcement agencies in the UK and overseas

The application of HyBeacons technology to rapid forensic testing at crime scenes was explored through active research with **LGC Forensics**; peer-reviewed papers between 2014 and 2018 introduced the portable ***ParaDNA Intelligence System***, a novel approach to DNA profiling [**5.1**]. It was developed in response to high demand for DNA evidence in criminal investigations, which can lead to large backlogs of samples requiring analysis and, due to budget constraints, a limited proportion of samples being processed for each case. This can result in delays to arrests and convictions, and potential DNA evidence being overlooked, meaning that criminals remain undetected. The ParaDNA system was shown to provide a straightforward, rapid and robust way to profile DNA samples at a crime scene in around 75 minutes, compared with a wait of that can sometimes be weeks for lab results.

The technology supported the creation of 25 jobs in the ParaDNA team (LGC Forensics) and the sale of forensic kits, instrumentation and software, and achieved revenues of GBP90,000,000 between 2017 and 2018. LGC Forensics, which became the largest player in UK forensics, was sold in 2017 to **Eurofins Scientific** for GBP30,300,000 (including employee transfer), which provided Eurofins with a European leadership position in forensics services [**5.2**]. Eurofins has

now become the largest forensic science service provider in the UK, working with police, crime enforcement agencies and private sector clients. According to Eurofins, the ParaDNA system has been used by the police and the legal sector, served by four Forensic Genetics and four Forensic Toxicology laboratories across Europe and Africa. Example use cases include the first deployment of the ParaDNA system at a crime scene in Leeds by West Yorkshire Police in October 2017. According to an NBC news report in July 2017, Plano Police Department in Texas began using the ParaDNA system when the Texas Department of Public Safety began charging law enforcement agencies for forensic testing, a move that would cost the Plano force USD110,000 per year [**5.3**]; after initial costs for acquisition, installation and training, Plano City Council instead budgets USD13,000 per year for screening test kits.

LGC also licensed HyBeacons to **Evogen**. Evogen incorporated HyBeacons into its rapid molecular diagnostics platform to increase the performance of the assays they were supplying to clinical, environmental and biodefense markets. In March 2013, Evogen entered into a license agreement with US-based **Focus Diagnostics** (Part of **Quest Diagnostics**), a world-leading provider of diagnostic information services and a specialist in the testing of infectious diseases (e.g. first on West Nile Virus, SARS and Influenza A H1N1), who incorporated HyBeacons into their ***Simplexa*** product line. According to the Evogen CEO at the time, the agreement highlighted the "*continued evolution of Hybeacons probe technology into an industry standard*". On 13 May 2016, Quest sold Focus Diagnostics (180 employees, annual revenues USD65m), including the Simplexa line, to Italian multinational biotech firm **DiaSorin** for USD300m (GBP208,000,000), giving DiaSorin access to 200 US hospitals served by Focus [**5.4**] and allowing the company to enter the molecular diagnostics market. Since then, DiaSorin has marketed four further products based on HyBeacons for PCR detection of Factor V Leiden and MTHFR mutations. Their molecular diagnostics platforms, which reports sales revenues of GBP3,300,000 in 2019, up 12.6% from 2018, provides 9% of the total income of DiaSorin (46% of which is from EU/Africa), where main customers are Health Departments and Organisations; acquisition of Focus boosted their revenues from GBP27,000,000 in 2016 to GBP50,000,000 in 2017, accruing a total of GBP170,000,000 to 2019 (figures calculated to 2 s.f. using exchange rate at year end).

4.2. Scorpion Primers: delivering commercial impact and more effective treatment pathways for cancer patients

The presence of certain genetic mutations in the tumours of patients with colorectal or non-small cell lung cancer can determine whether a patient will respond positively or negatively to a targeted drug treatment, compared with standard chemotherapy. The Scorpion Primer technology, co-developed by Southampton researchers, forms the basis of three *in vitro* diagnostic medical devices now marketed by Dutch company **Qiagen N.V.** (listed at the NYSE and FSE). Qiagen's ***therascreen*** kits are FDA-approved, qualitative real-time PCR assays for the detection of specific mutations in certain oncogenes. They function as companion diagnostic devices that, according to the FDA, provide information that is essential for the safe and effective use of a corresponding therapeutic product. The use of a companion diagnostic with a specific targeted therapy is stipulated in the instructions for use in the labelling of both the diagnostic device and the corresponding drug. This is the case for Qiagen's therascreen kits, meaning that, according to an associate director at the company, "*they have enabled the launch of numerous drugs (in the USA and beyond) for the treatment of patients with non-small cell lung cancer and colorectal cancer for which our 'companion diagnostic test' is required*." [**5.5**]

The two key CE-marked kits are the *therascreen* KRAS RGQ PCR kit and the *therascreen* EGFR RGQ PCR kit, detecting mutations in the KRAS and EGFR oncogenes respectively. The former allows the sale of two drugs, Erbitux and Vectibix, for the treatment of colorectal cancer; the latter allows the sale of three drugs, Iressa, Gilotrif and Vizimpro, for non-small cell lung cancer. Qiagen confirmed that the diagnostic kits are "*distributed globally so do make a real impact on patients across the globe*" [**5.5**]. By identifying whether or not patients are likely to respond positively to targeted therapies, the devices help prolong lives, reduce stress for patients and avoid large sums of money being spent by health services on ineffective treatments. The pairing of *therascreen* KRAS with Erbitux for the treatment of metastatic colorectal cancer was approved by the FDA in 2012 and sales have continued throughout the impact period. Its pairing with Vectibix for the same disease was approved in May 2014. The pairing of therascreen EGFR with Gilotrif for the treatment of metastatic non-small cell lung cancer received FDA approval in July 2013, with sales continuing

throughout the impact period. Its pairings with Iressa and Vizimpro for the same disease were both approved in 2018. They are registered in more than 40 countries.

The significance of the impact of the *therascreen* kits is captured through the global sales of the drugs to which they are paired and the prevalence of the diseases for which they aid treatment. Taking the US as an example, colorectal cancer is the third most common cancer diagnosed in both men and women, with an estimated 104,270 (colon cancer) and 45,230 (rectal cancer) new cases in 2021, according to the American Cancer Society. KRAS mutations occur in 40% of colorectal cancer patients, Qiagen reports. Eli Lily markets Erbitux in the US and Canada; Merck markets the drug outside of the US and Canada, for which Eli Lilly received royalties. Annual sales of Erbitux grew from GBP240,000,000 in 2014 to GBP410,000,000 in 2019, generating total revenues of GBP2,500,000,000 between 2014 and 2019. Merck earned total revenues of GBP4,300,000,000 over the same period. Vectibix is marketed by Amgen; the company generated GBP2,700,000,000 through global sales between 2014 and 2019 [**5.6**].

Non-small cell lung cancer accounts for 85% of all cases of lung cancer, the second most common cancer in both men and women. The American Cancer Society expects 235,760 new cases of lung cancer and 131,880 deaths from lung cancer in the US in 2021. Iressa is marketed by Astrazeneca and achieved GBP407,000,000 in sales in 2018, the first year in which it was paired with Qiagen's *therascreen* EGFR. Pfizer's Vizimpro was approved as a new treatment option for patients with EGFR-mutated non-small cell lung cancer in 2018; the approval for pairing with *therascreen* EGFR followed shortly afterwards. According to Pfizer's chief development officer for oncology, the *therascreen* kit was used in the "pivotal clinical trial for Vizimpro" and "will enable physicians to identify patients who may benefit from this medicine". Vizimpro's sales numbers are not disclosed but are likely to be included in the "other oncology" revenue of USD192bn for Q1 of 2020. Boehringer Ingelheim has not disclosed sales for its Gilotrif, although in 2017 GlobalData projected they would reach USD688m per year by 2022. [**5.6**]

The *therascreen* kits also play a crucial role in the expensive drug development process, as in the Vizimpro example above. Qiagen said: "*We also continue to support our drug development partners with ongoing clinical trials, using these kits (currently there are no other kits in development using Scorpions). Unfortunately, I cannot comment on these as we keep this work confidential.*" The *therascreen* devices are of high strategic importance to Qiagen; each FDA approval "*expands Qiagen's leadership in molecular companion diagnostics for personalized healthcare*", according to their press releases. [**5.5**]

Qiagen also licenses the Scorpion Primer technology for other applications. In 2010 LGC Biosearch Technologies, a biotech company with 2,400 employees headquartered in Hoddesdon, UK, entered into a licensing relationship with Qiagen for commercialisation rights in the fields of infectious diseases, environmental testing, animal identity and food testing, genetically-modified-organism (GMO) testing, industrial microbiology testing, bio-security, and forensic science. [**5.5**]

4.3. ATDBio: Working to change the landscape of DNA and COVID-19 testing

Originally built on Southampton research into the development of chemically modified novel oligonucleotide analogues, ATDBio has continued its strong commercial performance throughout the impact period. Turnover rose from GBP2,300,000 in financial year 2019 to GBP3,500,000 in 2020, with total revenues between 2014 and 2020 of GBP22,000,000. During 2020, ATDBio increased its workforce from 12 to 20 full-time staff in their Southampton and Oxford labs, including several Southampton Chemistry graduates and post-doctoral research assistants. [**5.7**]

Key customers include Oxford Nanopore Technologies, headquartered in Oxford and with nine offices around the world including in China, Japan and the US; it integrates ATDBio oligonucleotides into its novel DNA/RNA sequencing technology which is used for a wide range of applications including agriculture, biosecurity and food safety. Oxford Nanopore's Senior Director of Research said: "Since 2013, Oxford Nanopore has spent over GBP1,600,000 on DNA products from ATDBio and about 50% those orders fall within 2018-2019." [**5.8**]

In the summer of 2020 over GBP500,000 was spent by ATDBio on equipment for oligonucleotide synthesis and purification as part of a new initiative in large scale oligonucleotide production due to the demands for oligonucleotides for use in COVID-19 testing. ATDBio has supplied oligonucleotides to the key manufacturers of LAMP tests used in UK test and trace [text removed for publication] (LAMP = Loop-mediated amplification). In the weeks leading to Christmas 2020, ATDBio synthesised tens of grams of oligonucleotides for use in 100 million COVID-19 LAMP tests and this is ongoing. ATDBio has also been involved in the synthesis of modified

oligonucleotides for use in the development of new COVID-19 diagnostic tests, including HyBeacon probes [text removed for publication], as well as probes to differentiate between the original strain of SARS-COV-2 and new fast spreading variant VUI-202012/01 (which has 17 changes/mutations). [text removed for publication] It is noteworthy that on several occasions ATDBio was able to rapidly supply customers with oligonucleotides for COVID-19 research and diagnostics when other oligonucleotide suppliers failed to deliver.

4.4. Primerdesign: delivering commercial impact and novel diagnostics for bacterial and viral infections including Ebola, Swine Fever and COVID-19.

Primerdesign, where Brown was co-founder, produces diagnostic kits that utilise modified nucleic acids. These kits are sold to thousands of customers in more than 100 countries and have been used to diagnose infections including Ebola, swine flu and zika virus. In May 2016, the company, based in Southampton, was sold to French clinical diagnostics firm **Novacyt** for GBP12,000,000 [**5.10**], providing a major portfolio for the new parent company. Its flagship product range is its 550 ***Genesig PCR kits*** that are used for a wide range of purposes, from human pathogen detection and food testing, to veterinary testing and biothreat detection.

Based on Novacyt's 2019 annual report, Primerdesign has made a significant contribution to the Group's overall success. The Directors estimate that Primerdesign's core target molecular markets for RUO, IVD clinical and food pathogen testing are worth approximately EUR14.7bn per annum, with an estimated growth of over 4.3% per annum. The RUO market, alone, is estimated to be worth EUR1.3bn with the clinical market estimated at over EUR6.0bn. Sales for 2018 contribute EUR6,300,000 (12-2018), +3% compared to 2017. Novacyt's annual report 2019 says: "*Since its acquisition by the Company in May 2016, Primerdesign has continued to grow and is now a significant part of the Group … Following its first IVD CE Mark approval for Zika in July 2017, Novacyt produced a further two IVD CE marked products during 2018, further demonstrating the Company's ability to develop CEIVD assays … In follow-up to our largest single order for Primerdesign's Genesig® q16 instruments received in 2017, we received a further order in December 2018 for another 100 instruments, paid for in advance from a new customer in the Asia Pacific region … Primerdesign has now sold over 450 q16 units since its launch in 2015. As instrument sales grow, the Company expects a pull-through effect in relation to repeat Genesig® reagent sales*." [**5.10**] The report further states that the commercial reach of Primerdesign resulted in strong growth in international markets of 13%, with significant double-digit growth in the USA of 51%. Novacyt also states that they have invested in additional manufacturing space at the Southampton (Chandler's Ford) site to provide capacity for planned growth over the next few years, in particular for their COVID-19 test kit upscaling. The biggest selling product lines include Genesic detection kits for *Aspergillus* species, Hepatitis B virus and pig/pork meat speciation and the Genesic q16 PCR instrument; a test kit specific for the 2014 Ebola virus is sold at USD4 (containing 150 tests) and has received attention of the press (BBC 2014). Primerdesign has also launched an African Swine Flu assay in 2019 with significant demand in China, VietNam and Eastern Europe. The Genesig q16 real-time PCR system for Covid-19 detection and quantification has been listed by the **WHO as one of two tests for emergency use** [**5.11**]. As a result of Primerdesign's COVID-19 diagnostic test kit launch, Novacyt's share price rose by EUR2 per share in the first half of 2020, resulting in a net cash inflow of EUR2,400,000 (06-2020) [**5.10**].

5. Sources to corroborate the impact

5.1 ParaDNA publication
5.2 Supporting statement from LGC.
5.3 Supporting data for impact of the ParaDNA system in Plano Police Dept, USA.
5.4 Supporting information on HyBeacon technology transfer and impact
5.5 Supporting statement from Qiagen.
5.6 Summary report of global sales of drugs paired with Qiagen's *therascreen* diagnostic kits and media announcements of FDA approvals for *therascreen* diagnostic kits.
5.7 Supporting statement from ATDBio.
5.8 Supporting statement from Oxford Nanopore Technologies.
5.9 Supporting statement from Novacyt-Primerdesign.
5.10 Primerdesign sale, Ebola and Novacyt Group annual report for 2019.
5.11 Newsletter World Health Organisation, 07/04/2020.