Institution: Cardiff University		
Unit of Assessment: Allied Health Professions, Dentistry, Nursing and Pharmacy (3)		
Title of case study: Clinical translation of Cardiff's ProTide technology for new anti-cancer drug therapies		
Period when the underpinning research was undertaken: 2005 – 2020		
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:**
Chris McGuigan	Professor	01/07/1994 – 11/03/2016
Andrea Brancale	Professor	01/02/2001 – present
Magdalena Slusarczyk	Research Fellow	07/01/2008 – present
Michaela Serpi	Research Fellow	16/05/2011 – present
Period when the claimed impact occurred: August 2013 – Dec 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact (indicative maximum 100 words)

Chemotherapy using nucleoside-based drugs remains a fundamental part of modern cancer treatment, however drug resistance limits the effectiveness of chemotherapy in many cancer types. Cardiff researchers used their pro-nucleotide (ProTide) technology to develop new drug candidates that overcome key cancer resistance mechanisms within existing nucleoside drug treatments. NuCana, a biopharmaceutical startup company, licensed Cardiff's ProTide compounds as their only Intellectual Property assets. Since August 2013, via access to Cardiff's research and ProTide compounds, NuCana a) raised a total of US$251million (approximately £189million) in investment funding and b) spent £84M on research and development to deliver new clinical trials for three of Cardiff's anti-cancer ProTides.

2. Underpinning research (indicative maximum 500 words)

Chemotherapy drugs are used to stop the uncontrolled growth of cancer cells. Nucleoside analogues are a major class of anti-cancer chemotherapeutic agents used for treating a variety of tumours. They prevent tumour growth by inhibiting the replication of DNA, which leads to cell death. However, the effectiveness of nucleoside-based drugs is often reduced, due to innate or acquired cancer cell resistance, resulting in poor survival outcomes for patients. This resistance is due to various factors including poor drug uptake by tumour cells (deficiency in nucleoside transport proteins), poor activation (drugs must be phosphorylated to nucleotides to be effective) and rapid metabolic degradation of the drug. In addition, chemotherapy drugs target healthy cells, as well as cancer cells, resulting in severe side effects for patients. These limitations mean there remains an unmet need for more effective and tolerable chemotherapy drugs to treat a variety of cancers.

Cardiff University researchers pioneered research into the design and application of a novel phosphoramidate pro-nucleotide (ProTide) approach. Cardiff's ProTide technology provides a chemical platform on which to design and synthesise candidate compounds for drug development. This was outlined in a REF 2014 case study, focused on antiviral drugs. In 2005, the Cardiff team expanded the use of its ProTide technology to design potential anti-cancer drugs, focusing on thymectacin as the first target for modification in this way. This study found that activity against different tumour models in vitro could be 'tuned' according to the nature of the modifications, particularly the amino acid, ester and aryl chemical substituents **[3.1]**.

Over the REF period the Cardiff team continued their pro-drug research into new anti-cancer drugs as follows:

2.1 Developed drug candidates to overcome resistance to the nucleoside chemotherapy drug gemcitabine

The Cardiff team sought to develop a ProTide derivative of gemcitabine, a widely used but poorly effective chemotherapy agent in poor prognosis diseases such as pancreatic, bladder and non-small cell lung cancer. They designed and synthesised 80 different gemcitabine analogues for evaluation, finding six analogues that had greater potency than gemcitabine itself. Further evaluation revealed that one drug candidate in particular (NUC-1031) was highly potent and least affected by tumour cell resistance mechanisms **[3.2]**.

2.2 Addressed the limitations of the nucleoside chemotherapy drug 5-fluorouracil

5-fluorouracil (5-FU) is a widely used drug in the treatment of colorectal, breast and ovarian cancer. The effectiveness of 5-FU is often compromised because it depends on active nucleoside transporter uptake into cells and activating phosphorylation by kinases. The active drug is also vulnerable to breakdown by phosphorylases. 5-FU also undergoes rapid metabolism by the liver, and, in some cases, it is necessary for patients to carry an infusion pump to receive a slow but continual dose over two days.

These limitations led the Cardiff team to design and synthesise 39 ProTide analogues of 5-FU. Use of the ProTide approach meant that compounds were already delivered as masked phosphates (pro-nucleotides). By design and synthesis of the appropriate modifications, it was found that several of these candidate molecules had similar activity to 5-FU but could also enter cells independently of uptake mechanisms and were resistant to dephosphorylation **[3.3, 3.4]**.

2.3. Developed ProTide derivatives of additional anticancer nucleosides

The Cardiff research team has continued to develop ProTide derivatives of potential anticancer nucleoside analogues, including 3'-deoxyadenosine (cordycepin). Cordycepin has been identified as a potential cancer therapeutic but has failed in a clinical setting principally due to rapid intracellular deamination, coupled with poor uptake and lack of sufficient phosphorylation. The ProTide derivative of this molecule addresses these limitations and offers a potential therapeutic agent for kidney and haematological tumours **[3.5]**.

2.4 Cardiff's research collaboration and licence agreement with NuCana

This research, and that from the prior REF period, has led to Cardiff researchers being named as inventor(s) on 29 ProTide patent families in cancer therapy. In August 2009, a new UK-based biopharmaceutical company, NuCana, entered into a research collaboration and license agreement with Cardiff University based on the strength of Cardiff's ProTide research. NuCana was specifically set up to use Cardiff's ProTide technology to transform some of the most widely prescribed chemotherapy agents into more effective and safer medicines.

3. References to the research (indicative maximum of six references)

[3.1] Congiatu C, **Brancale A**, Mason MD, Jiang WG, **McGuigan C**. Novel potential anticancer naphthyl phosphoramidates of BVdU: Separation of diastereoisomers and assignment of the absolute configuration of the phosphorus center. *Journal of Medicinal Chemistry*, 2006, 49, 452-455. DOI:10.1021/jm0509896

[3.2] Slusarczyk M, Lopez MH, Balzarini J, Mason M, Jiang WG, Blagden S, Thompson E, Ghazaly E, **McGuigan C**. Application of ProTide technology to gemcitabine: a successful approach to overcome the key cancer resistance mechanisms leads to a new agent (NUC-1031) in clinical development. *Journal of Medicinal Chemistry*, 2014, 57, 1531-1542. DOI: 10.1021/jm401853a

[3.3] McGuigan C, Murziani P, **Slusarczyk M**, Gonczy B, Voorde J, Liekens S, Balzarini J. Phosphoramidate ProTides of the anti-cancer agent FUDR successfully deliver the pre-formed bioactive monophosphate in cells and confer advantage over the parent nucleoside. *Journal of Medicinal Chemistry*, 2011, 54, 7247-7258. DOI: 10.1021/jm200815w

[3.4] Slusarczyk M, Ferla S**, Brancale A**, **McGuigan C**. Synthesis and biological evaluation of 6-substituted-5-fluorouridine ProTides. *Bioorganic and Medicinal Chemistry*, 2018, 26, 551-565. DOI: 10.1016/j.bmc.2017.11.037

[3.5] Griffith H, **McGuigan C**, Ferrari V, **Serpi M** (2016) *New 2' and/or 5' amino-acid ester phosphoramidate 3'-deoxy adenosine derivatives as anti-cancer compounds*. WO2016083830 (A1), patent details (accessed 4th December 2020)

4. Details of the impact (indicative maximum 750 words)

As outlined in Section 2, the UK-based biopharmaceutical company, NuCana, was established with the specific purpose of using Cardiff's ProTide technology to enhance existing chemotherapy treatments **[5.1]**. In the company's 2017 Annual Report, it notes: "Our propriety ProTide technology was invented in the Cardiff University laboratory of our late Chief Scientific Officer, Professor Christopher McGuigan, who conceived of, and filed the original composition of matter patents for our initial ProTides" **[5.2**, p5**]**. Cardiff's ProTide compounds remain NuCana's only intellectual property asset. Since August 2013, NuCana has filed in excess of 800 patent applications on ProTides worldwide, with over 600 granted to date **[5.3]**. Within the REF2021 period, the collaboration between Nucana and Cardiff researchers has led to the following impacts:

4.1 NuCana's commercial growth, including $251M in investment funding

During the current REF period, NuCana has grown substantially as evidenced by the following:

Founder and CEO of NuCana, Hugh S Griffith, stated that *"Having a portfolio of ProTide compounds built on Cardiff University research has enabled NuCana to raise investment to progress a substantial discovery and clinical development programme"* **[5.3]**. In total **$251M** (almost £189M) has been raised through three major rounds of investment funding. This includes a $57M Series B funding round in April 2014, a further $114M when NuCana closed on the NASDAQ Initial Public Offering in September 2017 and $80M follow-on funding in a public offering in September 2020 **[5.3, 5.4]**.

NuCana has also increased in size since August 2013, from six to over 50 employees **[5.3]**. To enhance its growth, Nucana spent a total of £84M on research and development between 2015 and 2020 **[5.3]**. Expenditure levels have risen annually, from £4.9M in 2015 to £17.9M by the third quarter of 2020. This expenditure has been used to fund the clinical trials listed below, as well as support collaborative ProTide research at Cardiff University within the REF period (amounting to £1.4M) **[5.3]**.

4.2 Clinical studies for three Cardiff ProTide compounds

Securing the funding outlined in Section 4.1 enabled NuCana to invest £84M in research and development projects to target multiple hard-to-treat cancer types. As part of this programme, NuCana undertook clinical development of three of Cardiff's anti-cancer ProTides through Phase I to Phase III clinical studies **[5.3]** as follows:

a. Clinical Trials for NUC-1031 (Acelarin)

NUC-1031 – a ProTide derivative of gemcitabine **[3.2]** – is the most advanced Cardiff anticancer ProTide clinical candidate. Also known as Acelarin, this ProTide achieves its effect by generating and maintaining higher concentrations of the active nucleoside, gemcitabine triphosphate, inside tumour cells. Acelarin has undergone clinical trials to assess effectiveness in ovarian, pancreatic and biliary tract cancer. This latter cancer currently has no approved drug treatment **[5.5]**. These cancers are difficult to diagnose, so are often diagnosed late and are consequently poorly responsive to currently available therapies.

NuCana invested in Phase I (commenced 2012 and running through to the REF impact period), Phase Ib (ovarian cancer; commenced 2014) and Phase II (ovarian cancer; commenced 2017) studies **[5.6]**. Biliary tract cancer (a disease causing 340,000 new worldwide cases annually) has become the lead indication for this compound, resulting in investment in a global, multi-centre, randomised Phase III study of Acelarin for the first-line treatment of patients with biliary tract cancer. In June 2019 the U.S. Food and Drug

Administration granted orphan drug designation for Acelarin for the treatment of biliary tract cancer **[5.5]**.

b. Clinical Trials for NUC-3373

NuCana also invested in a Phase I trial (commenced 2016) for Cardiff ProTide NUC-3373, a ProTide derivative of 5-fluorouracil designed to target advanced solid tumours **[5.7]**. This trial demonstrated positive benefits in terms of activity and safety profile, compared to standard 5-fluorouracil treatment. A Phase Ib combination study, looking at how NUC-3373 can be combined with different colorectal cancer drugs other than 5-fluorouracil, is underway in patients with colorectal cancer (commenced 2018) with a further Phase III study planned **[5.7]**. These studies aim to establish the maximum clinically tolerated dose of this ProTide.

c. Clinical Trial for NUC-7738

NUC-7738 is the most recent Cardiff ProTide to enter clinical development through the NuCana collaboration. NUC-7738 is a ProTide transformation of 3'-deoxyadenosine (3'-dA), also known as cordycepin. Cordycepin has shown potent anticancer activity in pre-clinical studies but had not been developed as a chemotherapy drug due to its rapid metabolic breakdown. After successfully demonstrating the enhanced potency of NUC-7738 in pre-clinical studies compared to cordycepin (up to 185 times greater potency) **[5.8]**, NUC-7738 is currently undergoing a Phase I clinical study in patients with solid tumours (commenced 2019) **[5.8]**.

In summary, Cardiff's research has led to substantial growth of the biopharmaceutical company NuCana since August 2013. This growth has, in turn, enabled significant investment in new clinical trials focused on three of Cardiff's novel ProTide compounds designed to advance effective cancer treatments for patients who have been diagnosed with poor prognosis disease. NuCana's CEO stated that "*as a direct result of access to Cardiff anti-cancer ProTide technology, NuCana has been able to grow and develop as a company, attract world leading investors and advance an exciting pipeline of candidate drugs through development with the ultimate aim of addressing unmet clinical needs in the field of oncology*" **[5.3]**.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[5.1] NuCana Web site, homepage

[5.2] NuCana Annual report 2017

[5.3] Testimonial: Hugh S Griffith, NuCana's Founder and CEO

[5.4] Public offering of NuCana on NASDAQ

[5.5] NuCana webpage outlining the development and evaluation of Acelarin

[5.6] 3 posters presented at the American Society for Clinical Oncology (ASCO) - one on each clinical trial

[5.7] NuCana web page outlining clinical trials for NUC-3373

[5.8] NuCana web page outlining pipeline for NUC-7738