Institution: Cardiff University

Unit of Assessment: UoA3

Title of case study: ProTide Technology: Transforming drug discovery of nucleoside-based anti-viral and anti-cancer agents

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

ProTide technology, discovered by the McGuigan team at Cardiff University, is a pro-drug strategy with proven capacity to generate new drug candidates for nucleoside-based antiviral and anti-cancer indications. In the assessment period the McGuigan team has attracted more than £2 million direct research funding through sustained collaborations on ProTide technology with global pharmaceutical companies and smaller biotech firms in the USA and Europe. In the same period, either through working directly with Cardiff or by independent adoption of McGuigan's research, eight ProTide entities have progressed to clinical trials as cancer, HIV and hepatitis C treatments. The technology is demonstrating significant commercial impact for companies with ProTide-based drug candidates.

2. Underpinning research (indicative maximum 500 words)

ProTide discovery

From the mid-1990's the medicinal chemistry team of Professor Christpher McGuigan at Cardiff University (Reader 94-96 then Professor of Medicinal Chemistry 1996-present) began researching the design of novel chemically-protected phosphate prodrug groups or motifs, later to become known as 'ProTide' technology. In collaboration with the virology group of Professor Balzarini (Rega Institute of Katholieke Universiteit Leuven, Belgium) the McGuigan team investigated how the targeted attachment of ProTide motifs onto precursor nucleoside drugs enhanced drug entry into target cells and overcame nucleoside drug resistance.

In 1996 McGuigan and Balzarini first published the ProTide technology in the leading medicinal chemistry journal [3.1] and in a companion article in one of the world's most-cited multidisciplinary scientific journals[3.2]. McGuigan's team has contributed major reviews of progress and applications in the area, for example [3.3]. The McGuigan-led research showed the chemically-protected phosphate prodrugs to be stable to common extracellular deactivating enzymes that typically cause poor pharmacokinetics. Importantly the lipophilic character of the ProTide motif enables intracellular prodrug delivery by passive membrane permeation rather than relying on active transport. This is a highly desirable pharmaceutical feature for delivery of drugs to intracellular targets and for oral drug administration. Crucially the Cardiff team showed it could manipulate the design of the ProTide to realise selective metabolic bioactivation in target cells and hence maximise the efficacy of a drug while minimising its potential systemic toxicities. Further, the ProTide technology could also overcome the problem of cellular resistance to nucleoside-based drugs. Nucleoside drugs, which account for around half of all antivirals and a fifth of anticancer agents, are activated when they are transformed by phosphorylation through to their respective triphosphates by intracellular enzymes (nucleoside kinases). However, the absence or poor activity of nucleoside kinases in target cells is a frequent cause of clinical resistance to nucleosides. Applying the ProTide strategy effectively bypasses the reliance upon the initial, often rate-limiting, phosphorylation step.

ProTides in drug discovery

The research of the Cardiff team has continued to explore the chemistry, biology and reduction to practice of ProTide technology and its applications in drug discovery. The team have published (1994 - October 31st 2013) 85 original research papers on ProTides. Since 1994, McGuigan has been named inventor on 15 ProTide patents (Cardiff University). McGuigan's team has been at the forefront of candidate ProTide drug development particularly in anti-virals, including the design of a hepatitis C (HVC) drug candidate [3.4]. The ProTide technology has also been applied to modify and improve nucleoside-based anticancer agents [3.5].

The potential of ProTides to transform nucleoside therapeutics has attracted substantial commercial interest from the pharmaceutical industry. Between January 1993 and December 2007 the Cardiff team received £2.5M in research costs and licence fees from strategic collaborations

with multinational pharmaceutical companies to develop antivirals for the treatment of HIV, hepatitis B (HBV) and HCV. The Cardiff team partnered GlaxoSmithKline (USA) (1999-2003) to apply ProTide technology to GSK's nucleoside anti-retroviral agent abacavir and a second lead discovery candidate, LCd4A. ProTide technology delivered 10,000-fold increases in *in vitro* potency against HIV/HBV [3.6]. The research led to six collaborative publications with GSK scientists, including the first ever primate study of ProTides, which revealed key species-to-species variations in the activity of different ProTide motifs and informed future ProTide designs. A partnership with Roche (USA) (2003-2005) demonstrated for the first time the activity of ProTides against HCV. Potent anti-HCV activity at sub-micro Molar concentrations was achieved by ProTide modification of nucleoside parent molecules that were themselves inactive [3.7]. The research led to six collaborative publications with Roche scientists. The research arising from these partnerships helped the Cardiff team to better understand ProTide design to optimise target cell bioactivation and off-target stability, and has led to a major worldwide adoption of ProTides in the subsequent period.

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

[3.1] **McGuigan, C.**, Cahard, D., Sheeka, H.M., De Clercq, E. and Balzarini, J. Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. J. Med. Chem. (1996) 39: 1748-1753. http://dx.doi.org/10.1021/jm950605j

[3.2] Balzarini, J., Karlsson, A., Aquaro, S., Perno, C.F., Cahard, D., Naesens, L., De Clercq, E. and **McGuigan, C.** Mechanism of anti-HIV action of masked alaninyl d4TMP derivatives. Proc. Natl. Acad. Sci. USA (1996) 93: 7295-7299. http://www.pnas.org/content/93/14/7295.abstract

[3.3] **McGuigan, C.**, Mehellou, Y. and Balzarini, J. Aryloxy Phosphoramidate Triesters: A Technology for delivering mono-phosphorylated nucleosides and sugars into cells. ChemMedChem (2009) 4: 1779-1791. http://dx.doi.org/10.1002/cmdc.200900289

[3.4] **McGuigan, C., Madela, K.,** Aljarah, M., Gilles, A., **Brancale, A.,** Zonta, N., Chamberlain, S., Vernachio, J., Hutchins, J., Hall, A., Ames, B., Gorovits, E., Ganguly, B., Kolykhalov, A., Wang, J., Muhammad, J., Patti, J.M. and Henson, G. Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus. Bioorg. Med. Chem. Lett. (2010) 20: 4850-4854. *The publishers commended this paper as a "citation classic": it was the seventh most cited article from a total of 1632 during 2010.* http://dx.doi.org/10.1016/j.bmcl.2010.06.094

[3.5] **McGuigan, C.**, Murziani, P., **Slusarczyk, M.**, Gonczy, B., Vande Voorde, J., Liekens, S. and Balzarini, J. Phosphoramidate ProTides of the Anticancer Agent FUDR Successfully Deliver the Preformed Bioactive Monophosphate in Cells and Confer Advantage over the Parent Nucleoside. J. Med. Chem. (2011) 54: 7247-7258. http://dx.doi.org/10.1021/jm200815w

[3.6] **McGuigan, C.**, Harris, S.A., Daluge, S.M., Gudmundsson, K.S., McLean, E.W., Burnette, T.C., Marr, H., Hazen R., Condreay, L.D., Johnson, L., De Clercq, E and Balzarini, J. Application of phosphoramidate pronucleotide technology to abacavir leads to a significant enhancement of antiviral potency. J. Med. Chem. (2005) 48: 3504-3515. http://dx.doi.org/10.1021/jm0491400

[3.7] Perrone, P., Daverio, F., Valente, R., Rajyaguru, S., Martin, J.A., Leveque, V., Le Pogam, S., Najera, I., Klumpp, K., Smith, D.B. and **McGuigan, C**. First example of phosphoramidate approach applied to a 4'-substituted purine nucleoside (4'-azidoadenosine): conversion of an inactive nucleoside to a submicromolar compound versus hepatitis C virus. J. Med. Chem. (2007) 50: 5463-5470. http://dx.doi.org/10.1021/jm070362i

The research work described above was supported in part by commercial grants from F Hoffmann-La Roche Ltd, GlaxoSmithKline, Inhibitex (part of an overall £1.27 million investment into Cardiff laboratories, 2007-2013) and NuCana BioMed with McGuigan serving as PI on all grants. For example: McGuigan, C. Antiviral nucleotides. C. F Hoffmann-La Roche Ltd. 2002-2005. £566K.

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

ProTide technology embraced by global pharmaceutical companies in R&D programmes

Significant worldwide commercial impact has been delivered from Cardiff research with

multinational pharmaceutical companies and smaller drug discovery firms in the USA and Europe implementing the ProTide technology on candidate and existing therapeutics. The technology is transforming the prospects of antiviral and anti-cancer nucleoside agents. These users have invested in ProTide technology either through direct research partnerships with Cardiff University or by independently adapting the techniques described by the Cardiff team to their own proprietary agents. During the REF period eight novel ProTide entities have entered clinical trials worldwide for a range of diseases. Additionally, ProTide research income from commercial partnerships since January 2008 of ca. £2.2 million has been awarded to the Cardiff ProTide team who have supported the filing of 12 patent families and 28 collaborative Cardiff-industry-partner journal articles.

Partnership in ProTide R&D investment and wealth creation

Between 2007 and 2012 the US pharmaceutical company Inhibitex Inc. (employing 34 full time staff) collaborated with Cardiff University to develop the Cardiff designed ProTide, INX-189, a highly potent antiviral against the hepatitis C virus (HCV), an infectious disease affecting around 170 million people worldwide. The research investment from Inhibitex to Cardiff was £789,000 (for 2008-2012 period), excluding additional milestone payments, and supported five full time staff researchers throughout the period of the agreement. In 2011 successful Phase 1b/II clinical trials demonstrated the safety and efficacy of INX-189 in 50 HCV-infected patients. This ProTide was one of the only two assets of Inhibitex, the other asset, also designed in Cardiff's laboratories, was a non-ProTide phase II anti-viral agent against the varicella zoster virus or shingles which affects around 2 million people worldwide. Largely as a result of the success of INX-189, the NASDAQ capitalisation of Inhibitex rose to approximately $878 million (December 2011) [5.1]. In Feb 2012 Inhibitex was acquired by the multinational pharmaceutical company, Bristol-Myers Squibb (BMS), for $2.5 billion. This was the largest sum ever paid for a biotech with Phase IIa data, and the largest premium (>160%) on market price ever paid in the pharma sector for a company valued over $500 million [5.2]. This strategic exit for the board and shareholders of Inhibitex was afforded by the market confidence in ProTide technology. Due to late phase unexpected cardiotoxicity the clinical trials for INX-189 were suspended in 2012. The toxicity was recognised as a compound-specific idiosyncratic event and confidence in the technology remains with the continued clinical development of related ProTides (see below).

Cardiff ProTides Ltd was a spin-out company from the McGuigan laboratory established to develop ProTides of nucleoside-based anticancer drugs. In 2007 Cardiff ProTides Ltd was acquired by UK-based Morvus Technology Ltd. The following year (2008) Morvus, with UK based oncology medicines company NuCana BioMed, established research and license agreements worth £250,000 of research investment (for the period 2008-2013) to Cardiff (McGuigan). The collaboration supported four full-time staff researchers in Cardiff to work on anti-cancer ProTide technology as part of the development programme of NuCana BioMed. Anti-cancer ProTides from Cardiff are the single asset of NuCana BioMed and since 2008 four joint patents have been filed and one candidate, NUC1031, has entered phase I/IIa monotherapy clinical trials (October 2012) against a range of advanced and metastatic solid tumours including ovarian, breast or endometrial cancer [5.3]. Early, highly encouraging, safety and efficacy data in patients have very recently reported NUC1031 to induce stable progression-free disease in 6/11 patients. Based on these data Nucana are expanding the development of NUC1031[5.3] in collaboration with investment partners, such as the life science venture capital firm Sofinnova (Paris)[5.4], to include a cohort expansion study planned for Q4 2013. Another ProTide candidate (derived from fluorouracil) is in final preclinical candidate selection for primary colorectal cancer with a plan to enter human clinical trials in Q1 2014. As an unlisted company, NuCana BioMed has no current public valuation but with NUC1031 showing efficacy and safety in early trials and historic studies in the oncology market indicating ca. 30% success rate in a several $100 million market for solid tumours, the business of NuCana BioMed has been significantly enhanced by the Cardiff discoveries [5.3].

Independent adoption of ProTide research

Independent of any partnership with Cardiff the ProTide research of McGuigan's team has influenced global drug development, with examples of application including:

Pharmasset (USA) using Cardiff's public domain ProTide technology to generate its own anti-HCV

nucleoside (PSI-7851) [5.5]. Its pure isomer (PSI-7977, now GS-7977) began Phase III clinical trials for HCV in November 2011, which confirmed the highly effective and selective nature of this agent [5.6]. This ProTide agent is very likely to be the first nucleoside to be approved by FDA for HCV, an infectious disease affecting around 170M people worldwide. The biotech company Pharmasset was acquired by Gilead in January 2012. A significant proportion of its $11 billion market capitalisation was assigned to the Phase III anti-HCV ProTide PSI-7977 [5.7].

Gilead (USA) applied Cardiff's ProTide technology to acyclic nucleoside phosphonates, which led to GS-7340, a reverse transcriptase inhibitor in clinical trial Phase II for HIV. An adaptation of ProTide technology has also been applied to generate the anticancer agent GS-9219 which is also in clinical trial Phase 1b/2 and more recently licenced to VetDC for use in animal cancers [5.8].

The underpinning role played by Cardiff research and technology behind each of the above examples is evidenced by the extensive citation of the Cardiff work and the structural similarity of each of the phosphoramidate entities to INX-189 and related compounds. The ProTide platform technology is transforming nucleoside-based therapeutics in antivirals, with the capacity for impact in the anti-cancer arena[5.9].

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

[5.1] Contact - CEO, Biovitas, New York. Ex-Inhibitex board member and early investor in ProTides independent of Inhibitex. How the success of INX-189 raised the NASDAQ capitalisation of Inhibitex and drove the subsequent acquisition by BMS.

[5.2] Press release NY Times January 2012 BMS to acquire Inhibitex for $2.5 Billion. http://dealbook.nytimes.com/2012/01/07/bristol-myers-to-buy-inhibitex-for-2-5-billion/

[5.3] Statement from CEO NuCana BioMed, Edinburgh, investors in ProTides for cancer. Corroboration of the business model of NuCana in relation to ProTide platform technology. NuCana's collaboration with Cardiff on anti-cancer ProTide technology. The joint patenting of anti-cancer ProTides and the entry of NUC1031 into clinical trial with encouraging results. The further development of antio-cancer ProTides in 2013/2014.

[5.4] Statement from Managing Partner, Sofinnova Partners, Paris, major investor in Cancer Protides with NuCana BioMed. Corroborating an investment partnership with Nucana and encouraging clinical safety and efficacy data to support further development.

[5.5] Research article (2010) by Pharmasset showing application of ProTide technology to their proprietary nucleotide analogue PSI-7851. Lam, A.M., Murakami, E., Espiritu, C., Steuer, H.M., Niu, C., Keilman, M., Bao, H., Zennou, V., Bourne, N., Julander, J.G., Morrey, J.D., Smee, D.F., Frick, D.N., Heck, J.A., Wang, P., Nagarathnam, D., Ross, B.S., Sofia, M.J., Otto, M.J. and Furman, P.A. PSI-7851, a pronucleotide of beta-D-2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate, is a potent and pan-genotype inhibitor of hepatitis C virus replication. Antimicrob. Agents Chemother. (2010) 54: 3187-3196. http://dx.doi.org/10.1128/AAC.00399-10

[5.6] Press Release: PSI-7977 enters Phase III trials for Hepatitis C. http://www.hivandhepatitis.com/hepatitis-c/hepatitis-c-topics/hcv-treatment/3323-pharmasset-starts-phase-3-trials-of-psi-7977-without-interferon

[5.7] Press Release: Gilead $11 billion acquisition of Pharmasset which focuses on Pharmasset's main asset PSI-7977. http://www.reuters.com/article/2011/11/21/us-gilead-pharmasset-idUSTRE7AK0XU20111121

[5.8] Press release: VetDC exclusive North American license from Gilead to develop and commercialise GS-9219 for use in animal cancer. http://www.vet-dc.com/gs-9219-gilead-sciences.html

[5.9] Statement from academic clinician and opinion Leader in nucleoside therapeutics. NIHR/Wellcome Trust Imperial Clinical Research Facility and Department of Oncology, Imperial College London. A statement on how ProTide platform technology has the capacity to transform nucleoside-based therapeutics in the context of anti-cancer agents.

All documents, testimony and webpages saved as PDFs are available from the HEI on request.