Institution: University of Portsmouth

Unit of Assessment: 3 Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Research leads to the Commercial Development and Clinical Impact of a First-in-Class Anticancer Agent

1. Summary of the impact

A first-in-class anticancer agent discovered in Thurston's laboratory at the University of Portsmouth in the 1990s has been commercially developed and clinically evaluated over the last two decades. SJG-136 was successful in Phase I clinical trials and is completing Phase II clinical trials for the treatment of ovarian cancer and leukaemia, where significant patient benefit is observed. Related molecules based on this parent compound are in drug programmes being undertaken by Seattle Genetics Inc. and Genentech Inc., leading to additional clinical trials. A spin-out company, Spirogen Ltd, was established in 2000 to commercialise the intellectual property generated from the underpinning research, and the company has recently been sold to AstraZeneca for $200m.

2. Underpinning research

The naturally occurring pyrrolobenzodiazepines (PBDs) are tricyclic molecules with a unique shape that allows them to bind in the minor groove of DNA. They recognize specific DNA sequences, and form adducts that are difficult to repair by normal cell processes and thus have effective anti-tumour activity.

The drug development group at the University of Portsmouth was headed by David Thurston (Professor of Medicinal Chemistry: 1987-1999) who, during this time, developed close collaborations with colleagues working in the area of DNA structure and protein-DNA interactions (principally Geoff Kneale, Professor of Biomolecular Science & Matt Guille, Professor of Developmental Genetics). Thurston and co-workers initiated a program of medicinal chemistry to synthesise and evaluate analogues with novel or improved DNA-recognition properties.
Although the concept of joining two PBD monomer units together through a linker to create DNA cross-linking agents (i.e. "PBD dimers") had already been conceived, these dimers were joined through their C7'-positions and had poor DNA interactivity. Through molecular modelling, the Portsmouth group realised that joining PBD monomers through their C8'-positions instead should produce PBD dimers that match the shape of the DNA minor groove, a key feature of its mechanism of action.

Further modelling studies confirmed that C8'-linkage was preferable to C7'-linkage, and the Thurston laboratory produced the first example of a C8'-linked PBD dimer [**1, 2**]. This compound, DSB-120, was highly active as a DNA inter-strand cross-linking agent and highly cytotoxic to cancer cells. DSB-120 was progressed into human tumour xenograft studies. Unfortunately, DSB-120 had poor activity, probably due to high levels of protein binding. Prompted by some basic studies on the electrophilicity of the PBDs, the Portsmouth group realised that unsaturation at the C2-position of a PBD would allow the target DNA to be reached in cells *in vivo*.
This led to the design by Thurston's group of a new compound, SJG-136, with two methylene groups at the C2/C2'-positions [**3**]. Following the departure of Thurston in 1999, studies on the DNA binding activity of these and related compounds have continued at Portsmouth in collaboration with Guille (1995 – present). The key discovery in 2003/2004 that SJG-136 could inhibit protein binding to DNA in a sequence-selective manner was made in collaboration with Guille's laboratory [**4**].

SJG-136 has **a novel mechanism of action** which differentiates it from conventional cytotoxic and cross-linking agents. Adducts produced by commonly used chemotherapies such as cisplatin produce distortions in the DNA double helix. The majority of these adducts are subsequently recognized and removed by nucleotide excision repair (NER) factors. Cross-links formed by SJG-136 do not distort the DNA double helix and so are able to 'slip under the radar' of NER. Cross-

links induced by SJG-136 persist and permit the molecule to exert its cytotoxic effect for longer. This property makes SJG-136 highly active in refractory tumors, especially those where NER mechanisms are up-regulated (e.g., in cisplatin resistance).

3. References to the research

The underpinning research outlined above was published in leading international journals of the American Chemical Society (Journal of Medicinal Chemistry; Biochemistry) and the Royal Society of Chemistry (Chemical Communications). The research was funded by competitive peer-reviewed awards from SERC, CRC and the EU to University of Portsmouth (on all of which Thurston was principal investigator).

[1] Jenkins, T.C., Hurley, L.H., Neidle, S. and Thurston, D.E., "Structure of a Covalent DNA Minor Groove Adduct with a Pyrrolobenzodiazepine Dimer: Evidence for Sequence-Specific Interstrand Cross-Linking", Journal of Medicinal Chemistry, 37, 4529-4537 (1994).
DOI: 10.1021/jm00052a012 http://pubs.acs.org/doi/abs/10.1021/jm00052a012

[2] Puvvada, M.S., Forrow, S.A., Hartley, J.A., Stephenson, P., Gibson, I., Jenkins, T.C. and Thurston, D.E., "Inhibition of Bacteriophage T7 RNA Polymerase In Vitro Transcription by DNA-Binding Pyrrolo[2,1-c][1,4]benzodiazepines" Biochemistry, 36, 2478-2484 (1997).
DOI: 10.1021/bi952490r http://pubs.acs.org/doi/abs/10.1021/bi952490r

[3] Gregson, S.J., Howard, P.W., Jenkins, T.C., Kelland, L.R. and Thurston, D.E., "Synthesis of a Novel C2/C2′-exo Unsaturated Pyrrolobenzodiazepine Cross-linking Agent with Remarkable DNA Binding Affinity and Cytotoxicity" Chemical Communications, 9, 797-798 (1999).
DOI: 10.1039/A809791G
http://pubs.rsc.org/en/Content/ArticleLanding/1999/CC/A809791G#!divAbstract

[4] Gregson, S.J., Howard, P.W., Gullick, D.R., Hamaguchi, A., Corcoran, K.E., Brooks, N.A., Hartley, J.A., Jenkins, T.C., Patel, S., Guille, M.J., and Thurston, D.E., "Linker Length Modulates DNA Cross-Linking Reactivity and Cytotoxic Potency of C8/C8' Ether-Linked C2-Exo-Unsaturated Pyrrolo[2,1-c][1,4]benzodiazepine (PBD) Dimers", Journal of Medicinal Chemistry 47, 1161-74 (2004).
DOI: 10.1021/jm030897l http://pubs.acs.org/doi/abs/10.1021/jm030897l

Patents Arising from the Research
Thurston, D.E. and Howard, P.W. "PYRROLBENZODIAZEPINES", WO 0012508 (9th March 2000). Continued in Australia, Canada, Japan, New Zealand, USA and Europe.

Underpinning Research Grants awarded to D. Thurston / UoP (1990-1999)
SERC, Project Grant, (Molecular Recognition Initiative GR/F52675), "Molecular Recognition of DNA: Synthesis and Evaluation of Anthramycin-type Ligands" £63,100 (1990–1993).

SERC, Earmarked QUOTA Award (Molecular Recognition Initiative), Award No. 91306038 "Molecular Recognition of DNA: Investigation of the Covalent and Non-covalent Binding Components of the Interaction of Pyrrolo[2,1-c][1,4]benzodiazepines with DNA", £18,000 equiv. (1991–1994).

The Cancer Research Campaign, Project Grant (CRC SP1938/0201), "Investigation of the Relationship between Sequence-Selectivity and Antitumour Activity in DNA-Binding Pyrrolobenzodiazepines and Oxazolobenzodiazepines", £68,500 (1991–1994).

Cancer Research Campaign Technology Grant, "Synthesis and Evaluation of Novel DNA-Binding Cross-Linking Agents with Potential Antitumour Activity" £14,500 (1992–1994).

The Cancer Research Campaign, Project Grant (SP1938/0301), "Design, Synthesis and Evaluation of Pyrrolobenzodiazepine-based Antitumour Agents with Extended DNA Sequence-

Selectivity and Cross-Linking or Cleavage Potential" £235,809 (1993–1996).

EC, Biotechnology Programme Grant, "Studies on the Metabolism, Pharmacokinetics and Toxicology of the Novel Pyrrolobenzodiazepine Dimer Family of Anti-tumour Agents" £53,500 (69,563 ECU) (1993–1995).

The Cancer Research Campaign, Programme Grant (SP1938/0401) to fund the CRC Gene

Targeted Drug Design Research Group, £752,400 (1996–2001).

4. Details of the impact

There are four major impacts arising from this research:
(a) A highly successful and profitable spin-out company has been created and sustained;
(b) A new technology process has been adopted by the company's commercial partners;
(c) A new intervention has been trialled with patients;
(d) Clinical outcomes for patient groups have been improved.

In 1999, the University of Portsmouth and the Cancer Research Campaign (CRC; now CRUK) regarded a spin-out company as the best route for further development of SJG-136 towards Phase I clinical trials. Spirogen Ltd was subsequently formed in 2000, founded by Thurston and Dr Phil Howard (Senior Research Fellow at UoP until 1999, and now CSO at Spirogen), along with John Hartley (UCL) and Chris Martin (Xenva Ltd) **[1]**. The commercial success of the company is evident from the acquisition of Spirogen by Astra Zeneca for $200m in 2013 **[2]**.

A **new intervention** with SG-136 was **trialled with patients**, and significant **clinical benefits** were observed **[3, 4, 5]**. SJG-136 entered a number of Phase I clinical trials **[6-8]** in the mid-to-late 2000s in the USA through the National Cancer Institute (Vanderbilt [Rothenburg / Puzanov], Sloan Kettering [Rizvi], MD Anderson [Ravandi] and Ohio State University [Byrd]), and in the UK through Cancer Research UK (Edinburgh [Jodrell] and Royal Free London [Hochhauser]). The results showed evidence of clinical efficacy and the side-effects were relatively non-toxic (e.g. lower limb oedema and fatigue, which were transient and reversible). Significant patient benefit in the form of partial responses and stabilisation of disease was observed in ovarian and bowel cancer, melanoma and leukaemia.

To date, over 69 patients have been treated in multiple Phase I clinical trials. There were15 cases leading to disease stabilisation and three notable Partial Responses recorded in patients heavily pre-treated and resistant to other chemotherapy agents. On the basis of these Phase I data, SJG-136 (subsequently renamed SG2000) entered Phase II clinical trials in ovarian cancer through the US National Cancer Institute in early 2011 **[3ii]**, and the trial is close to completion. A further Spirogen-sponsored Phase II clinical trial in leukaemia with two centres in the USA started recruiting in 2013.

During the mid-2000s, Spirogen Ltd approached the US Biotech company, Seattle Genetics Inc., with an interest in attaching a PBD dimer to an antibody to generate targeted therapies. This relationship has developed to the point where a CD33 targeting antibody-PBD conjugate (ADC) is in Phase I clinical trials **[9,10]**. Since 2011, Spirogen has also had research collaborations and license agreements with Genentech Inc., a member of the Roche Group. These biotechnological and clinical developments were instrumental in convincing MedImmune, a subsidiary of Astra Zeneca, of the commercial potential of SG2000 conjugates in anti-cancer drug therapy **[1,2]**. Commenting on the recent deal, Spirogen CEO Dr. Chris Martin said: "This deal reflects the very significant progress made by our scientists, most notably over the last two years, as we have applied our warhead and linker technologies to the development of highly potent and specific ADCs. We believe that PBD-armed antibody-drug conjugates will emerge as a critical component in the next generation of cancer biologics with the potential to make a difference for oncologists and their patients. We look forward to combining our world class capabilities in this area with MedImmune's ability to develop this exciting class of oncology drugs."

(www.spirogen.com/news/latest.php?id=1071)

5. Sources to corroborate the impact

[1] The formation of Spirogen and its subsequent development can be found at www.spirogen.com/spirogen/history.php. Letters are available from;
(a) the Principal Investigator and Scientific Advisor to Spirogen;
(b) the CEO of Spirogen, both stating that the research at Portsmouth was key to the creation and subsequent success of the company.

[2] News of the acquisition of Spirogen by AstraZeneca in October 2013
www.reuters.com/article/2013/10/15/us-astrazeneca-spirogen-idUSBRE99E03Y20131015

[3] Key Examples of Clinical Trials of SJG-136
(i) National Cancer Institute (NCI) Website for recruitment of patients Phase I Clinical Trials in Ovarian Cancer: SJG-136 in Treating Patients With Relapsed or Refractory Acute Leukemia, Myelodysplastic Syndromes, Blastic Phase Chronic Myelogenous Leukemia, or Chronic Lymphocytic Leukemia. Conducted 2005-2009. http://clinicaltrials.gov/show/NCT00301769
(ii) National Cancer Institute (NCI) Website for recruitment of patients Phase II Clinical Trials in Ovarian Cancer: "Clinical Trial for SJG-136 in Treating Patients With Epithelial Ovarian, Primary Peritoneal, or Fallopian Tube Cancer That Did Not Respond to Previous Treatment With Cisplatin or Carboplatin". Conducted 2010-2012. http://clinicaltrials.gov/show/NCT01200797

[4] Article in Pharmaletter describing partial response in Phase I: The Pharmaletter, "SJG-136 achieves partial response in OC patient", 9th June 2008
www.thepharmaletter.com/article/sjg-136-achieves-partial-response-in-oc-patient

[5] Information on SJG-136 (SG2000) on Spirogen Website:
http://www.spirogen.com/products/development.php?id=126 and
http://www.spirogen.com/pdf/SG2000-Highlights.pdf

[6] Hochhauser, D., et al., (2009) Phase I Study of Sequence-Selective Minor Groove DNA Binding Agent SJG-136 in Patients with Advanced Solid Tumors.
Clinical Cancer Research, 15, 2140-2147.

[7] Janjigian, Y.Y., et al., (2010) A Phase I Trial of SJG-136 (NSC#694501) in Advanced Solid Tumors. Cancer Chemotherapy and Pharmacology, 65, 833-838.

[8] Puzanov, I., et al., (2011) Phase I Pharmacokinetic and Pharmacodynamic Study of SJG-136, a Novel DNA Sequence Selective Minor Groove Cross-linking Agent, in Advanced Solid Tumors.
Clinical Cancer Research,17, 3794-3802.
[doi: 10.1158/1078-0432.CCR-10-2056]

[9] Jeffrey, S.C., et al., (2013) A Potent Anti-CD70 Antibody-Drug Conjugate Combining a Dimeric Pyrrolobenzodiazepine Drug with Site-Specific Conjugation Technology. Bioconjugate Chemistry, 24(7): 1256-1263.

[10] Sutherland, M.S.K., et al., (2013) SGN-CD33A: A Novel CD33-targeting Antibody-Drug Conjugate Using a Pyrrolobenzodiazepine Dimer is Active in Models of Drug-Resistant AML.
Blood, 122(8): p. 1455-1463.