Institution:
University of Cambridge

Unit of Assessment:
UoA5

Title of case study:
Collagen Peptides and Toolkits for diagnostics and drug discovery.

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

The Farndale group have identified fragments of collagen, synthesised and assembled in active, triple-helical conformation, for use as ligands to manipulate platelet function. As a result of this work, the fragment Collagen-Related Peptide (CRP) is included in British Committee for Standards in Haematology guidelines as a platelet agonist for the diagnosis of platelet defects. The group has also synthesised triple-helical collagen peptide libraries and used them to map binding of cells or proteins to collagen more widely. The peptides are made and distributed by the Farndale lab, generating income through sales and licencing, and are used internationally by companies and hospitals to develop diagnostics and for high-throughput drug discovery. Prof Farndale also acts as a consultant for companies developing diagnostics.

2. Underpinning research (indicative maximum 500 words)

Cell-collagen interactions are involved in a plethora of clinical conditions, including the two leading causes of death and disability in the Western world, heart attack and ischemic stroke, as well as tumour biology, bacterial infections, bleeding disorders, wound healing, immune-regulation and inflammatory diseases such as rheumatoid arthritis. However, they have been difficult to characterise because intact collagens bind many different receptors and matrix components. In 1994 Richard Farndale (Department of Biochemistry, then Senior Research Fellow; Lecturer from 1997, Reader from 2000, and Professor of Matrix Biochemistry since 2008) began his underpinning research on collagenous peptides and their interaction with cells, in collaboration with Michael Barnes (MRC External Staff, first at Strangeways Research Lab, Cambridge, and since 1995 at the Department of Biochemistry).

First application of triple-helical peptides to regulate platelet function: identification of GPVI-binding motif 'CRP': Between 1994 and 1995 Farndale and Barnes discovered that a self-assembling triple-helical model peptide (Collagen-Related Peptide, CRP) bound platelets in both monomeric (mCRP) and cross-linked (CRP-XL) form. Furthermore, that CRP-XL was a potent agonist in platelets, rapidly inducing platelet activation and aggregation (Ref. 1, Section 3). This work was the first application of a triple-helical peptide to human platelets, and indicated that CRP regulated platelets through an unidentified collagen receptor distinct from integrin □2□1. From 1995 to 1998 Farndale and Barnes collaborated with B. Kehrel (University of Muenster) and M. Okuma (University of Kyoto), to identify the receptor for CRP. They evaluated the response of platelets (provided by Okuma) with defined functional deficiencies in Glycoprotein VI (GPVI). Only GPVI-deficient platelets were unresponsive to CRP, indicating that GPVI was the receptor for CRP (Ref. 2, Section 3).

First mini-Toolkit: identification of integrin-binding motif 'GFOGER': In parallel, Farndale and Barnes pursued their long-standing interest in integrin □2□1, the only collagen-binding integrin expressed on platelets. Between 1996 and 2000 they devised and synthesised a set of seven overlapping peptides, which they used to map the main integrin-binding motif in collagen I. Subsequent truncation and substitution within these peptides identified the amino acid sequence GFOGER, in triple-helical conformation, as the high-affinity motif that bound integrin □2□1, securing platelet and other cell adhesion to collagen (Ref. 3, Section 3).

First full Toolkit: identification of VWF-III: The next step towards the development of a "full toolkit" was taken between 2003 and 2006, when the Farndale lab synthesised a set of 57 peptides for collagen III spanning the entire triple-helical domain of human collagen III. In collaboration with E. Huizinga and P. de Groot at the University of Utrecht, who provided purified human plasma von Willebrand Factor (VWF), they used this toolkit to locate a high affinity binding site for VWF, named VWF-III (Ref. 4, Section 3).

First combination of triple-helical peptides to reconstitute full collagen activity: Between 2007 and 2010, research in the Farndale lab pulled the previous discoveries together, using CRP, GFOGER

and VWF-III to investigate, with confocal imaging, the effects of each receptor-ligand interaction on platelet binding and activation during thrombus formation (Ref. 5, Section 3).
First use of Toolkits to identify a novel collagen receptor (OSCAR): Using the toolkit approach between 2009 and 2011, the group of Farndale together with Dr Alex Barrow and Prof John Trowsdale (respectively: Marie Curie Fellow from 2009 to 2012; Professor of Immunology since 1997; both at the Department of Pathology) discovered a novel collagen-binding immune receptor, OSCAR, closely related to GPVI. OSCAR is expressed on several leukocyte populations, and the group showed, leading an international research consortium, that osteoclasts can be derived from peripheral blood mononuclear cells after stimulation with one of the OSCAR-specific peptides identified in the Farndale lab (Ref. 6, Section 3).

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

1. Morton LF, Hargreaves PG, **Farndale RW**, Young RD, Barnes MJ (1995) Integrin α2β1-independent activation of platelets by simple collagen-like peptides: collagen tertiary (triple helical) structure and quaternary (polymeric) structure are sufficient alone for α2β1-independent platelet reactivity. Biochem J **306**: 337-344. http://www.biochemj.org/bj/306/0337/3060337.pdf
2. Kehrel B, Wierwille S, Clemetson KJ, Anders O, Steiner M, Knight CG, **Farndale RW**, Okuma M, Barnes MJ (1998) Glycoprotein VI is a major collagen receptor for platelet activation: it recognizes the platelet activating quaternary structure of collagen, whereas CD36, GPIIb/IIIa and VWf do not. Blood 91: 491-499. http://bloodjournal.hematologylibrary.org/content/91/2/491.full.pdf+html
3. Knight CG, Morton LF, Peachey AR, Tuckwell DS, **Farndale RW**, Barnes MJ (2000) The collagen-binding A-domains of integrin α1β1 and α2β1 recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. J Biol Chem 275: 35-40. DOI: 10.1074/jbc.275.1.35
4. Lisman T, Raynal N, Groeneveld D, Maddox B, Peachey AR, Huizinga EG, de Groot PG, **Farndale RW** (2006) A single high-affinity binding site for von Willebrand Factor in collagen III, identified using synthetic triple-helical peptides. Blood, **108**, 3753-6. DOI:10.1182/blood-2006-03-011965
5. Pugh N, Simpson AM, Smethurst PA, de Groot PG, Raynal N, **Farndale RW**. (2010) Synergism between platelet collagen receptors defined using receptor-specific collagen-mimetic peptide substrata in flowing blood. Blood 115, 5069-79 DOI:10.1182/blood-2010-01-260778
6. Barrow AD, Raynal N, Andersen TL, Slatter DA, Bihan D, Pugh N, Cella M, Kim T, Rho J, Negishi-Koga T, Delaisse J, Takayanagi H, Lorenzo J, Colonna M, **Farndale RW**, Choi Y, Trowsdale J. (2011) OSCAR is a collagen receptor that costimulates osteoclastogene-sis in DAP12-deficient humans and mice. J Clin Invest 121, 3505-16 DOI:10.1172/JCI45913

Grants relevant to this Case:

MJ Barnes and RW Farndale. The molecular mechanisms of cell-collagen interaction. MRC: Special Project 1994-1999. £1.2m

RW Farndale and CG Knight. Recognition motifs for cell surface receptors and matrix proteins within the triple-helical domains of collagen. Wellcome Trust: Project. 2003-2005 £143k

RW Farndale. Design and synthesis of peptide agonists for platelet receptors. MRC: Project. 2005-2008 £326k

RW Farndale and WH Ouwehand. Platelet receptors for collagen; activatory pathways. British Heart Foundation Programme Grant 2004-2009. £822k.

RW Farndale. The collagen-binding integrins: structure and regulation. MRC: 5-year Research Grant 2006-2011. £882k

RW Farndale. Collagen Toolkits and related triple-helical peptides. Wellcome Trust: Biomedical Resource Grant 2011-2014. £472k *(Designed to propagate the Toolkit project)*

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

Farndale's triple-helical peptides and peptide toolkits have been in continuous demand since their first publication, ie from 1995 and 2006 respectively. They have been synthesized and distributed widely by Farndale to industry and hospitals for diagnostic development and drug-discovery, leading to the following impacts:

Impact on health and well-being:

Healthcare guidelines

Since the discovery of CRP by Farndale, CRP is included as one of the new extended panel of platelet agonists for the diagnosis of rare platelet defects using light transmission aggregometry in the BCSH "Guidelines for the Laboratory Investigation of Heritable Disorders of Platelet Function" of August 2011 (Ref. 1, Section 5).

Diagnostics

A CRP-peptide (CRP-18/i) has been included as a *platelet agonist to activate GPVI* in a Phase 4 clinical trial, sponsored by Karolinska University Hospital, Sweden, and completed in 2010, on platelet function in diabetic patients (NCT01035320) (Ref. 2, Section 5).

Using CRP-XL from the Farndale lab the University Medical Center Utrecht, NL, have developed a *novel platelet activation test* that uses anticoagulated unprocessed blood to determine platelet function, which has now been tested in blood of thrombocytopenic patients (Ref. 3, Section 5).

A number of hospitals are using CRP in routine evaluations of patients with bleeding disorders: At the University Hospital Linkoping, Sweden, CRP is included in the *flow cytometry protocol* for patients' blood samples; this is most important because collagen preparations do not work in flow cytometry. Since 2008 50 patients have been tested per year.

Both at the University Hospital Vienna (Panzer et al), and at the Department of Cardiovascular Sciences, KU Leuven (Freson et al), patients with a GPVI mutation that explains a history of bleedings were identified using CRP. In Vienna, CRP has also been used successfully to assess the responsiveness of platelets that were induced by treating patients with chronic immune thrombocytopenia with Eltrombopag, a recently FDA approved medication for these patients (Ref. 4, Section 5).

The company BioData Corp sells CRP as a diagnostic reagent (see also *Impact on Commerce*, Ref. 11, Section 5).

Drug development

Portola Pharmaceuticals (San Francisco, US) have used peptides from the Farndale lab in the development of a *novel assay to monitor the pharmacodynamic activities of novel platelet inhibitors* for in-house drug development (Ref. 5, Section 5).

The biopharmaceuticals company Trigen Holdings AG purchased CRP-XL material from the Farndale lab in 2006 for their drug discovery programme. Trigen's follow-on company, AdvanceCor, currently conducts Phase II trials (NCT01042964) on Revacept, a dimeric form of the extracellular domain of GPVI, as an anti-thrombotic. The key discoveries on Revacept had occurred before Trigen bought the CRP peptide from the Farndale lab; however, the AdvanceCor CEO testifies to the value the research findings on CRP and GPVI from the Farndale lab have had for the further development of the drug since 2008 (Ref. 6, Section 5).

The biopharmaceutical company Regado Biosciences (Durham, US) have used Farndale's CRP-XL as a tool to determine the pharmacology and specificity of some of their preclinical platelet receptor antagonist leads. One of these leads, REG3, a specific GPVI inhibitor, is planned to enter Phase 1 clinical trials in 2014 (Ref. 7, Section 5).

Impact on commerce:

Commercial income has been generated in the University (Ref. 8, Section 5)

a. through services provided: The Farndale lab operates a small Research Facility, which produces and distributes specific peptides to the academic and commercial research community worldwide. The major products are CRP, GFOGER and related sequences, and Collagen Toolkits. Since 2008 £137k of income has been generated through the sale of the above peptides by this facility.

b. through consultancy: Farndale has non-exclusive consultancy contracts with the companies Biokit (Barcelona) and Diagnostica Stago (Paris), acting as an advisor for the development of an assay for a VWF diagnostic kit. Since 2008 over £20k has been generated through this consultancy work.

c. through licensing of patents: The key OSCAR-binding motifs were protected with a patent (WO2009GB02382 20091006) and licensed exclusively to NovoNordisk in 2010 for high throughput screening and drug development, generating to date over £105k in licensing fees.

Industry has invested in R&D, has adopted a new technology or process and/or has

commercialised a new product: *Development of drugs, reagents and assays since 2008*
The drug development company **Portola Pharmaceuticals** (San Francisco, US) have used Farndale's CRP peptide for research into the structure and function of GP VI on platelets (Ref. 2, Section 5). The developer and distributer of clinical diagnostic systems **Biokit SA** (Barcelona, Spain) have invested into the development of an assay for VWF for the diagnosis of the bleeding disorder von Willebrand disease, based on the interaction between the VWF A3 domain and a collagen sequence identified in the Farndale lab. They have used collagen peptide samples synthesised in (or commercially synthesised by CRB Billingham, UK, and evaluated by) the Farndale lab (Ref. 9, Section 5). The global healthcare company **Novo Nordisk A/S** (Måløv, Denmark), in collaboration with Farndale, is using peptides derived from the Toolkit program for characterisation of potential therapeutic targets and as a tool for establishing CMC (Chemistry-Manufacturing-Controls) assays (Ref. 10, Section 5). The company **BioData Corp** sells CRP under the brand name Collagen SRP™ (Ref. 11, Section 5).

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

1. BCSH guidelines: "Guidelines for the Laboratory Investigation of Heritable Disorders of Platelet Function British Committee for Standards in Haematology" August 2011; http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2141.2011.08793.x/full
2. Almquist T, Jacobson SH, Lins PE, Farndale RW, Hjemdahl P. Effects of lipid-lowering treatment on platelet reactivity and platelet-leukocyte aggregation in diabetic patients without and with chronic kidney disease: a randomized trial *Nephrol Dial Transplant*. 2012 27:3540-6. doi:10.1093/ndt/gfs183 NCT01035320 clinicaltrials.gov/ct2/show/NCT01035320
3. Roest M, van Holten TC, Fleurka GJ, Remijn JA (2013) Platelet Activation Test in Unprocessed Blood (Pac-t-UB) to Monitor Platelet Concentrates and Whole Blood of Thrombocytopenic Patients *Transfus Med Hemother* 40:117–125; doi:10.1159/000350688
4. Haselboeck J, Kaider A, Pabinger I, Panzer S. Function of eltrombopag-induced platelets compared to platelets from control patients with immune thrombocytopenia. *Thromb Haemost*. 2013: 109/4, 569-768. doi:10.1160/TH12-07-0522
5. Testimonial Chief Scientific Officer at Portola Pharmaceuticals
6. Testimonial Managing Director at AdvanceCOR
7. Testimonial Executive Director at Regado Biosciences
8. Spreadsheet showing income generated to the University
9. Testimonial Chief Technical Officer at Biokit
10. Testimonial Principal Scientist at Novo Nordisk
11. http://www.biodatacorp.com/platelet-aggregation/aggregation-reagents/aggregation-related-products/item/126-collagen-srp-synthetic-reactive-peptide.html