Institution: University College London / Birkbeck College

Unit of Assessment: 5 - Biological Sciences

Title of case study: Antibody sequence and structure analysis assists biologic drug design

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

Research by Dr Andrew Martin at the UCL Research Department of Structural & Molecular Biology has led to a series of antibody-related tools being made available for free use over the Web. One of these, Abysis, has been visited over 360,000 times by over 8,000 users. Abysis has also been released under a commercial license and has been purchased by companies ranging from small biotechs to large pharma for use in their antibody therapeutic development pipelines, allowing them to identify unusual features of their sequences and to improve strategies for humanisation. Martin has also acted as an expert witness for drug companies in patent disputes.

2. Underpinning research (indicative maximum 500 words)

Martin's group is one of the only groups specialising in computational analysis and prediction related to antibodies. Since the invention of monoclonal antibodies, their potential as 'magic bullet' drugs has been realised. However, it is only relatively recently that problems in their use have been overcome and antibodies now represent approximately a third of all drugs in development. So-called 'fully human' antibodies from phage libraries have had mixed success in the clinic and the research in the Martin lab is a valuable contribution to developing antibodies as drugs. In particular, his research has led to the development of numerous widely used tools and databases for analysis of antibody sequence and structure, contributions to modelling antibodies, an understanding of VH/VL packing, and a measure of 'humanness' of antibodies.

Between 1994 and 1999, Andrew Martin and Janet Thornton worked on the application and related analysis of a novel method for modelling antibody complementarity determining region (CDR) loops. In particular, Martin developed a new method for modelling long antibody loops using machine learning methods, and analysed binding site topography **[1]**. This work provided a quantitative evaluation of the shape of antibody combining sites, correlating this with the antigen class. It also identified which amino acids of the binding site interact with different classes of antigen. He also developed a new database 'Kabatman' allowing access to Kabat antibody sequence data and analysed CDR loop conformations, updating the manual analysis of Chothia's group in Cambridge with a new automated technique, expanding the analysis of key residues responsible for CDR conformation and therefore allowing better modelling of their structure **[2,3,4]**.

Martin continued this work at Reading between 1999 and 2004, where he analysed antibody sequences and collaborated with Rybak's group at the National Center for Biotechnology Information (Bethesda, MD) leading to grant of a patent on anti-CD22 antibodies (US Patent 7,456,260; 2008).

Back at UCL in 2004 (as Lecturer, and later Senior Lecturer), he developed a new automated method for applying standard numbering schemes to antibody sequences. Various such schemes have been devised, the most popular being the Kabat and Chothia schemes, but there had been no tools that applied these schemes automatically. Applying standard numbering to antibody sequences and structures, as allowed by Martin's research, is fundamental to analysing their properties **[5]**. In addition, he analysed the variation in VH/VL domain packing, demonstrating that this can vary by some 30^{o}, and developed a machine learning method to predict the packing angle – again allowing improved prediction and modelling of antibody structure **[6]**. He developed methods for assessing the 'humanness' of antibodies by evaluating how similar they are to the expressed human repertoire **[7]**. Combining a number of these tools and resources, he developed the Abysis antibody database, an integrated resource based around a relational database of sequence and structure data together with a set of tools for analysing new sequences.

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

[1] Reczko M, Martin AC, Bohr H, Suhai S. Prediction of hypervariable CDR-H3 loop structures in antibodies. Protein Eng. 1995 Apr;8(4):389-95. http://dx.doi.org/10.1093/protein/8.4.389

[2] Martin AC, Thornton JM. Structural families in loops of homologous proteins: automatic classification, modelling and application to antibodies. J Mol Biol. 1996 Nov 15;263(5):800-15. http://dx.doi.org/10.1006/jmbi.1996.0617

[3] Martin AC. Accessing the Kabat antibody sequence database by computer. Proteins. 1996 May;25(1):130-3. http://doi.org/bkn87w

[4] MacCallum RM, Martin AC, Thornton JM. Antibody-antigen interactions: contact analysis and binding site topography. J Mol Biol. 1996 Oct 11;262(5):732-45. http://doi.org/dfv7rj

[5] Abhinandan KR, Martin AC. Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains. Mol Immunol. 2008 Aug;45(14):3832-9. http://dx.doi.org/10.1016/j.molimm.2008.05.022.

[6] Abhinandan KR, Martin AC. Analysis and prediction of VH/VL packing in antibodies. Protein Eng Des Sel. 2010 Sep;23(9):689-97. http://dx.doi.org/10.1093/protein/gzq043.

[7] Abhinandan KR, Martin AC. Analyzing the "degree of humanness" of antibody sequences. J Mol Biol. 2007 Jun 8;369(3):852-62. http://dx.doi.org/10.1016/j.jmb.2007.02.100

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

Provision of antibody-related tools and databases to the pharmaceutical industry

Martin has been making antibody-related tools available for free use over the Web since 1996 when KabatMan made the Kabat antibody sequence available in a manner that let people perform automated global analyses of antibody sequences for the first time.

The initial development of Abysis, a new integrated database of antibody sequences and structures, was funded by biopharmaceutical company UCB (2006-9) in return for which they received a version for in-house use. This is now their primary resource for analysis of antibody sequence and structure and is used as a repository for their own in-house antibody sequence data **[a]**.

Abysis has been made freely accessible over the web since 2009. Between October 2009 and December 2012, Martin's antibody-related pages (including Abysis) have been visited >570,000 times (Abysis >360,000) by nearly 43,000 distinct users (Abysis nearly 8,000). This represents an average of >14,600 visits per month (Abysis >9,200) from >1,100 distinct users (Abysis >200) per month. Commercial users of the online Abysis and the antibody web site include: [Text removed for publication].

Abysis has also been released under a commercial license since the end of 2009 through UCL Business for in-house use by commercial customers. It has been purchased by companies ranging from small biotechs to large pharma for use in their antibody therapeutic development pipelines and has grossed [Text removed for publication] of sales on annual licences **[b]**. Commercial users with company installations of Abysis system include *[Text removed for publication]*. (All have been purchased on annual licences, most of which have renewed each year.) UCB and the other companies that have purchased Abysis use it to store their in-house sequence data and compare it with public data. This enables them to identify unusual features of their sequences and to improve strategies for humanisation **[c]**. [Text removed for publication]

[Text removed for publication] also used the measure of humanness available within Abysis to show that Humira, the first 'fully human' antibody (produced by phage display), did not appear especially human in nature. Recent results have shown that almost 30% of patients develop anti-

antibody responses within three years, suggesting that this humanness measure may well be a useful tool to help predict immunogenicity, and it is being used within Abysis for this purpose **[f]**.

In recognition of the commercial impact of the Abysis software, the BBSRC funded a 1-year project under its 'Follow-On' scheme for further commercial development and marketing of Abysis starting May 2013 **[g]**. This grant is allowing various improvements focused on the needs of customers such as easier installation, an improved interface, easier input of proprietary sequence data and interfacing with protein modelling software. In addition, it includes funding for marketing including market research and commercial stalls at key industry-attended conferences.

Another of Martin's programs, his ProFit software for protein structure least squares fitting has over 75 commercial users including [Text removed for publication] **[h]**.

Drug development

Collaborations with other UCL groups include work on anti-DNA antibodies in systemic lupus erythamatosus (Kalsi et al., 1996; Ravirajan et al., 1998). Here, expertise in antibody modelling led to structural models of antibodies interacting with DNA that were tested though mutations. Other collaborations include work on features of fine specificity (Lie et al., 1999) and expression (Kipriyanov et al., 1997; Saldanha et al., 1999), the latter suggesting mutations that significantly improved expression and/or stability without affecting binding.

A review of the patent literature has shown that over 800 patents were published in the REF period where the patent applications cite the publications given above, indicating significant commercial relevance of Martin's work. The list of assignees that hold patents citing Martin's research papers include: Hoffman-La Roche, Novartis, AstraZeneca, GlaxoSmithKline, Abbott Laboratories and many more, around topics including antitumor antibodies, fibronectin cradle molecules, amyloidogenic disease and recombinant anti-interleukin-9 antibodies **[i]**.

Recently, a collaboration with the French Centre de Recherche du Service de Santé des Armées (CRSSA) **[j]** led to Idec no longer maintaining their patent restricting the use of macaque antibodies as therapeutics (US5693780). Martin's measure of humanness, available within Abysis, has shown that, contrary to claims in the Idec patent, macaque antibodies can be distinguished from human antibodies. This has therefore given freedom to operate, in the use of macaque antibodies as therapeutics, to all commercial companies as well as for development by the CRSSA.

Provision of expert witness in court cases

Martin's expertise in antibodies was recognized by UCB who asked him to act as an expert witness in a series of related patent disputes from 1998 onwards, while his general expertise in bioinformatics was recognized by Human Genome Sciences and GlaxoSmithKline who asked him to be an expert witness in cases related to gene patenting. Those within the impact period of 2008-13 are described below.

2007-8 – Lilly vs. HGS (EP0939804B1, Ebner et al) **[k, l]**. Lilly challenged the validity of HGS's patent on neutrokine alpha (also known as Blyss or BAFF) on the grounds of industrial application and obviousness. This was a very important case for Biotech in the UK. Lilly claimed that it was obvious that this gene/protein was a member of the TNF family and therefore there was no inventive step. Martin argued for HGS in the UK courts that it was not obvious what this gene would do, showing the complexity of the bioinformatics involved in identifying its function. This is an important area for all genes/proteins whose function is determined through Bioinformatics approaches. The judge in the initial UK hearing was fully convinced by Martin's evidence and this area was never questioned again during the subsequent appeals **[m]**.

2010-11 – Interference 105,705, US Application 10/938,117 (Adair et al.) v US6180370 (Queen et al.). UCB counter-attacked PDL claiming that PDL's patent interfered with their own. While it has stood up in court, the PDL patent is open to certain interpretations that mean that (depending on the priority date to which it is entitled), it may be pre-empted by various pieces of prior art. A

settlement was reached out of court **[n]**.

2011 – Appeal on refusal to grant patent – Colombian patent application [Text removed for publication]. While UCB have been successful in gaining patents in most countries, the Columbian patent office refused to grant a patent and Martin was asked to give an expert opinion on matters related to the grounds for refusal **[n]**.

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

[a] Director, V-Region Discovery & Engineering, UCB. Can corroborate company's engagement with Abysis. Contact details provided.

[b] A report on Abysis income from UCL Business is available on request.

[c] Abysis is available from http://www.e-lucid.com/i/software/bioinformatics/Abysis.html and through Ebisu (UK) http://www.chemogenomix.com/chemogenomix/Abysis.html. The latter website corroborates details of how the software is used.

[d] [Text removed for publication]

[e] [Text removed for publication]

[f] Bartelds GM, Krieckaert CL, Nurmohamed MT et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA. 2011 Apr 13;305(14):1460-8. http://jama.jamanetwork.com/article.aspx?articleid=896649

[g] "Commercializing Abysis - An Integrated Resource For Storing And Analyzing Antibody Sequence And Structure" BB/K015443/1, £161,293.42, 13/05/2013 to 12/05/2014.

[h] http://www.bioinf.org.uk/software/profit/index.html. Report on downloads and users available on request.

[i] Report by Cambridge IP Ltd available on request.

[j] Thullier P, Huish O, Pelat T, Martin AC. The humanness of macaque antibody sequences. J Mol Biol. 2010 Mar 12;396(5):1439-50. http://dx.doi.org/10.1016/j.jmb.2009.12.041.

[k] Details of the court case are available from: http://www.bailii.org/ew/cases/EWHC/Patents/2008/1903.html

[l] Letter of support from the legal representatives of HGS available on request.

[m] Full discussion of subsequent appeals is available at http://ipkitten.blogspot.co.uk/2011/11/human-genome-sciences-v-eli-lilly.html

[n] Can be verified by Associate General Patent Counsel, UCB. Contact details provided.