Institution: University Cambridge

Unit of Assessment: UoA1

Title of case study: Engineering of recombinant therapeutic antibodies to optimize effectiveness

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

Research into modified Fc regions for therapeutic antibodies has resulted in the development of antibodies with novel and optimised functions. An aglycosylated anti-CD3 antibody called otelixizumab has reached phase 3 clinical trials with GSK and a novel antibody for treatment of fetomaternal alloimmune thrombocytopenia has been tested in human volunteers. The patented technology has been licensed to Pfizer and to GSK for incorporation into their therapeutic antibody programmes with four of these already in clinical trials (tanezumab, ponezumab, RN316 & RN564). Licensing revenue totalling £3.2 million has been returned to the University's company Cambridge Enterprise Ltd in the impact period. In addition, consultancy and advisory services on antibody engineering have been provided to a number of other biopharma companies.

2. Underpinning research (indicative maximum 500 words)

Dr Mike Clark was a graduate student of the inventor of monoclonal antibodies Dr Cesar Milstein between1978-1981, and then joined Herman Waldmann's group to work on therapeutic antibodies as a Post-doctoral Research Associate (1981-1990). In 1990 Dr Clark was appointed to a Lectureship and in 2007 was promoted to Reader in Therapeutic Immunology in the Department of Pathology. During his post as a Senior Research Associate in Waldmann's group and in collaboration with Greg Winter's group (MRC LMB) he was a co-inventor of the first fully humanized antibody Campath-1H (alemtuzumab) specific for the CD52 antigen on lymphocytes. Campath was approved for treatment of BCLL by both the FDA and EMA in 2001. More recently (2013) it was approved by the EMA for treating multiple sclerosis. Campath's properties and therapeutic success formed the basis of subsequent translational research at Cambridge.

In the research period, Clark and colleagues continued the theme of research into therapeutic applications of antibodies, in particular investigating the structural features that determine the effector functions of the different human IgG subclasses, and determining how to exploit these structural differences through the generation of optimised and novel mutant Fc regions. The CD52 specific Campath-1H (alemtuzumab) antibody was used as one of the key model systems in this research from Clark's laboratory. Work by Clark's group (Redpath et al 1998) demonstrated that it was an ideal choice for complement activation. Subsequent work published by Armour et al (1999 & 2003) using alemtuzumab as the wildtype example emphasised the importance of the IgG1 isotype in binding to human FcgRI and FcgRII receptors. The results of this research have underpinned the ideal choice of the IgG1 subclass for cytotoxic cell-depleting antibodies such as alemtuzumab. However for some therapeutic applications it became clear to Clark that such antibodies could exhibit severe side-effects through cross-linking of Fc receptors. This was particularly evident in the use of anti-CD3 antibodies for immunosuppression in renal allograft rejection where severe side effects resulting from cytokine release syndrome were encountered that could not be completely avoided even with a monovalent CD3 antibody (Abbs et al 1994). This study indicated to Clark and colleagues that further modifications to reduce Fc receptor binding and cross-linking might lead to improved efficacy and with reduced side-effects In 1993 a first step in that direction was taken in collaboration with Waldmann's group when a therapeutic CD3 antibody was rendered non-depleting and non-mitogenic through mutation of the conserved N-linked glycosylation site in the Fc region (Bolt et al 1993).

In September 1995, Clark began a long term research programme in collaboration with Drs Lorna Williamson and Willem Ouwehand of the National Blood and Transplant Service and University Department of Haematology, based at Addenbrookes Hospital, to try to develop a treatment for fetomaternal alloimmune thrombocytopenia (FMAITP). The disease results from maternal IgG alloantibodies directed towards platelets crossing the placenta and causing platelet destruction in the developing foetus. The research programme sought to develop a recombinant antibody with novel and desired properties: high affinity for the platelet alloantigen HPA-1a, the ability to cross

the human placenta via the receptor FcRn, inability to trigger any complement activation or Fc mediated cytotoxicity. The antibody also needed to appear as human as possible so as to avoid an antiglobulin response. Ideally the antibody should block the killing of platelets by natural allo-antibodies produced by the mother during pregnancy. The first stage of the research programme was to produce new mutant antibodies by exchanging residues between the natural human subclasses IgG1, IgG2 and IgG4. These new mutants were tested in model systems using the lymphocyte antigen CD52 (Campath-1H) and the red cell antigen RhD as target antigens (Armour et al 1999 & 2003). In progressing from the pre-clinical to the clinical phase human volunteer studies were conducted of the best candidate Fc region using the red cell antigen RhD as the test system The RhD antigen was selected because of the extensive clinical experience and research literature on the in-vivo properties of anti-D antibodies. This work was successful and identified a mutation called G1-delta-ab as the lead candidate with the desired properties listed above and thus ideal for development of a therapeutic anti-HPA1a antibody (Armour et al 1999, 2003, 2006).

In 2004 Clark and colleagues began work on expressing and developing the HPA-1a specific antibody for use in a second human volunteer study to demonstrate the in-vivo effectiveness of the antibody in prolonging the survival of platelets. In April 2004, Dr Cedric Ghevaert of the Department of Haematology was recruited as the clinical researcher to help carry out this clinical study. However following the bad clinical experience in volunteers with TeGenero's antibody TGN1412 (March 2006), the MHRA tightened up the regulations of first in man antibody based trials which required Clark and colleagues to do extra testing in-vitro and in-vivo in animal models to minimise any risks of adverse reactions (Ghevaert et al 2008). The results proved satisfactory and the MHRA granted approval for the human volunteer study, which was then completed in late 2012; the results have recently been published (Ghevaert et al 2013).

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

1. Bolt S, Routledge E, Lloyd I, Chatenoud L, Pope H, Gorman SD, **Clark M**, Waldmann H. (1993) The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties. Eur J Immunol. 23: 403-411.
2. Abbs IC, **Clark M**, Waldmann H, Chatenoud L, Koffman CG, Sacks SH (1994) Sparing of first dose effect of monovalent anti-CD3 antibody used in allograft rejection is associated with diminished release of pro-inflammatory cytokines. Ther Immunol 1: 325-331
3. Redpath S, Michaelsen T, Sandlie I, **Clark MR**. (1998) Activation of complement by human IgG1 and human IgG3 antibodies against the human leucocyte antigen CD52. Immunology 93: 595-600
4. Armour KL, **Clark MR**, Hadley AG, Williamson LM (1999) Recombinant human IgG molecules lacking Fc receptor I binding and monocyte triggering activities Eur J Immunol 29: 2613-2624
5. Armour KL, Van De Winkel JGJ, LM Williamson LM, **Clark MR** (2003) Differential binding to human FcgRIIa and FcgRIIb receptors by human IgG wildtype and mutant antibodies Molecular immunology 40: 585-593
6. Armour KL, Parry-Jones DR, Beharry N, Ballinger JR, Mushens R, Williams RK, Beatty C, Stanworth S, Lloyd-Evans P, Scott M, **Clark MR**, Peters AM, Williamson LM (2006) Intravascular survival of red cells coated with a mutated human anti-D antibody engineered to lack destructive activity. Blood 107: 2619-2626
7. Ghevaert C, Wilcox DA, Fang J, Armour KL, **Clark MR**, Ouwehand WH, Williamson LM (2008) Developing recombinant HPA-1a-specific antibodies with abrogated Fcg receptor binding for the treatment of fetomaternal alloimmune thrombocytopenia J Clinical Invest 118: 2929-2938
8. Ghevaert C, Herbert N, Hawkins L, Grehan N, Cookson P, Garner SF, Crisp-Hihn A, Lloyd-Evans P, Evans A, Balan K, WH Ouwehand WH, Armour KL, **Clark MR**, Williamson LM (2013) Recombinant HPA-1a antibody therapy for treatment of fetomaternal alloimmune thrombocytopenia: proof of principle in human volunteers Blood 122:313-320

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

Impact on health: Work by Clark on producing a non-depleting and non-mitogenic Fc modified antibody through use of an aglycosylated IgG1 Fc region in the anti-CD3 antibody (Bolt et al 1993) led to the development of otelixizumab, which was licensed to GSK in October 2007. It has since been tested in 8 clinical trials, including phase 3 trials for Type 1 Diabetes Mellitus (NCT00678886 13/5/2008 & NCT01123083 11/5/2010) and on-going Phase 1 trials in Rheumatoid Arthritis (NCT01077531 25/2/2010 & NCT01101555 8/4/2010). The two phase 3 trials for Type 1 diabetes both failed to reach significance for the primary end points and a current on-going trial is now exploring a different dosing and delivery route (NCT00946257 23/7/2009).

The published human clinical volunteer studies by Clark and colleagues have demonstrated that antibodies with selected modifications to the Fc regions have the desired properties for the intended purposes (Armour et al 2006, Ghevaert et al 2013). These volunteer studies showed that cells coated with the Fc modified antibody had longer survival times than cells coated with wild type IgG1 and that this could extend to situations where there was a mixture of the two antibodies on the cell surface as would be encountered in an affected foetus. These results identify an alternative strategy for the treatment of FMAITP, a disorder that currently is usually treated with high doses of intravenous IVIg and prednisone, an expensive therapy that also is associated with serious side effects and potential risks of infection (IVIg is a prepared from pooled human plasma donations).

The pharmaceutical company Pfizer has taken a license (agreement dated Dec 18th 2009) to develop a number of therapeutic antibodies that incorporate the mutations described in Armour et al 1999, protected by world patent application WO1999058572 and US7597889. Pfizer had a clinical requirement that their antibodies would be non-depleting and non-activating, with a low in-vivo toxicity profile, which could be provided by incorporation of our modified Fc regions into their products. They initiated clinical trials with several licensed antibodies within the period 2008-2013 that are listed on the US NIH website ClinicalTrials.gov. These include 25 listed trials with the anti-nerve growth factor antibody tanezumab, several of which have been in phase 3 (NCT00744471 29/8/2008, NCT00809783 16/12/2008, NCT00733902 11/8/2008, NCT00863304 13/4/2009). Another antibody ponezumab to the beta-amyloid protein has been tested in 8 listed trials for the treatment of Alzheimer's disease including three phase 2 trials (NCT00722046 23/7/2008, NCT00945672 22/7/2009, NCT01821118 4/3/2013). RN316 specific for Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) is a therapeutic antibody aimed at lowering cholesterol levels and has been used in 7 listed trials, including two at phase 2 (NCT01342211 25/4/2011, NCT01592240 3/5/2012). A fourth antibody RN564 has recently entered phase 1 clinical trials for treatment of osteoporosis (NCT01293487 9/2/2011). The constant region used in these antibodies have behaved as expected in that the antibodies had extended half-lives, normal biodistribution, and no reported side-effects attributable to Fc mediated functions. Full results of the Phase III trial with Tanezumab in osteoarthritis have just been published (Spierings et al 2013) and this reports significant efficacy in pain relief and no additional safety issues with the antibody.

Commercial impact: The modifications that have been identified in Dr Clark's research have been protected by a number of patent families with patents that are still in force and that have been assigned by the inventors and Cambridge University to BTG plc.

The aglycosylated IgG1 CD3 antibody otelixizumab is protected by the world patent family WO1993019196 (Bolt et al 1993) and a recently granted patent US RE43898 (Gorman et al 2013) and US6767996 (Bolt et al 2004). These have all been assigned to BTG plc. Other intellectual property relating to this antibody has been created by Waldmann and colleagues at Oxford University and this too was assigned to BTG plc. In an agreement dated 18th Sep 2012 the two Universities of Oxford and Cambridge entered into a joint revenue sharing arrangement with BTG plc under which all the income arising from the various IPRs is to be pooled and then shared equally. Between 2008 and 2013 £2.8 million has been received by Cambridge under these arrangements.
Mutations resulting in non-depleting and non-activating Fc regions but retaining low

immunogenicity and also binding to the neonatal Fc receptor FcRn have been protected by the world patent family WO1999058572 (Armour, Clark and Williamson1999) with a granted US patent US7597889 (Armour, Clark and Williamson 2009). This patent has been licensed to Pfizer for use in multiple products, four of which are already in clinical trials (see above) with others still at the pre-clinical stage (e.g. RN 307). In an agreement dated 20th Feb 2013, GSK have taken out a research license to explore the applicability of this technology to one of their research programmes in order to reduce the unwanted toxicity that they have encountered with their antibody. Between 2008 and 2013 these licenses have returned £382,148 in revenue to Cambridge Enterprise Ltd. Vectors encoding these mutations for research use are available from the San Diego based company Invivogen under license and a number of preclinical and research studies have made use of the mutations (e.g. Richter et al 2013)..

Recent research by Clark and colleagues has led to further findings identifying residue changes from a human pseudo-gamma sequence that, when introduced into human IgG1, result in increased binding affinity for the inhibitory human Fc receptor FcgRIIb. This receptor is of interest because it plays a role in dampening down and regulating immune responses, such as inhibiting mast cell degranulation mediated by IgE, thereby preventing allergic hypersensitivity reactions. World patent applications have been made under WO2012146934 (Armour and Clark 2012) and the potential to further exploit this technology by commercial licensing and incorporation into therapeutic antibodies is being explored.

Specialist Advisory roles: In addition to the licensing of patents and know-how to industry, further commercial impact arises from consultancy and advisory roles of Dr Clark to several established international biotech companies and to several smaller start-up and early stage biotech companies operating in the UK that are developing therapeutic antibody based programmes. In the period 2009-2013 Dr Clark has been a member (and since 2012 Chair) of the Scientific Advisory Board of the Centre d'Immunologie Pierre Fabre in France. In 2013 he joined the Scientific Advisory Board of UCB. Smaller biotech companies with which he has worked as an advisor/consultant during the period 2008-2013 include Antitope Ltd, Crescendo Biologics Ltd, Kymab Ltd & VHsquared Ltd. Dr Clark also provided advice and assistance to licensees of the patents and know-how derived from his laboratory research to the companies: BioAnalab (Millipore), BTG plc, Genzyme, GSK and Pfizer.

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

1. Bolt SL, **Clark MR**, Gorman SD, Routledge EG, Waldmann H (1993) Anti-CD3 Aglycosylated IgG Antibody World Patent WO/1993/019196
2. Bolt SL, **Clark MR**, Gorman SD, Routledge EG, Waldmann H (2004) Humanized anti-CD3 specific antibodies. US Patent 6,706,265
3. Gorman SD, **Clark MR**, Cobbold SP, Waldmann H (2013) Altered antibodies and their preparation. US Patent RE43,898
4. Armour KL, **Clark MR**, Williamson LML (2009) Binding molecules derived from immunoglobulins which do not trigger complement mediated lysis US Patent 7,597,889
5. Armour KL, **Clark MR** (2012) Binding molecules with biased recognition. World Patent application WO/2012/146,934
6. Ghevaert C, Herbert N, Hawkins L, Grehan N, Cookson P, Garner SF, Crisp-Hihn A, Lloyd-Evans P, Evans A, Balan K, WH Ouwehand WH, Armour KL, **Clark MR**, Williamson LM (2013) Recombinant HPA-1a antibody therapy for treatment of fetomaternal alloimmune thrombocytopenia: proof of principle in human volunteers Blood 122:313-320
7. Spierings ELH, Fidelholtz J, Wolfram G, Smith MD, Brown MT, West CR (2013) A phase III placebo- and oxycodone- controlled study of tanezumab in adults with osteoarthritis pain of the hip or knee. Pain 154: 1603-1612
8. Richter F, Liebig T, Guenzi E, Herrmann A, Scheurich P, Pfizenmaier K, Kontermann RE (2013) Antagonistic TNF Receptor One-specific antibody (ATROSAB): Receptor binding and in vitro bioactivity. PLoS ONE 8(8):e72156. doi:10.1371/journal.pone.0072156
9. Letter of corroboration from the CSO of Antitope Ltd
10. Letter of corroboration from the CEO of Crescendo Biologics Ltd.