Institution: University of Birmingham

Unit of Assessment: A1

Title of case study: The development of HLA-peptide tetramers and their application as a novel form of cell therapy for immune suppressed patients suffering from cytomegalovirus infection

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

T lymphocytes recognise antigens in the form of an HLA-peptide complex. HLA-peptide tetramers consist of a fluorescent HLA protein and peptide which together bind to, and therefore identify, T cells that recognise this HLA-peptide complex. As such they have proven to be a revolutionary reagent in immunology. Professor Paul Moss at the University of Birmingham has played an integral role in the clinical and commercial application of tetramers, particularly around the cytomegalovirus (CMV)-specific immune response in the context of monitoring immune recovery after transplantation and pioneering a new approach for cellular immunotherapy**. The impact of this research relates to the clinical management of CMV infection in immunosuppressed patients and the creation of Cell Medica, a UK Biotech company pioneering tetramer-based cell therapy, thus demonstrating impact on clinical practice and the UK economy.**

2. **Underpinning research** (indicative maximum 500 words)

Tetramers are protein based molecules which are used to quantify and isolate antigen-specific T cells, especially CD8+ T cells. CD8+ T-cells (or T-lymphocytes) are a type of lymphocyte that play a central role in cell-mediated immunity, to pathogens such as viruses. The first tetramers were developed in the 1990s, by a team that involved Professor Paul Moss in his previous appointment at the University of Oxford. Tetramers are formed from four peptide-major histocompatability complex (MHC) molecules that are specific for a given population of T cells. These molecules are folded with the peptide (antigen) of interest and complexed with fluorescently-labeled streptavidin around a biotin core. The tetramer will specifically label T cells that express T cell receptors that are specific for a given peptide-MHC complex. The strength of antigen-specific responses can be measured as the percentage of CD8+ tetramer+ T cells as a fraction of all CD8+ lymphocytes in the blood.

Following his appointment as Chair of Haematology at the University of Birmingham in January 1998, Professor Paul Moss used Medical Research Council Programme Grant support to investigate the use of tetramers in relation to the clinical challenge of CMV infection following bone marrow (stem cell) and solid organ transplantation. His group in Birmingham was the first to develop a tetramer containing a CMV peptide and used this reagent to demonstrate the extraordinary high frequency and phenotypic heterogeneity of CMV-specific T cells within peripheral blood of healthy donors (1). This work caused a paradigm shift in scientific understanding of adaptive immunity. Previously it was understood that the CMV-specific T cell immune response was present at a frequency of around 1 in 100,000 CD8+ T cells. This paper showed that the true frequency was well over 1% of the peripheral T cell repertoire, making CMV the most immunodominant antigen that is encountered by the human immune system. This work has itself had considerable impact, including leading directly to the vaccine team in Portland developing the most promising approach for HIV infection using CMV as a vaccine vector (Hansen et al, 2011).

The team in Birmingham, under the continued leadership of Professor Paul Moss, then went on to address CMV infection in stem cell and liver transplantation. Here, they were the first to use tetramers to demonstrate that the CMV-specific immune response was very weak in these patients and a direct explanation for the high degree of morbidity and mortality related to CMV infection (2, 3). The Moss group then developed a direct use for tetramers as a means for antigen-specific T cell therapy, whereby tetramers were used to isolate populations of CMV-specific T cells for future administration to patients. Following the first publication of magnetic separation of these cells in the laboratory (4), they went on to complete the first clinical trial using the direct isolation of

antigen-specific T cells from transplant donors followed by infusion into patients. Again, this groundbreaking paper was the first report of the direct selection of antigen-specific T cells from human volunteers followed by their immediate infusion into patients for any disease category (5). This approach has been widely replicated and adopted in many clinical protocols.

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

Grant Support:
- Rickinson & Moss - MRC Programme Grant 2000-2005
- Moss - Leukaemia & Lymphoma Research Programme Grant 2000-2005

1. Gillespie GM, Wills MR, Appay V, O'Callaghan C, Murphy M, Smith N, Sissons P, Rowland-Jones S, Bell JI, **Moss PA**. Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. J Virol. 2000 Sep;74(17):8140-50. (352 citations) *DOI 10.1111/j.1469-0691.2009.02899.x*
2. Cwynarski K, Ainsworth J, Cobbold M, Wagner S, Mahendra P, Apperley J, Goldman J, Craddock C, **Moss PA**. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. Blood. 2001 Mar 1;97(5):1232-40. (219 citations) *DOI 10.1182/blood.V97.5.1232*
3. Singhal S, Shaw JC, Ainsworth J, Hathaway M, Gillespie GM, Paris H, Ward K, Pillay D, **Moss PA,** Mutimer DJ. Direct visualization and quantitation of cytomegalovirus-specific CD8+ cytotoxic T-lymphocytes in liver transplant patients. Transplantation. 2000 Jun 15;69(11):2251-9. (63 citations) *PMID 10868622*
4. Keenan RD, Ainsworth J, Khan N, Bruton R, Cobbold M, Assenmacher M, Milligan DW, **Moss PA**. Purification of cytomegalovirus-specific CD8 T cells from peripheral blood using HLA-peptide tetramers. Br J Haematol. 2001 Nov;115(2):428-34. (56 citations) *DOI 10.1046/j.1365-2141.2001.03106.x*
5. Cobbold M, Khan N, Pourgheysari B, Tauro S, McDonald D, Osman H, Assenmacher M, Billingham L, Steward C, Crawley C, Olavarria E, Goldman J, Chakraverty R, Mahendra P, Craddock C, **Moss PA**. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after slection by HLA-peptide tetramers. J Exp Med. 2005 Aug 1;202(3):379-86. (280 citations) *DOI 10.1084/jem.20040613*

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

The impact of the application of HLA-peptide tetramers to the study of cytomegalovirus infection can be categorized within **changing clinical practice** and **commercial development**.

The development of tetramer technology, with which Professor Paul Moss was involved, has had an enormous impact on immunology research in industry and academia. However, the specific focus of this case study is on Professor Paul Moss' development of a clear clinical application for these important reagents.

HLA-peptide tetramers have transformed the ability to interrogate the function of the immune system and have had an enormous impact on the understanding of CMV infection. A PubMed search for 'tetramer' and 'cytomegalovirus' identifies 148 individual papers which is representation of the widespread adoption of this technique since the original paper 12 years ago. Tetramers have also been used widely in clinical applications over this timeframe, specifically in relation to clinical monitoring and cellular therapy. As indicated above, in 2001 the Birmingham team were the first to use tetramers to monitor the reconstitution of CMV-specific immune responses following stem cell transplantation. This work led to a plethora of similar publications which correlated such reconstitution with factors such as level of T cell depletion and control of viral reactivation. This work proved so important that Beckman Coulter went on to develop a set of HLA-peptide reagents which are used and sold as a tool to monitor T cell reconstitution in order to guide clinical management of the risk and clinical significance of viremia in the post-transplant period (1). Studies have established the value of this approach which is now used in specialist haemopoietic transplantation centres and is also finding application in solid organ transplant (2).

In relation to cellular therapy, the manuscript of Cobbold *et al* (2005) opened the potential for HLA-peptide tetramers, and other forms of multimeric reagents, to be used as an approach for accelerating the transfer of antigen-specific T cells between patients. This publication was followed by similar trials within Europe that demonstrate the value of such an approach in the treatment in patients with antiviral-resistant CMV reactivation. This approach has proved therapeutically beneficial in several reports (3) and is now used as a therapeutic approach in the treatment of refractory disease. Indeed, the use of cellular immunotherapy as a prophylactic means of suppress the initiation of viremia is also appealing and is subject to large scale clinical trials sponsored by commercial organisations (see below).

It is within the area of the clinical application of multimer technology that the University of Birmingham's contribution has been most dominant in a commercial setting. Specifically, the UK biotechnology company Cell Medica was established in London in 2006 with the aim of commercializing antigen-specific cellular therapy within the clinical arena (4) and has had a range of impacts through the current assessment period. The company is pioneering tetramer-based therapy and Professor Paul Moss has served on the Scientific Advisory Board of Cell Medica since its foundation. In July 2012 the company successfully completed series A financing (£17m) and expanded its operations to include sites in Texas and Berlin (5). Cell Medica is currently sponsoring three multi-centre clinical trials, enrolling exclusively within the United Kingdom, which will ascertain the value of CMV-specific T cell therapy, performed using multimer selection and the prophylaxis or management of CMV reactivation in patients undergoing haemopoietic transplantation (6). The first trial started in 2008 and the second in 2011, with a third treating children launched in 2013. Over 100 patients within the UK have entered clinical trials to assess multimer-based CMV-specific T cell adoptive therapy. The 'health and wealth' contribution of tetramers to UK medicine has therefore been significant. In 2013 the company opened a GMP cell therapy product facility in Berlin, its European commercial manufacturing facility, initially focusing on adoptive cellular treatments. The commercial launch of the product is planned in early 2014.

In conclusion, the University of Birmingham and in particular Professor Paul Moss has made a central contribution to the development of clinical and commercial application of HLA-peptide technology, particularly around the study of CMV infection and its management. This has been translated to considerable scientific, clinical and commercial opportunities within the UK and beyond.

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

1. Gratama JW et al. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. Blood. 2010 116(10):1655-62. DOI 10.1182/blood-2010-03-273508
2. Sund F et al. CMV-specific T-cell immunity, viral load, and clinical outcome in seropositive renal transplant recipients: a pilot study. Clin Transplant 2010 24 (30 401-9. DOI 10.1111/j.1399-0012.2009.00976.x
3. Schmitt A et al. Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation. Transfusion. 2011 Mar;51(3):591-9. DOI 10.1111/j.1537-2995.2010.02940.x
4. http://www.cellmedica.co.uk
5. http://www.cellmedica.co.uk/news/cell-medica-secures-17-million-265-million-equity-investment/.
6. http://www.cellmedica.co.uk/news/cell-medica-announces-completion-patient-recruitment-randomi /