Institution: Cardiff University

Unit of Assessment: UoA5 Casestudy6

Title of case study: Improved Diagnostic Technology with the first genome standard for Pathogenic Human Cytomegalovirus.

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

Cytomegalovirus (CMV) causes life-threatening disease, particularly in immunocompromised individuals. CMV antivirals are toxic and before 2010 there was no standard for quantifying patients' viral load to enable precise use of these drugs. Research at Cardiff University led to the isolation and characterisation of wild-type CMV strain Merlin. The strain was recognised by the WHO in 2010 as the best source of the CMV genome and adopted as the international prototype strain and PCR standard. All major pharmaceutical companies offering CMV testing swiftly recalibrated their kits, and now market the assays as standardised against the strain. As a consequence, the Standard is improving clinical CMV disease definition and regulation of antiviral therapy, aiding the management of toxicity and resistance worldwide.

2. Underpinning research (indicative maximum 500 words)

Establishing the CMV Prototype Strain

CMV has the largest genome (235kb) of any human virus. Virologists used a range of heavily-attenuated CMV strains that replicated efficiently in culture but did not fully represent the clinical agent; either in genetic content or biological properties. Moreover, Cardiff research demonstrated that laboratory-adapted strains had lost much of their capacity to evade host immunity[3.1-3.3]. Crucially, no genetically-intact strain was available to support CMV research.

Between 1997-99, Prof Gavin Wilkinson (Cardiff University) obtained urines of congenitally-infected neonates from the Cardiff National Public Health Service (NPHS)-Wales diagnostic laboratory and propagated clinical isolates *de novo*. Between 2001-3 purified genomic viral DNAs were sent to Dr Andrew Davison (MRC Unit, Glasgow), who sequenced the complete genomes of three Cardiff isolates. Wilkinson and Davison selected Strain Merlin for future research in 2003 based on its excellent growth properties, stability and genomic integrity. This characterised Cardiff isolate provided the first complete CMV sequence and was designated the prototype CMV clinical strain by the National Center for Biotechnology Information (NCBI)[3.4].

Cloning Strain Merlin Genome

During growth *in vitro,* CMV mutates rapidly and in a reproducible manner[3.5]. To avoid this predictable, progressive deterioration with strain Merlin, Dr Richard Stanton (Cardiff University, 2005-current) cloned the entire Merlin genome into a bacterial artificial chromosome (BAC)[3.5]. The production of this infectious plasmid provided a:

1. reliable source of CMV genome and genes.
2. reproducible source of genetically-defined, clonal virus in perpetuity.
3. viral vector enabling tailored mutation, transgene insertion and vaccine development.

Characterising Strain Merlin

In 2011 Davison (Glasgow) and Wilkinson (Cardiff University) deep-sequenced the strain Merlin transcriptome; the first time this has been performed for a DNA virus, then the most extreme definition of gene-usage to date[3.6]. Wilkinson and Stanton characterised the expression of all 170 Merlin protein-coding genes using a viral vector, providing detailed characterisation of CMV gene usage and enabling high-throughput screening of CMV gene function. Thus, Merlin is the best-characterised and only genetically-intact CMV strain that can be cultured *in vitro*.

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

3.1 **Tomasec, P.**, Braud, V.M., **Rickards C.**, **Powell, M.B.**, **McSharry, B.P.**, Gadola, S., Cerundolo, V., **Borysiewicz, L.K.**, McMichael, A.J. and **Wilkinson, G.W.G.** (2000). Surface Expression of HLA-E, an Inhibitor of Natural Killer Cells, Enhanced by Human Cytomegalovirus gpUL40. Science, 287. 1031-1033. (Citations=264, according to Scopus) DOI: 10.1126/science.287.5455.1031

3.2 **Wang, E.C.Y.**, **McSharry, B.**, Retiere, C., **Tomasec, P.**, **Williams, S, Borysiewicz, LK**, Braud,

V.M., and **Wilkinson, G.W.G**. (2002). UL40-mediated NK evasion during productive infection with human cytomegalovirus. Proc. Natl. Acad. Sci. USA. 99, 7570-7575. (Citations=69) DOI: 10.1073/pnas.112680099

3.3 **Tomasec, P**., **Wang, E.C.Y**., Davison, A.J., Vojtesek, B., **Armstrong, M**., **Griffin, C.A**., **McSharry, B.P**., **Morris, R.J.**, **Llewellyn-Lacey, S**., **Rickards, C**., Akio Nomoto, A., Sinzger,C. and **Wilkinson. G.W.G**. (2005). Down-regulation of NK Cell Activating Ligand CD155 by Human Cytomegalovirus UL141. Nature Immunol. 6, 181-188. (Citations=91) DOI:10.1038/ni1156

3.4 Dolan, A., Cunningham, C., Hector, R. D. Hassan-Walker, A.F., Lee, L., Addison, C., Dargan, D.J., McGeoch, D.J., Gatherer, D., Emery, V.C., Griffiths P.D., Sinzger, C., **McSharry, B.P., Wilkinson G.W.G**. and Davison A.J. (2004). Genetic content of wild type human cytomegalovirus. J. Gen Virol. 85, 1301-1312. (Citations=188) DOI: 10.1099/vir.0.79888-0

3.5 **Stanton, R.J**, Baluchova, K, Dargan, D.J., Cunningham, C., Sheehy, O, **Seirafian, S**., **McSharry, B.P**., **Neale, M.L**., **Davies, J.A**., **Tomasec, P**., Davison, A.J. and **Wilkinson, G.W.G**. (2010). Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. J. Clin Invest. 120, 3191-208. (Citations 14) DOI: 10.1172/JCI42955

3.6 Gatherer, D., **S. Seirafian**, C. Cunningham, M. Holton, D. J. Dargan, K. Baluchova, R. D. Hector, J. Galbraith, P. Herzyk, **G. W. Wilkinson**, and Davison, A. J.. 2011. High-resolution human cytomegalovirus transcriptome. Proc Natl Acad Sci U S A 108:19755-19760. DOI: 10.1073/pnas.1115861108

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- Wellcome Trust Programme Grant awarded to GWG Wilkinson, P Tomasec, and ECY Wang. (£765,717). Immune Modulation by Human Cytomegalovirus. 2003-2009. GO65811.
- Wellcome Trust Programme Grant awarded to LK Borysiewicz, M Rowe and GWG Wilkinson (£599,048). Evasion of MHC class I restricted cytotoxic T cells by human cytomegalovirus and Epstein Barr virus. 1997-2002. G046655
- MRC Project Grant: An Analysis of Wildtype human cytomegalovirus. GWG Wilkinson, ECY Wang, and P Tomasec. (FEC: £901.468). 2010-14. G1000236
- MRC Project Grant awarded to GWG Wilkinson, ECY Wang, and P. Tomasec. Modulation of Natural Killer cells by human cytomegalovirus. 2007-2010. (FEC £701,175). G0700142

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

The Virus. Cytomegalovirus (CMV) is the major viral cause of congenital malformation. Associated care costs $4.027 billion/year in the US alone. Infection is associated with life-threatening disease in the immunocompromised (most significantly transplant recipients and HIV-AIDS patients), and more than 95% of glioblastoma multiforme - the most common and aggressive form of brain tumour. Moreover, CMV is an important cause of infectious mononucleosis, hepatitis, colitis, retinitis, arteriosclerosis and sensorineural hearing loss. CMV also induces changes to the immune repertoire that contribute to autoimmune disease (arthritis) and immunosenescence.

The Problem: Lack of consistent measurement of CMV load. Since CMV disease takes many forms, it lacks specific symptoms and diagnosis is absolutely dependent on laboratory testing. With more than 50% of the adult population carrying a persistent infection, the presence of antibodies does not define disease. PCR is therefore the key diagnostic test for defining CMV disease, and accurate quantification of virus load can be used to direct antiviral therapy and patient management (e.g. removal/alleviation of immunosuppression in transplant recipients). The CMV antivirals, such as Ganciclovir and Foscarnet, are associated with significant toxicity and resistance can arise rapidly. Clinicians therefore need to use them with guidance from precise quantification of CMV load.

Before the Cardiff impact, CMV Q-PCR assays employed a diverse range of standards, including cloned fragments and assorted laboratory strains with varying experimental definitions of titre. This lack of universal PCR test calibration prevented consensus or cooperative management of life-

threatening infections across clinical centres. So, in 2004, the International Herpesvirus Management Forum identified the urgent need for:

"an international quantitation standard…to compare studies using different PCR-based systems and to facilitate patient management at multiple care centres" [5.1].

The Path to Impact – Providing a Genome Standard for the Pathogenic Herpesvirus Human Cytomegalovirus. The importance of defining the genome of a CMV clinical isolate is evidenced by the adoption of this virus as the global PCR standard as well as more than 250 citations of the original 2004 sequence paper (although commonly only the accession number is referenced).

Since 2004, the annotated strain Merlin genome has been available through the World Health Organisation (WHO) reference library NIBSC. Additionally, the viruses were deposited in virus banks run by the American Type Culture Collection and the European Collection of Cell Cultures, and thereby freely available to users worldwide. This enabled resource distribution beyond academia, informing commercial development of vaccines and medicinal chemistry.

Impact: WHO adopts Strain Merlin as PCR Standard. Following the warning by the International Herpesvirus Management forum, the WHO recognised the lack of CMV Standard as globally significant. In 2008, the development of the first WHO International Standard for CMV was discussed at the Standardisation of Genomic Amplification Techniques Clinical Diagnostics meeting. Participants agreed then that the optimal standard would "*comprise a whole virus preparation of the prototype clinical HCMV strain Merlin".* [5.2a].

Subsequently, the WHO Expert Committee on Biological Standardization supported this recommendation: "*the strain [Merlin] was chosen as it is well-characterised and more likely to represent a clinical virus than other laboratory-adapted strains"* [5.2b].

In 2010, Wilkinson and Stanton (Cardiff) were formally acknowledged to have provided the WHO reference laboratory (NIBSC) with HCMV strains AD169 and Merlin, the Merlin-BAC clone and technical and logistical advice on working with these reagents [5.2c, 5.3]. Merlin and other test candidate strains were evaluated in 32 diagnostic, commercial and research laboratories in 14 countries evaluated test candidates. The recommendation stated: *"…the results of the [collaborative] study indicate the suitability of the candidate HCMV Merlin standard as the proposed 1st WHO International Standard for HCMV. It is therefore proposed that the candidate standard (NIBSC code 09/162) be established as the 1st WHO International Standard for HCMV…."* [5.2d]

Impact: Adoption of Merlin as calibration standard by the pharmaceutical industry. Following adoption by the WHO, the CMV Merlin strain was then distributed by NIBSC as an international standard. Between January 2011 and April 2013, NIBSC shipped 960 vials of the standard to 270 diagnosis centres in 43 countries.

In the next two years following WHO adoption, Merlin was evaluated and implemented as a calibration standard in commercial Q-PCR testing assays by Cobas Roche, Abbott, ABI Taqman, Altona Diagnostics (Germany) Biomerieux Argene, Chiron, and J&J Diagnostics/Amersham Amerlite (Qiagen). Promotional material for these devices now states they have been standardized to the first WHO international standard[5.4]. The Roche assay has now been approved by the FDA as a result of its calibration[5.6]. The Abbott assay has been approved for evaluation of a trial of a first-ever CMV vaccine trial[5.5]. The Merlin strain is also regularly distributed from Cardiff to support commercial research. Since 2010, 35 Material Transfer Agreements have been signed.

Impact: Benefits of Merlin in clinical management of CMV. In 2011, a single London hospital (Royal Free) carried out approximately 10,000 assays at a cost £0.5M (Dr R. Milne, personal communication). In the UK alone in the same year, 58 centres carried out 252,303 CMV diagnostic PCR tests, all now calibrated to the WHO standard; a market increasing 22% annually[5.7]. The global market is proportionately larger.

The Cardiff research has therefore enabled greater validation and consistency in millions of tests conducted since 2010, improving the clinical management of CMV disease. The commercial PCR assays, now calibrated against Merlin, allow more accurate assessment of viral load and thereby more precise use of antiviral therapy, reducing the risk of toxicity and resistance. These clinical

benefits of Merlin as a CMV standard have been recognised in a growing number of studies. One international multicenter evaluation [5.8], assessing the performance of the Roche assay against non-calibrated tests, stated: *"The implementation of an international standard and the availability of commercial QNAT [quantitative nucleic acid testing] with broad interlaboratory agreement that are traceable and colinear to the first WHO CMV international standard represent a much needed advancement."* An editorial to the same paper quotes: "*With the availability of this test and the WHO International Standard for recalibrating LDTs [laboratory developed tests], the tools are now on hand to conduct multicenter clinical studies to establish clinical cutoffs for determining the risk of disease, diagnosing disease, and monitoring response to antiviral therapy*."[5.9] http://cid.oxfordjournals.org/content/56/3/374.full.pdf+html (page 375) Another concludes: "*Patients with pretreatment CMV DNA of <18 200 (4.3 log10) IU/mL are 1.5 times more likely to have CMV disease resolution. CMV suppression (<137 [2.1 log10] IU/mL), as measured by a test calibrated to the WHO Standard, is predictive of clinical response to antiviral treatment*" http://cid.oxfordjournals.org/content/56/11/1546.full.pdf+html (page 1546, 'Conclusions' paragraph on first page).

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

5.1 Razonable RR, Emery VC; 11th Annual Meeting of the IHMF (International Herpes Management Forum). Management of CMV infection and disease in transplant patients. 27-29 February 2004. Herpes. 2004;11(3):77-86. http://www.ncbi.nlm.nih.gov/pubmed/15960905 confirms the international need for a standardised CMV strain3

5.2 Fryer, J.F., Heath, A.B, Anderson, R. Philip D. Minor, P.D. and the Collaborative Study Group. A report of the Expert Committee on Biological Standardization, Geneva, 18 to 22 October 2010. Collaborative Study to Evaluate the Proposed 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-Based Assays. http://www.nibsc.org/PDF/HCMV_IS.pdf demonstrates the evidence presented to WHO of Merlin being the optimal candidate for an international standard. (Quote 5.3a page 3, paragraph 4; 5.3b page 11, paragraph 2 of Discussion and conclusions; 5.3c page 13, Acknowledgements; Quote 5.3d page 2, paragraph 2 of Summary).

5.3 CMV International Standard. Confirms the adoption of Merlin as WHO standard http://www.nibsc.org/documents/ifu/09-162.pdf

5.4 http://www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/realtime-cmv.html is one example of many international companies referring to the international standard in marketing material for their calibrated assay kits.

5.5 Evidence of the Abbott PCR assay, based on the Merlin standard, being used to monitor HCMV vaccine trial http://www.marketwatch.com/story/abbott-to-collaborate-with-astellas-in-cmv-vaccine-trial-2012-09-06 (screenshot taken on 22nd July 2013 and available from HEI on request)

5.6 Evidence of FDA approval for Roche COBAS® AmpliPrep/COBAS® TaqMan® CMV Test - P110037 diagnostic assay based on Merlin standard. http://www.accessdata.fda.gov/cdrh_docs/pdf11/P110037b.pdf

5.7 Dii -data information intelligence -Continuous Molecular Market Assessment Wave 2, Europe 2011 confirms the size of the UK CMV testing market (data available on request from HEI).

5.8 Hirsch, H.H., Lautenschlager, I., Pinsky, B.A., Cardenoso, L., Aslam, S., Cobb, B., Vilchez, R.A., and Valsamakis, A. (2012). An International Multicenter Performance Analysis of Cytomegalovirus Load Tests. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. http://dx.doi.org/10.1093/cid/cis900 states the clinical benefits of standardised CMV testing, page 372.

5.9 Caliendo, A (2013) The Long Road Toward Standardization of Viral Load Testing for Cytomegalovirus. Clincal Infectious Diseases – Editorial commentary 2013:56 (1February) http://cid.oxfordjournals.org/content/56/3/374.full.pdf+html Editorial to 5.8 that confirms the tools are now on hand for use in multicentre clinical studies.