Institution: University of Glasgow

Unit of Assessment: Unit 1; Clinical Medicine

Title of case study: Developing the first international diagnostic standard for human cytomegalovirus

1. Summary of the impact

Human cytomegalovirus (HCMV) infection can lead to life-threatening disease in people with weakened immune systems. Research at the University of Glasgow has genetically characterised a strain of HCMV, known as 'Merlin'. This research directly led to the adoption of this strain as the first diagnostic standard by the World Health Organisation (WHO). The standard has been distributed to 43 countries and is used in major commercial diagnostic test kits, including the first standardised test approved by the United States Food & Drug Administration. The standard provides consistency across healthcare centres in relation to the diagnosis of HCMV-associated disease and the clinical management of patients treated with HCMV antiviral drugs.

2. Underpinning research

Human cytomegalovirus (HCMV) is one of nine herpes viruses known to infect humans. Work in Glasgow on the genomics of this pathogen was initiated in the groups of Dr Andrew Davison and Prof Duncan McGeoch in 2001. This was taken forward latterly by Davison's group, with Dr Katarina Balachova and Dr Derek Gatherer as key players. Davison has researched the content, expression, function and evolution of herpesvirus genomes since 1976. He is a world leader in the genomics of these viruses, and his 121 research articles provide a frame of reference for much of the research being conducted worldwide. The key work underpinning this case study involved the characterisation of the genome of wild-type HCMV strain Merlin.

Characterisation of a definitive HCMV strain

HCMV has the largest genome of the known human viruses. For decades, researchers have grown ('passaged') laboratory strains of HCMV in human tissue culture cells. However, such high-passage strains have mutated and are now unrepresentative of original wild-type viruses. For example, they have lost the capacity to evade the host immune response.[1] Indeed, substantial subsets of genes have been deleted from the two most widely used laboratory strains, AD169 and Towne.[2,3] Davison's and McGeoch's groups at Glasgow initiated a collaboration with Professor Gavin Wilkinson (Cardiff Institute of Infection & Immunity, Cardiff University), to characterise the wild-type HCMV genome using three low-passage strains. The complete genome of one of these strains, known as Merlin, was sequenced by the Glasgow groups, leading to the first description of a clinically relevant HCMV genome.[4] The Merlin sequence has been designated the official reference for HCMV by the National Center for Biotechnology Information, thereby providing a key resource for medical, functional and diversity studies.[5]

Further characterisation of Merlin in Davison's group determined the HCMV transcriptome. In 2011, this study produced the latest map of functional protein-coding regions in the HCMV genome, and provided researchers with the most complete information resource available.[6]

Generation of a stable source of HCMV

To derive a stable source of wild-type virus, Dr Baluchova (Glasgow) and Dr Richard Stanton (Cardiff) jointly reported their generation and characterisation of a bacterial artificial chromosome (BAC) containing the Merlin strain genome, the structure and sequence of which was determined by Davison's group.[7] The few mutations present were repaired to generate a BAC that recapitulated the wild-type Merlin genome. This reagent is an important research and clinical tool: it provides a stable source of HCMV genes; a means of generating genetically defined viruses in perpetuity by transfection of BACs into human cell lines; and a tool for characterising the virus further and contributing to vaccine development. Merlin is now the most well characterised low-passage HCMV strain. The Merlin BAC is the only current, manipulable source of wild-type HCMV.

Key University of Glasgow researchers: Dr Andrew Davison (MRC Virology Unit, 1988–2013;

Honorary Lecturer, 2000–2013), Dr Derek Gatherer (MRC Senior Investigator Scientist, 2003–2013), Dr Katarina Baluchova (MRC Career Development Fellow, 2005–2010) and Prof Duncan McGeoch (MRC Virology Unit, 1977-2010). ***Key external collaborators:*** (Cardiff University)**:** Prof Gavin Wilkinson (Professor, Cardiff Institute of Infection & Immunity) and Dr Richard Stanton (Lecturer, Cardiff Institute of Infection & Immunity)

The MRC virology Unit in Glasgow was one of the MRC's intramural units, and all staff, including these key researchers, were employed by the MRC. In 2010, the MRC-University of Glasgow Centre for Virus Research (CVR) was established, bringing together the MRC virology Unit with University virologists under a single management structure. The formal transfer of MRC staff to the University of Glasgow took place on 1 May 2013. Permission has been granted by HEFCE to include researchers within the former MRC Virology Unit within the REF2014.

3. References to the research

1. Tomasec, P. *et al.* Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus UL141. *Nat. Immunol.* 2005; 6, 181–188 doi:10.1038/ni1156.
2. Davison, A.J. *et al.* The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J. Gen. Virol.* 2003; 84, 17–28 doi:10.1099/vir.0.18606-0.
3. Bradley, A. *et al.* High-throughput sequence analysis of variants of human cytomegalovirus strains Towne and AD169. *J. Gen. Virol.* 2009; 90, 2375–2380 doi:10.1099/vir.0.013250-0.
4. Dolan, A. *et al.* Genetic content of wild type human cytomegalovirus. *J. Gen. Virol.* 2004; 85, 1301–1312 doi:10.1099/vir.0.79888-0.
5. Human herpesvirus 5 strain Merlin, complete genome. National Center for Biotechnology Information Reference Sequence: NC_006273.2 (link)
6. Gatherer, D. *et al.* High resolution human cytomegalovirus transcriptome. *Proc. Natl Acad. Sci. USA* 2011; 108, 19755–19760 doi:10.1073/pnas.1115861108.
7. Stanton, R.J., *et al.* Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. *J. Clin. Invest.* 2010; 120, 3191–3208 doi:10.1172/JCI42955.

4. Details of the impact

Roughly half the human population is infected with human cytomegalovirus (HCMV). In general, HCMV is kept in check by healthy immune systems, but is the major viral cause of birth defects and developmental disabilities such as hearing loss, visual problems and other neural impairments. HCMV is also the most significant complication in solid organ transplantation (affecting 15–60% of patients) and a major complication of bone marrow transplantation (20–35% of patients), leading to life-threatening disease due to immune system impairment. HCMV also presents a significant risk to patients with HIV/AIDS. The care costs associated with these high-risk groups in the USA alone have been estimated at over $4 billion per year.

Improved screening and management of HCMV

HCMV has poorly defined clinical symptoms, so diagnosis of congenital HCMV or HCMV-related neurological, eye or respiratory illness (and monitoring of immunosuppressed patients) is accomplished by laboratory testing. Antibody-based qualitative tests struggle to distinguish between active disease and latent infection. However, quantitative techniques, based on polymerase chain reaction (PCR), amplify viral DNA markers in a specific manner and in proportion to the amount of HCMV present. The amount of HCMV correlates with the appearance of disease, and the quantity of virus in blood or urine (the 'viral load') provides a reliable way to identify HCMV infection. Quantitative PCR-based tests can help clinicians decide when to initiate treatment and how to manage HCMV with antivirals, the benefits of which must be balanced against their toxicity.

Prior to research at the University of Glasgow, HCMV quantitative-PCR assays developed in individual laboratories used different reference standards (controls to check diagnostic assay performance), which often included only parts of the viral genome or clinically irrelevant laboratory strains. This lack of consistency meant that there was no consensus agreement that could

establish the viral load in the blood to indicate when to start, stop or modify treatment. The International Herpesvirus Management Forum recognised this in 2004, advising in a statement that:

> '*an international quantitation standard distributed by an external quality control organisation is required to compare studies using different PCR-based systems and to facilitate patient management at multiple care centres*.'[a]

Merlin as a reference standard

WHO International Standards are the highest order of reference for biological substances. They facilitate the calibration of secondary references used in routine laboratory assays and provide a uniform measure for comparison between laboratories, regardless of instrumentation or reaction conditions. The development of the 1st WHO International Standard for HCMV was initiated in June 2008, at a meeting of the Standardisation of Genomic Amplification Techniques Clinical Diagnostics group at the National Institute for Biological Standards and Control (NISBC), UK. The participants agreed that an international standard for HCMV would come from a well-characterised '*laboratory-cultured strain similar to circulating clinical isolates, and containing all potential PCR gene targets.*'[b] The work of the Glasgow groups, together with Cardiff collaborators, to sequence and characterise Merlin, had established Merlin as the prototype HCMV virus, so the participants decided that '*the candidate standard would comprise a whole virus preparation of the prototype clinical HCMV strain Merlin.*'[c] The whole virus was chosen to standardise the complete assay process (extraction of viral DNA and subsequent amplification).

This recommendation was adopted into the WHO biological standardisation programme by the WHO Expert Committee. The Merlin strain was chosen because it was '*well characterised and more likely to represent a clinical virus than other laboratory-adapted strains'.*[c] The standard was tested in a collaborative study coordinated by NISBC, involving clinical and commercial laboratories in 14 countries that agreed that the international standard represented a much needed advance. WHO approved Merlin as the 1st WHO International Standard for CMV in November 2010. The standard is held in the NIBSC as the nominated WHO reference laboratory and distributed under the designation '09/162'.[d] Between January 2011 and July 2013, NIBSC shipped 409 vials of the standard to 274 laboratories in 43 countries.[e]

Commercial and clinical impact

The International Standard is now used for calibration in commercial quantitative-PCR assays by leading international biotechnology companies, including Altona Diagnostics GmbH (Germany)[f], Abbott Molecular Inc. (USA)[g], Argene/bioMérieux SA (France)[h] and Roche Diagnostics Ltd. (Switzerland).[i] In July 2012, the Roche diagnostic assay was the first DNA test approved by the US Food & Drug Administration for monitoring patients undergoing CMV antiviral therapy, and was '*based on information that included an assessment of the test's accuracy in measuring viral load and its ability to accurately measure variations in the amount of [human] CMV virus*'.[j] This was an important step forward to standardize treatment of HCMV disease, and facilitated by the WHO International Standard. A calibrated assay produced by Abbott Molecular Inc. was also approved for evaluation of the first HCMV vaccine trial to reach phase III, undertaken by Astellas Pharma Inc.[k]

Clinical laboratories can adopt standardised commercial tests or recalibrate their own to the WHO International Standard. The West of Scotland Specialist Virus Centre, which was also one of the UK centres involved in the WHO/NISBC validation study, uses its own International Standard-recalibrated test and performs 5,000 assays per year. The majority of these tests are to monitor patients who are at high-risk of HCMV infection or undergoing anti-viral treatment, and occasionally for diagnostic purposes, to identify underlying cause for neurological, eye or respiratory illness.[l]

The report of the international multicentre performance analysis of HCMV tests stated that implementation of an international standard, together with the availability of such standards in commercial tests, will improve the reporting of meaningful clinical data on HCMV. This includes monitoring of viral load, and establishing the cut-off values that represent different disease stages in different patient groups. Thus, by providing a means to enable validated and consistent PCR

tests for HCMV, research at the University of Glasgow has facilitated the crucial first step towards improved '*management guidelines that should significantly clarify decision making for clinicians and improve infection outcomes in at-risk patients.*'[m]

5. Sources to corroborate the impact

a. 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*, 11, 77–86 (2004), p78.
b. Fryer J & Morris C. International working group on the standardisation of genome amplification techniques (SoGAT) for clinical diagnostics, NIBSC UK, 24-25 June 2008. (PDF, p8)
c. Fryer, J.F., *et al.* 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.
d. CMV International Standard.
e. Data from the National Institute for Biological Standards and Control; available on request.
f. Altona RealStar® CMV PCR Kit 1.0, 2012 (p10)
g. Abbott RealTi*m*e CMV
h. Argene CMV R-gene®
i. Roche COBAS® AmpliPrep/COBAS® TaqMan® CMV
j. FDA approval for Roche COBAS® AmpliPrep/COBAS® TaqMan® CMV Test - P110037 diagnostic assay based on Merlin standard.
k. Abbot PCR assay based on Merlin standard used to monitor HCMV vaccine trial
l. Consultant Clinical Scientist, Head of Molecular Development and Specialist Typing, West of Scotland Specialist Virology Centre (available on request)
m. Hirsch, H.H. *et al.* An International Multicenter Performance Analysis of Cytomegalovirus Load Tests. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2012; 56, 367–373