- 1 Title Sequencing drug-resistant cytomegalovirus in paediatric patients: towards
- 2 personalised medicine
- 3 Author
- 4 Charlotte Houldcroft<sup>1</sup>\*
- 5 \*c.houldcroft@ucl.ac.uk

# 6 Affiliation

7 1. UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK

# 8 Keywords

- 9 1. Herpesviruses
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- 14 6. TORCH infection
- 15 7. Next-generation sequencing

# 16 **Summary** (117 words)

Cytomegalovirus is an ubiquitous herpesvirus that causes silent-to-mild infections in 17 18 healthy individuals, and potentially fatal infections in the immunocompromised, especially paediatric patients. CMV reactivation during periods of intense immune 19 20 suppression is associated with significant economic costs and poor patient outcomes. With a limited range of drugs licensed to treat CMV reactivation, managing antiviral 21 22 resistance is vital. In research settings, high-throughput sequencing is superseding PCR-based monitoring of resistance mutations, revealing a more complicated – and 23 24 more informative - picture of emerging mutation profiles in clinical samples. In the next decade, it is foreseeable that CMV whole genome sequencing for management of 25 26 antiviral drug resistance will become as important for personalised patient care as qPCR monitoring of virus loads is today. 27

#### 28 **Main text** (1459/1500 words)

Cytomegalovirus (CMV) is a common human pathogen causing life-long infection. In immunocompetent individuals, this double stranded betaherpesvirus causes a silent or mild primary infection, occasionally causing an infectious mononucleosis-like illness. In individuals with iatrogenic, acquired or inborn immunodeficiency, CMV has the capacity to cause a range of diseases. These include pneumonitis, colitis, CNS disease, and ocular manifestations such as retinitis and uveitis; the more severe forms of CMV disease can be fatal.

Paediatric patients may be at risk of severe CMV infections for a number of reasons. There is data to suggest that while the paediatric CD8<sup>+</sup> T cell response to primary CMV infection is similar to that of adults [1], the CD4<sup>+</sup> response is not as effective as the adult response [2]. Primary CMV infections in infants leads to prolonged shedding of the virus in urine and saliva which is not seen in adults [3], with the result that infants may present a significant infection risk to their siblings and other children they interact with closely, as well as CMV-negative adults.

43 There are four major categories of paediatric patient in whom serious CMV disease is commonly reported. Congenital CMV infection occurs in ~0.5% of live births, with the 44 45 greatest risk found in mothers who are infected with CMV for the first time during pregnancy. Children and adults with HIV are at significant risk of CMV disease due to 46 47 poor T cell control of this virus. Reduced T cell function contributes to the increased risk of CMV disease in organ transplant recipients, as intense immune suppression is 48 49 required to prevent graft rejection. Finally, inborn errors of immunity and conditions leading to poor thymus development also put children at risk of serious CMV infections 50 and disease. CMV is therefore a significant pathogen in many areas of paediatric 51 medicine. 52

### 53 The burden of drug resistance in cytomegalovirus disease

There are five drugs available for treatment of CMV (ganciclovir, cidofovir, foscarnet, maribavir and letermovir), and a number of other drugs used off-label to treat CMV disease, such as leflunomide and artesunate. These drugs may be given

prophylactically to prevent CMV reactivation, or pre-emptively to treat emerging CMV disease, but either approach requires weeks to months of antiviral therapy. These drugs have significant side-effect profiles of their own, creating a risk that antiviral treatment may need to be reduced or stopped. Some CMV antivirals poorly penetrate certain tissue compartments. Both factors increase the likelihood of paediatric patients being exposed to sub-therapeutic drug doses, a risk factor for emergence of antiviral resistance.

The economic burden of CMV reactivation in the setting of paediatric transplantation, 64 65 especially haemopoetic stem cell transplant (HSCT), has been calculated as up to £22,500 (~\$34,000) per patient, as well as CMV disease increasing the risk of graft 66 67 rejection in solid organ transplant recipients [4, 5]. A recent study found that pre-emptive antiviral treatment led to 14.5% of CMV-positive haplo-HSCT recipients developing 68 69 antiviral resistance mutations [5]. Ensuring timely and effective delivery of antivirals and 70 preventing resistance is also important for long-term patient outcomes in congenitally 71 infected neonates. Antiviral therapy can reduce or prevent many of the long-term sequelae of congenital CMV infection, including sensorineural hearing loss [6]. Given 72 the small pool of antiviral options currently available to clinicians, there are compelling 73 clinical and financial reasons to better manage CMV drug resistance. 74

### 75 Monitoring CMV drug resistance

Unlike many pathogenic bacteria, it is time consuming and expensive to culture CMV and test its drug-resistance phenotype by plaque assay. Fortunately, there is a growing catalogue of known drug resistance mutations [7, 8] in the genes targeted by ganciclovir, foscarnet and cidofovir: UL54 and UL97.

The primary approach to genetically confirming a clinical suspicion of antiviral resistance has been to PCR amplify and Sanger sequence small regions of these genes, which can be achieved directly from clinical samples. This method has successfully identified hundreds of mutations which may convey resistance to one or more drugs (and their prodrugs or oral derivatives). Traditional small sequencing of these PCR products is relatively inexpensive, but cannot be easily scaled over the

growing number of genes, widely distributed over CMV's large (~230kb) genome, on which new anti-CMV drugs act. It is also limited to detecting resistance mutations which reach frequencies of greater than ~20%.

PCR amplification of CMV fragments has increasingly being combined with highthroughput sequencing technologies. Amplicons are sequenced to high depth using a variety of next-generation sequencing platforms. This approach detects resistance mutations sooner, at lower frequencies, than Sanger sequencing of PCR products, with studies reporting detection of resistance mutations at frequencies of 3% [9, 10]. Unfortunately, as with Sanger sequencing, this approach still relies on labour-intensive PCR for the growing number of genes targeted by new antivirals and other therapies.

Novel approaches are being developed which can sequence whole CMV genomes, or 96 genes of interest, without the need for PCR or virus isolation and culture. Using 97 technology developed for human whole-exome capture and pull down, methods such as 98 SureSelect (Agilent) or SeqCap (Nimblegen) can be used to sequence virus genomes 99 to high depth directly from clinical samples. SureSelect uses custom baits to capture 100 CMV sequences and enrich them in the DNA that forms a sequencing library. This 101 approach has been very successful in sequencing relatively low virus load samples for 102 other herpesviruses [11, 12], and allows antiviral resistance mutations to be monitored 103 104 even as circulating CMV populations in blood, urine or CSF decline to low levels.

### 105 The future of CMV disease management

106 It is recognised that there is no unified strategy in place for monitoring the emergence of 107 CMV drug resistance in immunocompromised paediatric patients [13]. Clinical trials of 108 antiviral drugs are typically performed in adults rather than children. Choosing which 109 drugs to treat with, for what duration and dose, and managing clinically suspected drug 110 resistance is therefore even more challenging in paediatric patients than in adults [14].

Further studies are needed to establish at what frequency a resistance mutation must be present to warrant clinical action. Published data suggests that resistance mutations seen at high frequencies at the point of clinical diagnosis of resistance can be detected weeks earlier by deep sequencing, at frequencies <10% [10]. Researchers and

clinicians need data on whether, in the presence of a low-frequency drug resistance mutation, modifying an existing treatment schedule to increase the dosage or change drug delivery method to achieve better tissue penetration is a viable treatment option.

Deep-sequencing studies of the evolution of antiviral resistance in CMV disease also 118 highlight the need for new drugs which target different parts of the CMV genome to 119 avoid the emergence of cross-resistant variants. Given the occurrence of single 120 121 nucleotide variants which convey resistance to more than one drug, multi-drug resistant CMV is a known phenomenon [10]. At present, clinical sequencing may reveal that a 122 123 patient's circulating CMV strain or strains are already resistant to all available treatment options. In the future, if drugs such as artesunate, leflunomide and letermovir are more 124 125 widely used [15], sequence data could inform a clinical decision to switch to drugs which act on different CMV genes. By using these new drugs, clinicians may be able to avoid 126 the problem of pre-existing cross-resistance in previously treated patients, a significant 127 problem for drugs which target UL54 and UL97. 128

Adoptive immunotherapy is a further weapon in the CMV management arsenal, infusing 129 the host with CMV-specific cytotoxic T cells, and it may one day reduce reliance on 130 antivirals [16]. It is also likely to drive CMV evolution, selecting for CMV epitopes which 131 escape the infused T cells. These mutations will expand the number of genes which 132 need to be sequenced in order to manage CMV infection in immunocompromised 133 patients. It will rapidly become intractable to use PCR-based methods of CMV 134 sequencing, if this point has not already been reached. The future of CMV management 135 136 will require whole-genome sequencing and analysis of circulating CMV strains within 137 each patient. This will in turn create a need for automated, scalable solutions for monitoring CMV genome variation, as the current bioinformatic burden of sequence 138 139 analysis is a limiting factor in reducing the turn-around times from sample to personalised drug-resistance profile. 140

### 141 Future perspective

While the majority of immunocompromised paediatric patients with CMV infections are successfully treated with standard approaches to CMV load monitoring (qPCR) and

prophylactic or preemptive antiviral treatment, clinicians cannot currently predict which 144 patients will fail first-line therapies due to pre-existing resistance mutations or progress 145 to multidrug-resistant CMV infections. Prospective and regular sequencing of CMV, 146 particularly in patients who have already been treated with drugs with anti-CMV activity, 147 is likely to become the new gold standard in virological monitoring within the near future. 148 To achieve this goal, existing sequencing and analysis methods must be made scalable 149 and automatable to reduce costs and turn-around times, and bring this technology into a 150 151 healthcare laboratory setting.

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# 154 Abbreviations

- 155 CNS Central nervous system
- 156 CMV Cytomegalovirus
- 157 CSF Cerebrospinal fluid
- 158 HCST Haemopoetic stem cell transplant
- 159 HIV Human immunodeficiency virus
- 160 PCR Polymerase chain reaction
- 161 qPCR Quantitative polymerase chain reaction
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