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**Therapeutics to prevent congenital cytomegalovirus during pregnancy: what is available now and in the future?**

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**Human cytomegalovirus (CMV)** is the leading non-genetic cause of fetal malformation in developed countries. Congenital CMV infection can cause serious clinical sequelae, and in severe cases result in fetal or neonatal death. Despite the clinical and social importance of congenital CMV there is currently no standardised management strategy to prevent or treat maternal/fetal CMV infection during pregnancy and no evidence-based therapeutic for prenatally diagnosed CMV infection or disease. For pregnant women with a primary CMV infection during pregnancy, standard medical practise remains to offer no treatment at all or the option to terminate pregnancy. If intervention is requested, pregnant women may be offered a narrow range of medical therapies with limited evidence for efficacy and some with high risks of toxicity. However, there are several experimental and novel anti-CMV therapeutics currently being investigated that may provide a safe and effective therapeutic for use during pregnancy to prevent both fetal infection and reduce the risk of congenital CMV disease developing in the fetus once infected *in utero*.

**Established anti-CMV therapeutics**

**Valaciclovir**

To date, the only established CMV antiviral to undergo investigations during pregnancy is valaciclovir, the prodrug of the nucleoside...
analogue aciclovir. The converted active form of acyclovir is incorporated into the replicating viral DNA causing premature chain termination. Valaciclovir is used as a CMV prophylaxis in organ transplant recipients. A pilot observational study showed maternal treatment with oral valaciclovir to treat confirmed cases of fetal CMV infection was well tolerated and decreased viral load in fetal blood. Furthermore, treatment resulted in a modest decrease in adverse fetal outcomes (52% of 21 infants) compared with the untreated group (58% of 24 infants). These results led to a French multicentre, nonrandomised, single group assignment, phase IV clinical trial evaluating the efficacy of valaciclovir in the treatment of confirmed fetal CMV infection, which has recently completed. Results demonstrated a high safety profile with no liver or renal toxicity observed and met the primary endpoint in reducing the number of symptomatic children at birth and number of terminations of pregnancy for fetal anomalies using the Optimal Two-Stage Simon design. The outcome of valaciclovir treatment was 34/43 (79%) asymptomatic neonates, 2/41 (5%) terminations of pregnancy and 7/43 (16%) symptomatic neonates. Due to the toxicity associated with the other established CMV antivirals (ganciclovir, valganciclovir, cidofovir and foscarnet) it is unlikely these will ever be investigated in any clinical trials involving pregnant women.

Hyperimmune globulin (HIG)

Immunoglobulin therapy has been used safely during pregnancy for passive immunisation against a wide variety of viruses including; cytomegalovirus, rubella, varicella, measles and hepatitis A and B. To date, there have been several clinical trials, observational studies and case series reports on the prophylactic and therapeutic use of HIG during pregnancy, which has been recently reviewed in detail. While all the various studies on using HIG to prevent or treat congenital CMV to date have shown a beneficial trend, the lack of data from properly designed randomised clinical trials limits the conclusions that can be drawn and prevents recommendation as a treatment for pregnant women. Hyperimmune globulin (HIG)
standard therapeutic for congenital CMV during pregnancy. Of all the established and experimental CMV antiviral therapies currently available, only HIG and valaciclovir have been investigated in pregnant women (Table 1).

**Novel therapies with clinical drug candidates**

**Brincidofovir**

Brincidofovir (or CMX001) is an alkoxyalkyl ester analogue of cidofovir (a nucleoside analogue of cytosine), which acts by competitive inhibition of cysteine incorporation into the viral DNA strand and thus causing premature chain termination. While no data are available for the safety and efficacy of brincidofovir to prevent or treat congenital CMV, several studies in animal guinea-pig models show cidofovir and brincidofovir have potential benefits in preventing CMV transmission during pregnancy6–8. A recent phase II study on the efficacy of brincidofovir to prevent CMV disease in haematopoietic-cell transplant (HSCT) recipients showed promising results9. Patients who received brincidofovir twice weekly had significantly fewer incidence of CMV events than those who received placebo (10% v. 37%; P = 0.002). Brincidofovir has now entered phase III trials in HSCT recipients.

**Maribavir**

Maribavir is an orally bioavailable benzimidazole antiviral that binds to the CMV-encoded protein kinase pUL97 (a viral orthologue of cellular cyclin-dependent kinases) and inhibits viral nuclear egress and the efficiency of virus production in CMV-infected cells. Despite early phase I and II clinical trials showing promising anti-CMV activity observed10,11, this activity could not be confirmed in two subsequent phase III prophylaxis trials performed in allogeneic HSCT12. Resistance against maribavir has been reported at times, but the clinical significance has still to be investigated in the ongoing Phase II studies. Interestingly, the residues subject to resistance mutation within the pUL97 kinase are distinct from those of ganciclovir/valganciclovir resistance, although all contained within the kinase domain (Figure 1). Notably, other pUL97 inhibitors, presently under intense investigation at the experimental level, do not show detectable resistance formation15,16.

**Letermovir**

Letermovir (AIC246) represents a new class of non-nucleoside CMV inhibitors known as the 3,4-dihydro-quinazolines. Letermovir acts by targeting the CMV terminase complex and thus interfering with viral DNA concatamer maturation and subsequent cleavage and packaging of CMV progeny DNA into capsids. As there is no mammalian counterpart of the viral heterodimeric terminase enzyme, target-related toxicities, which are observed with the current anti-CMV polymerase inhibitors, are not expected17. Furthermore, the novel mode of action should provide new treatment options for resistant variants; however, the putative frequency of viral drug resistance to letermovir has not been addressed in detail18. Letermovir exhibits potent anti-CMV activity in vitro and in vivo and retains high efficacy against resistant variants. Letermovir has shown promising anti-CMV activity in an open-label, proof-of-concept phase IIa trial involving kidney/pancreas transplant recipients with...
CMV viremia. It also met all primary endpoints as a prophylactic drug in a recent phase Ib clinical trial in HSCT recipients and is currently entering phase III trials.

**Artesunate**

Another novel approach focuses on CMV inhibitors derived from natural resources or semi-synthetic derivatives like the antimalaria drug artesunate. Artesunate is derived from artemisinin, a natural product from the Chinese herb *Artemisia annua*. Meta-analyses of malaria patients treated with artemisinins demonstrated the safety of this class of drugs. For pregnant women with severe malaria, artemisinins are recommended by the World Health Organization as first-line therapy during the second and third trimester whereas less certainty about the safety is given in the first trimester. In addition to antimalaria activities, artesunate possesses a strong and broad antiviral activity and is particularly efficacious against CMV. Based on promising data obtained in vitro and from an experimental animal model, a small number of clinical antiviral investigations in transplant recipients have been conducted so far. In some cases, treatment of patients with standard drug-resistant CMV led to an efficient control of infection, whereas other case reports showed an unsatisfying outcome with poor benefit or complete treatment failure. A recent investigation using an ex vivo model of first trimester placental CMV infection showed a reduction of infection by 40% in the presence of artesunate. Although the mode of action is not fully elucidated, the targeting of artesunate to cellular proteins was demonstrated and may act as an inhibitor of cellular activation pathways, in particular the NF-κB pathway.

**Experimental therapies presently under early development**

**Kinase inhibitors**

Small molecules inhibiting cyclin-dependent kinases (CDKs) or other virus-supportive cellular kinases show promise as a potential tool for host cell-directed antiviral intervention. CDKs are serine/threonine protein kinases characterised by the formation of heterodimeric complexes with cyclins thereby regulating substrate recognition and phosphorylation activity. An obvious advantage of the targeting of CDKs or other cellular kinases over direct antivirals (e.g. viral kinase inhibitor maribavir) may be the reduction, or even complete suppression, of the induction of drug-resistant virus as it is generally difficult to select for resistance to inhibitors that target cellular functions. This may improve the therapeutic quality of a novel drug candidate. The recent identification of a potent selective CDK7 inhibitor with broad anti-herpesviral activity and low cytotoxicity profiles nicely substantiates this concept and now awaits proof-of-concept investigations in animal models. Thus, the kinase inhibitor strategy may lead to a novel option for antiviral therapy approaches that already proved to be highly potent in current anticancer therapy.

**Inhibitors of viral nuclear egress**

*In vitro*, HCMV production is largely co-regulated by the interaction between viral and cellular proteins and by the formation of virus-host multi-protein complexes. Recently, the viral ‘nuclear egress complex’ (NEC) has attracted the deep interest of researchers, since it represents a regulatory key position of viral replication and a

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**Figure 2.** The postulated cytomegalovirus nuclear egress complex (NEC) and potential novel therapeutic targets. Recruitment of viral and cellular components leads to phosphorylation (P) and partial disassembly of the nuclear lamina, allowing viral capsids to be packaged and exported to the cytoplasm through the nuclear envelope. Potential therapeutic targets include: (1) blocking formation and/or expression of the core viral NEC components pUL50 and pUL53; (2) preventing subsequent recruitment of viral/cellular components of the NEC; and (3) inhibiting NEC phosphorylation and subsequent disassembly of the nuclear lamina.
putative target for novel antiviral strategies. During the nuclear phase of HCMV replication, viral capsids are packaged and exported to the cytoplasm by transition through the nuclear envelope (nuclear egress) for further virion maturation. Nuclear egress is a multi-step process that involves a phosphorylation-triggered distortion of the nuclear lamina. Pivotal for nuclear egress is the role of the two viral nuclear egress proteins pUL50 and pUL53 that heterodimerise and form a core for the multimeric viral capsid scaffold.

In the absence of a CMV vaccine or an evidence-based therapeutic option available to prevent or treat fetal CMV infection during pregnancy, further scientific data is urgently needed on the safety and efficacy profiles of experimental and novel antiviral compounds. These investigations are extremely problematic given potential drug toxicities and the fact that CMV tropism and placental physiology are both highly host-specific. We have therefore begun investigating these experimental compounds in our established ex vivo placental explant model systems to better inform future directions in clinical trials.

Conclusion

References


**Biographies**

**Dr Stuart Hamilton** is a postdoctoral scientist in the Virology Research Laboratory, SEALS Microbiology at the Prince of Wales Hospital and University of New South Wales. His research is focussed on: (1) understanding transmission modes of intrauterine CMV infection; (2) examining the adverse effects CMV infection has on placental development/function and subsequent pregnancy outcomes; and (3) investigating safety and efficacy profiles of experimental CMV antiviral compounds in human ex *vivo* placental explant histolab and cell culture models.

**Dr Corina Hutterer** is a senior postdoctoral scientist at the Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg, with a strong interest in drug development and novel intervention strategies directed against herpesviruses. She completed her PhD in virology at the Institute of Medical Virology at the Humboldt University of Berlin in 2010 and then joined the research laboratory of Manfred Marschall focusing on virus-host interactions and the development of better therapeutic options for the treatment of cytomegalovirus infections.

**Professor Manfred Marschall** is group leader and international expert in herpesviruses at the Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg. His major research interest is human cytomegalovirus and is particularly focussed on three major topics: (1) the functional and structural characterisation of the CMV-encoded protein kinase pUL97 and CMV nuclear egress complex; (2) the cross-talk between herpesviral and cellular kinases (i.e. the regulation of viral nuclear egress, the virus-CDK interregulation, as well as questions of virus-induced cellular signalling and pathogenesis); and (3) the development of kinase inhibitors (and others) as putative broad-spectrum antiviral therapeutics in collaboration with international biotech and pharmaceutical companies.