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Review

Refractory Cytomegalovirus Infection in Renal Transplant Recipients: Current Knowledge and Future Perspective

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Abstract

Introduction. Resistant cytomegalovirus (CMV) in kidney transplant recipients represents an emerging issue. Prolonged hospitalization, increased utilization of resources, limited treatment options, and high toxicity of available treatment make the resistant disease a significant burden for the patients and a challenge for the treating physician.
Methods. This is a narrative review of the epidemiology, diagnosis and treatment options regarding resistant cytomegalovirus.
Discussion. Significant research has identified the main resistance mechanisms to the guideline-recommended treatment of resistant disease and for salvage and novel treatment options. The high toxicity of high dose ganciclovir and foscarnet pose an independent risk of treatment failure and graft loss. Several antiviral drugs and cytomegalovirus-specific T cells are being evaluated for their role in treating resistant cytomegalovirus disease.
Conclusion. Resistant CMV infection should be promptly recognized in kidney transplant recipients. Novel therapeutic approaches seem promising.

Key words: CMV, resistant, treatment, foscarnet, ganciclovir, letermovir

Introduction

Cytomegalovirus (CMV) is considered the most important pathogen after kidney transplantation. CMV disease can affect any organ system, including lungs, intestines, liver, brain, retina and the transplanted graft. Disease surveillance and treatment increase the costs of post-transplant care and increase the number of diagnostic and therapeutic procedures, creating a higher burden on the patient. Successful prevention via prophylaxis or preemptive treatment has significantly reduced the disease burden among kidney transplant recipients (KTRs). Exposure to antivirals has contributed to the development of mutations leading to resistance to treatment, particularly ganciclovir. Ganciclovir resistance is associated with more extended hospitalization, higher morbidity, and mortality in solid organ transplants [1,2]. Another increasingly recognized subpopulation of patients is those unresponsive to ganciclovir but without identifiable genetic mutations, termed treatment refractory CMV [3]. Additionally, second-line treatment options come with significant side-effect profiles, which themselves pose a risk for acute graft rejection, systemic toxicity and worse outcomes. Rates of reported ganciclovir-resistant CMV and treatment-refractory CMV are increasing. The novel treatment is under extensive research and is mainly used as salvage treatment when other options fail. Herein, we review resistant CMV epidemiology, genetics, diagnosis and treatment options.

Material and methods

This article is written as a narrative review based on relevant articles containing the terms "resistant cytomegalovirus" or "refractory cytomegalovirus" and "kidney transplant" or "solid organ transplant". Most relevant articles in PubMed were screened and selected. Subthemes involved were treatment resistance, refractory CMV, resistance monitoring, treatment of resistant disease, ganciclovir resistance. Previous reviews and references were screened for additional studies involving the following subthemes: cytomegalovirus resistance genetics, diagnostic methods, novel treatment.

Epidemiology of resistant CMV

CMV in the general population infects up to 60-100% of people. Similar to other members of its family, Herpesviridae, after primary infection, establishes lifelong latency. After impairment of the host's immune response, CMV can cause invasive disease and other indirect immunological effects [4]. CMV disease occurrence

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in KTRs without prophylaxis is dependent on the CMV serological match between the host and the donor, use of antilymphocyte antibodies, use of mTOR inhibitors and the existence of other risk factors [5-7]. Without prevention, the majority KTRs develop CMV infection and up to two-thirds develop CMV disease [5,8,9]. CMV resistance to treatment, primarily to ganciclovir, is sporadic in patients not treated with ganciclovir or valganciclovir [10]. CMV resistance is promoted in CMV D+/R- cases, prolonged ganciclovir exposure, high viral load and severe immunosuppression. A recent large meta-analysis has estimated the genotypic CMV resistance among solid organ transplants (SOT) to be around 12% and indicated that resistance rates are growing [11]. Resistance among patients on ganciclovir prophylaxis occurs 5-10% of cases [12,13]. Resistance has been described in patients on valganciclovir prophylaxis as well as preemptive treatment [14]. Two studies have associated suboptimal valganciclovir dosing with the development of ganciclovir resistance in solid organ transplant recipients [3,15]. Foscarnet is used as a second-line agent for treating ganciclovir resistant CMV, and although genotypic resistance to foscarnet has been described, there are no reports of treatment failure due to phenotypic resistance. In cases of failed viremia clearance on foscarnet treatment, subtherapeutic dosing has been identified as the presumed cause [3]. A recently published case series investigating the use of letermovir after foscarnet as a step down treatment for ganR-CMV disease described two cases of possible development of letermovir resistance, successfully treated with the addition of valganciclovir to the step-down regimen [16].

Mutations leading to treatment resistance

The list of gene mutations conferring CMV resistance is constantly increasing. Genotypic analyses in vivo and in vitro have elucidated the significance of many genetic mutations. Mutations conferring resistance to the main drugs used in CMV treatment are mutations in the UL97 and UL54 genes. The two genes encode a critical protein kinase and DNA polymerase, respectively [17]. Ganciclovir requires intact UL97 kinase function to become active and exhibit antiviral properties; therefore, UL97 gene mutations lead to ganciclovir and valganciclovir resistance. The DNA polymerase encoded by the UL54 gene is needed for the function of three common antivirals, ganciclovir, foscarnet and cidofovir, and its mutation confers resistance to these drugs. Sohrabi *et al*. published a cross-sectional study involving 58 KTRs with CMV and performed sequencing and analysis of the UL97 and UL54 genes. Although mutation in the UL97 gene vastly outnumbers mutations in the UL54 gene, mutation in UL97 alone confers low-level resistance compared to simultaneous mutations in UL97 and UL54 [17,18]. Most high-level GCV resistance is observed in dual UL97 and UL57 mutations [19]. UL56, UL51, and UL89 gene complex can affect viral terminase function and confer varying degrees of letermovir resistance. Mutations in UL89 and UL51 have only been shown to affect letermovir sensitivity in vitro. These studies have shown a low genetic barrier for the development of letermovir resistance, with in vitro resistance occurring sooner than resistance to foscarnet [20]. Cases of breakthrough infection and disease with letermovir-resistant virus have been reported in a phase 2 letermovir-prophylaxis study in stem cell recipients and adult and pediatric hematopoietic cell transplant recipients receiving letermovir for primary or secondary prophylaxis [21,22]. During maribavir selection pressure, UL27 mutations have been identified to cause low-grade resistance to maribavir, but these have not yet been isolated in vivo [22-24]. Additionally, most specific UL97 mutations which lead to maribavir resistance do not lead to ganciclovir mutations and vice versa [22,25]. Only one specific UL97 mutation was described, which resulted in maribavir resistance after prolonged ganciclovir exposure [26].

Diagnosis

Resistant or refractory CMV should be suspected in patients who display stable or progressive CMV viral loads or with persistent clinical symptoms despite adequate antiviral treatment for two weeks [10]. Two methods are most commonly used for monitoring CMV disease: reverse-transcriptase polymerase chain reaction (RT-PCR) and antigenemia assays. RT-PCR is more expensive but superior, as it has a higher sensitivity and is particularly more reliable at lower viremia levels (<1000 DNA copies/mL) [5]. After a suspected resistant or refractory disease, genotypic analysis of the UL97 and UL54 genes must be performed, and further management is guided based on these results. In situations where genotype analysis is unavailable, treatment should be changed empirically [10]. An important note is that rising viral loads within the first two weeks of treatment are not predictors of resistance development, and resistance studies are not recommended in these cases [27]. The sample of choice for genotypic studies is plasma, as there were reports of inconsistent findings between analyses of cerebrospinal fluid, leukocytes, bronchoalveolar lavage, and tissue samples [10,28]. Several limitations regarding the use of genotypic tests exist. There is a lack of standardization of genotypic assays. They may not target all the genetic loci of interest and may report mutations that have not been definitely linked to confer phenotypic resistance. To detect resistance, the strain must represent a certain proportion of the total viral population to be detectable [29].

Treatment of resistant disease

After the establishment of the diagnosis of ganciclovir resistant CMV, several established treatment options exist. There are reports of off-label use of leflunomide, artesunate, letermovir, and other antivirals as salvage therapy for more refractory cases. Additionally, adoptive immunotherapy has been reported as a successful treatment in several cases. Due to the severe and limiting side-effect profile of these drugs, factors that need to be considered when choosing the appropriate agent include the results of genotypic analyses, presence of neutropenia, degree of renal function, or renal failure, immunosuppressive regimen, and comorbidities present.

Ganciclovir

Ganciclovir is a 20-deoxyguanosine analog, and serves as a competitive substrate for a CMV DNA polymerase encoded by the UL54 gene. To act as a substrate for the polymerase, it requires phosphorylation mediated by the UL97 protein kinase, transforming it to ganciclovir-monophosphate. The subsequent bi- and tri-phosphorylation by host kinases results in the active form ganciclovir triphosphate. It acts by incorporation into the DNA chain, thereby terminating the synthesis of CMV DNA [20,30]. High or intermediate-dose ganciclovir (up to 10mg/kg/12h if normal renal function) can be used in patients with the non-severe disease and in whom the use of foscarnet is not recommended [10]. There is increasing evidence that even when high-grade resistance mutations are confirmed, treatment by increasing the ganciclovir dose is sufficient for viral replication control [31].
Interestingly, the IMPACT trial studied six asymptomatic or low-level disease patients with resistant CMV strains and demonstrated viral load clearance in half of the patients without antiviral therapy [31,32]. Despite these findings, treatment of documented CMV viremia is recommended to prevent the indirect effects of the virus. There are proposed benefits of allowing viremia for the development of virus-specific immunity. However, the significance of this immunity, particularly in patients with proven resistant CMV, is uncertain [10]. It must be noted that all patients in the study had very low viral loads and the cases were non-severe. Considering the myelosuppressive effects of high dose ganciclovir, caution is advised in using high dose ganciclovir for CMV with high-level resistance mutations [33].

Foscarnet

Foscarnet is a guideline-recommended agent for proven ganciclovir resistant CMV disease and as first-line empiric therapy for suspected ganciclovir resistant CMV in situations when genotypic studies are not available [10,20,34,35]. Foscarnet is a pyrophosphate analog that inhibits a CMV DNA polymerase encoded by the UL54 gene, resulting in the termination of CMV DNA synthesis [20]. Unlike ganciclovir, it does not require phosphorylation for its antiviral activity and is therefore not affected by UL97 mutations. Limiting its use are its side effects, notably nephrotoxicity, electrolyte disturbances and genitourinary infections. As with many antivirals, nephrotoxicity can occur via calcium crystal deposition. Specific for foscarnet is crystal deposition in the glomerular capillaries rather than in the tubules [36]. Kidney functional and pathological changes are reversible if the fibrotic changes are not severe and the patient receives adequate hydration [36,37]. Notably, treatment failure due to foscarnet toxicity is not uncommon in KTRs, as they are receiving other nephrotoxic drugs, including calcineurin inhibitors, and are at risk for other electrolyte imbalances [14,33].

Combination treatment with foscarnet and ganciclovir

Indications of a synergistic effect when using foscarnet and ganciclovir simultaneously exist [33,38]. The mechanism of synergy is not elucidated. However, the combined nephrotoxicity and effects on the bone marrow decrease the utility of this approach. No data regarding the use of this protocol exclusively in KTRs exist, and no studies directly compared foscarnet or high dose ganciclovir alone versus a combination of the two.

Letermovir

Letermovir targets the CMV terminase complex and inhibits the cleavage of CMV DNA and its package into capsids [39]. Considering its different mechanism of action, mutations in UL54 and UL97 do not affect letermovir susceptibility, and there is no cross-resistance with ganciclovir, foscarnet, maribavir, or cidofovir [40]. Primarily used and investigated in hematologic stem cell transplant recipients, it is approved as primary CMV prophylaxis in HSCT recipients. Combination treatment of letermovir with ganciclovir or cidofovir has resulted in additive effects and an additive/minor antagonistic effect when used concurrently with foscarnet against CMV in cell cultures [39]. As mentioned earlier, the genetic barrier to letermovir resistance development is low, and monitoring for resistance may be necessary. In a study of resistant CMV retinitis where letermovir was used as salvage treatment, three of four patients failed to clear CMV viral loads, and two developed genotypically confirmed resistance. Of note, letermovir was used after treatment with ganciclovir, valganciclovir, foscarnet and CMV immunoglobulins [41]. Letermovir has significant drug interactions with cyclosporine, and half dosing of letermovir is necessary when co-administered with cyclosporine [42]. Advantages of letermovir include its good oral availability and good patient tolerance. Currently, it is only used as off-label salvage therapy for treatment-resistant CMV. Further studies will elucidate its potential in

concurrent treatment with other novel drugs, including artesunate, which may have synergistic effects [43].

Cidofovir

Cidofovir is an acyclic monophosphate deoxycytidine analog that causes premature termination of CMV DNA synthesis. It acts as a nuclear analog substrate for the UL54 polymerase, and the reduced sensibility to cidofovir has been mapped to UL54 [20]. Mono-resistance to cidofovir is rare, and it frequently appears with cross-resistance to ganciclovir. It is currently used as an alternative agent for resistant CMV in solid organ transplant recipients, and its use is limited by high nephrotoxicity [40].

Brincidofovir

Brincidofovir is an oral lipid conjugate formulation of cidofovir, mainly investigated as CMV prophylaxis in HSCT recipients. Its lipid formulation enabled lower renal toxicity and increased in vitro toxicity compared to cidofovir [44]. A phase 3 study of brincidofovir prophylaxis in CMV HSCT recipients failed to meet its primary endpoints, and currently, oral brincidofovir is not developed as a treatment for CMV [22,40,45].

Leflunomide

Leflunomide is an isoxazole-derivative drug primarily used to treat rheumatoid arthritis and prevent and treat solid organ rejection [46]. It has shown antiviral activity against CMV, BK virus, and herpes simplex virus. Leflunomide does not share the mechanism of action of other antivirals, including ganciclovir, foscarnet and cidofovir, making cross-resistance an unlikely occurrence [47]. Hepatotoxicity, bone marrow suppression and its long half-life represent disadvantages of leflunomide [29,46]. Definitive evidence of clinical benefit in the treatment of resistant CMV is lacking. However, case reports and case series of its use have been published [29,46,48,49].

Maribavir

Maribavir is an inhibitor of the viral pUL97 kinase activity and interferes with nascent viral particles' morphogenesis and nuclear egress [39]. Maribavir does not require phosphorylation for conversion to an active form, rendering it particularly useful in UL54 mutations [40]. Maribavir has a favorable safety profile, good oral bioavailability and lower toxicity than currently approved drugs [50]. Tacrolimus and sirolimus levels increased in 10% of patients treated with maribavir, and dose adjustments are recommended [39,51]. Maribavir cannot be administered concomitantly with ganciclovir. A recent study evaluating the appropriate maribavir dose for refractory or resistant CMV reported discontinuation of the drug due to adverse effects (including CMV infection or disease) in 34% of the involved patients. The most common reported adverse effects are dysgeusia, nausea, and vomiting. Neutropenia is dose-independent. Treatment success was reported in two-thirds of treated patients [52].

Artesunate

Artesunate is an artemisinin derivative used primarily as an antimalarial agent. It has been shown to decrease CMV DNA replication by a pathway independent of commonly used antivirals. Studies demonstrated a synergistic effect combined with ganciclovir and additive effects with foscarnet and cidofovir [33]. Case reports of its use against resistant CMV show mixed outcomes [29]. In the treatment of malaria, artesunate has been shown to be well tolerated [33].

Filociclovir (Cyclopravir)

Filociclovir is a guanosine analog and acts by terminating DNA synthesis. Similar to ganciclovir, it requires phosphorylation by UL97 kinase [33]. In vitro studies have shown that it is five times more potent than ganciclovir against CMV, which may result from it being a better substrate for UL97 than ganciclovir [22]. Filociclovir shows activity against HHV-6. Currently, no available data of its use against CMV in humans exist. Clinical trials are underway [33].

Adoptive T cell therapy

Adoptive immunotherapy involves using HLA matched transfused donor T cells (CD4+ and CD8+) to restore adequate immunity without the occurrence of side effects associated with antivirals. The primary experiences draw from hematologic stem cell transplant recipients, in whom this therapy was used as an adjunct to preemptive antiviral therapy and the treatment of refractory CMV infections [22]. The main presumed benefit of this approach for KTRs is the presumed allograft stability. In SOTs, this approach was less explored, presumably due to the lack of HLA-matched donors and due to the T cell response atenuation by the immunosuppressive treatment used. However, this became a more explored field after successfully treated severe CMV disease in a KTR using third-party CMV-specific T cells [53]. The creation of third-party cell banks and cell registries could help create readily available treatment [54].

Currently, several clinical trials involving both HCTs and SOTs are evaluating the use of T-cell transfusions for treatment of CMV infection or severe CMV disease (NCT03266640, NCT04364178, NCT03665675). Furthermore, protocols for the in vitro selection and expansion of CMV-specific T-cells have been developed [29]. A

recent report from a prospective study reported treatment of recurrent or resistant CMV disease in 13 SOT recipients with autologous T-cell transfusions involving 4 KTRs. Improvement in symptoms was reported in 11/13 enrolled patients, including reduction of viremia and/or reduction or cessation of antivirals [55]. None of the patients who received adoptive CMV-specific T-cell therapy showed moderate or severe treatment-related adverse effects.

CMV intravenous immune globulin

The role of intravenous immunoglobulins (IVIG) in the treatment of CMV disease is unclear, and there are conflicting reports. The treatment is well tolerated and is currently mainly used as an adjunct treatment for resistant or refractory disease [40]. Benefits for CMV pneumonia are poorly defined, and extensive studies fail to show treatment effects [22]. However, a German team recently published a case report of a successfully treated KTR with multi-drug resistant CMV by immunosuppression change and high dose CMV specific IVIG. It is postulated that the combined effects of mTOR inhibitors and induction of specific CMV immunity contributed to viral control in their patient [56]. In addition, the opsonizing activity of immunoglobulins may favor uptake of CMV antigens into antigen-presenting cells and thereby increase antigen-presentation and their stimulatory capacity toward CMV-specific T cells.

Conclusion

Resistant CMV infection remains a major challenge after kidney transplantation. It should be promptly recognized in kidney transplant recipients. Novel therapeutic approaches seem promising.

Conflict of interest statement. None declared.

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