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Consensus document

## GESITRA-SEIMC/REIPI recommendations for the management of cytomegalovirus infection in solid-organ transplant patients

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### ABSTRACT

Cytomegalovirus infection remains a major complication of solid organ transplantation. In 2005 the Spanish Transplantation Infection Study Group (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) developed consensus guidelines for the prevention and treatment of CMV infection in solid organ transplant recipients. Since then, numerous publications have clarified or questioned the aspects covered in the previous document. These aspects include the situations and populations who must receive prophylaxis and its duration, the selection of the best diagnosis and monitoring technique and the best therapeutic strategy. For these reasons, we have developed new consensus guidelines to include the latest recommendations on post-transplant CMV management based on new evidence available.

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For the diagnosis of central nervous system disease, CMV detection by culture or PCR in cerebrospinal fluid samples is accepted. The diagnosis of retinitis is based on the presence of typical lesions observed during ophthalmological examination. In these cases, the diagnostic value of viral load in blood or plasma or in other laboratory tests as predictors of ocular CMV disease is low, although they may be positive before or at the same time as diagnosis.

The presence of CMV in the urine of patients with renal dysfunction or micturition syndrome is insufficient for diagnosing organ disease.

“Universal prophylaxis” consists of administering an effective antiviral drug to prevent the development of CMV infection and/or disease in risk patients, if there are no clinical suspicion of and microbiological data indicating infection. “Pre-emptive treatment” or “pre-emptive therapy” consists of starting pre-emptive antiviral treatment in patients displaying asymptomatic CMV replication (detected by regular monitoring of blood DNA or viral antigenemia).

### Diagnosis and virological monitoring

Solid-organ transplant (SOT) patients must be monitored virologically in order to detect CMV infection. Although great progress has been made, especially in molecular tests, none of the techniques currently available are universally valid<sup>6,7</sup>; hence, each technique must be selected according to the specific clinical objective, patient characteristics, the technical specifications of the method and the possibilities of the laboratory.

#### Pre-transplant assessment

The aim in this stage is to assess the risk of complications in the post-transplant period. Therefore, it is necessary to determine the immune status to CMV of both the donor and the recipient, since the latter conditions the risk of active CMV infection and disease post-transplant. For this purpose, a specific and sensitive serological method must be used, based on the detection of IgG-class antibodies (AII). Techniques that simultaneously detect IgM antibodies do not add sensitivity and in contrast may produce false positives or incorrect values.<sup>8</sup> In general, commercial enzyme immunoassay techniques are reliable, although not all are equivalent and must be validated beforehand by the laboratory. As regards recent transfusions, the normal precautions must be taken in pre-transplant serological screening, due to the possibility of hemodilution or passive antibody transfer.

In CMV-seronegative recipients, serological tests must be repeated in intervals close to the transplant date.

Other virological tests (cultures, antigen detection, nucleic acid amplification) are not useful in this context and should not be used prior to transplant (EII), except in exceptional circumstances.

#### Virological diagnosis post-transplant

Virological diagnosis post-transplant aims to detect both asymptomatic viral replication and CMV disease in transplanted patients. In this sense, serological tests have no use whatsoever in this period and must not be used to monitor these patients. In contrast, cultures, antigen detection and molecular methods are particularly useful in this period.<sup>6,7</sup> As a general rule, quantitative techniques are recommended, given the known relationship between replication intensity, disease development and relapse risk.

#### Diagnosis of CMV disease

Viral syndrome is the most common clinical expression of CMV disease. Quantitative virological tests are required to diagnose this

condition. Systematic monitoring with urine or saliva samples is not recommended since they have little value for predicting disease. Blood cultures (including rapid shell vial cultures) are not useful because they take a long time to produce results and due to their low sensitivity. These techniques are only useful either for obtaining strains for epidemiological characterization or for the performance of phenotypic resistance studies.

The antigenemia test detects the pp65 antigen of the virus in peripheral blood leukocytes by indirect immunofluorescence. This test has been shown to be useful for diagnosing CMV disease, particularly in viral syndrome cases<sup>9</sup>; evidence has been published indicating that sudden increases in antigenemia values predict the appearance of symptoms.<sup>10</sup>

The advantages of this test are that it is easy to perform and economical. However, it does have certain limitations, e.g. low antigen stability, which means samples must be processed within 6–8 h, it cannot be used in patients with less than 1000 neutrophils/ $\mu$ L, and its lack of standardization, which means that results from different laboratories cannot be compared and makes it difficult to establish a universally valid reference value. Consequently, no threshold value can be recommended. This value must be established in each center and individual threshold values may even be established for each patient according to his/her risk specific factors.

When interpreting antigenemia values, consideration must be given to the type of transplant (lower values for pulmonary or intestinal transplants and higher values for cardiac transplants), as well as the immunosuppression regime used. Although a threshold value of 20–50 CMV<sup>+</sup> cells/ $10^5$  leukocytes generally correlates well with the presence of symptoms, in the case of digestive disorder or retinitis, lower or even negative antigenemia values may be recorded.

Molecular tests, especially those based on PCR techniques, are the main alternative to antigenemia for making a diagnosis, starting pre-emptive treatment and monitoring response to treatment.<sup>7,9</sup> The results of these tests are not affected by spontaneous degradation of viral DNA and are therefore more sensitive and robust than antigenemia, and better for quantifying viral kinetics. In recent years, the technical advantages (sensitivity, speed, large linearity interval and reduced risk of contamination) of quantitative methods based on real-time PCR technology have resulted in more widespread use of these techniques for monitoring transplant patients. For this reason, although antigenemia is still acceptable, it would seem to be reasonable to recommend the use of these molecular methods (BIII).

Viral load values may be determined in both plasma and whole blood samples, and both are well correlated. However, it is much easier to determine these values in plasma. Whether using whole blood or plasma samples, the recommended anticoagulant is ethylenediaminetetraacetic acid (EDTA) as heparin interferes with PCR. Since the viral load values in whole blood are higher than those obtained in plasma, around 1 logarithmic unit,<sup>11</sup> individual patients should always be monitored using the same type of sample (AII).

Since DNAemia is subject to substantial biological variability, three- to five-fold increases in the initial value (0.5–0.7 logarithmic units) may not be significant.<sup>12</sup>

Although DNAemia is more robust, the variability between different PCR techniques or between different laboratories is very important. This has prevented its standardization and the determination of common cut-off points; hence, no recommendation can be made in this respect. For this reason, specific patients can be monitored using a specific reference and in the same laboratory (AII). Recently, an international reference standard was introduced similar to the one available for other viruses, and this may be a significant advance in standardization,<sup>13</sup> reducing inter-laboratory variability. However, the introduction of this new standard will not

eliminate differences in viral load, either among centers or among different types of SOT.

#### *Diagnosis of focal disease*

Antigenemia or DNAemia values may be negative or low, especially in patients with gastrointestinal involvement or retinitis. Therefore, diagnosis should always be performed on tissue samples whenever possible.<sup>3</sup> Qualitative PCR is not recommended for diagnosing the disease in organs as, except in the case of central nervous system involvement, accurate diagnoses cannot be made.<sup>3</sup>

#### *Virological monitoring for pre-emptive treatment*

Many transplant groups have used pre-emptive treatment as a preventive strategy instead of universal or risk factor-based prophylaxis. This strategy must only be performed in centers with viral load quantification methods. Although both antigenemia and DNAemia are useful for this purpose, DNAemia detected by PCR is recommended and efforts should be made to obtain the outcomes of these tests within 24 h.

One weekly determination is recommended during the period of greatest risk. A value close to the cut-off point should enable closer patient monitoring.<sup>7</sup> Due to the lack of standardization of either antigenemia or DNAemia, the establishment of a cut-off point for starting pre-emptive therapy is controversial. For this reason, the threshold values of these techniques must be determined by each transplant group and may be individualized for each patient.

#### *Monitoring of treatment response and the appearance of relapses*

Except in exceptional circumstances, such as in cases of central nervous system disease, quantitative tests are necessary to monitor the response to treatment. Given the characteristics of diagnostic techniques, molecular methods seem to be more appropriate than antigenemia in this context, and testing at least weekly once is recommended during treatment. Increases in antigenemia levels 24–48 h after the start of treatment do not necessarily mean that treatment has failed.<sup>7</sup> The aim must be achieving negative antigenemia or negative DNAemia results at the end of the second or third week of treatment. An increase or maintenance in antigenemia or DNAemia levels during therapy may indicate resistance, although it may also be due to host-dependent factors.

#### *Consensus recommendations*

1. Patients undergoing SOT must be monitored virologically to detect CMV infection (AI).
2. To determine CMV immune status, a serological method is recommended based on the detection of IgG class antibodies (AII). Except in exceptional circumstances, the performance of other virological tests (cultures, antigen detection, amplification of nucleic acids) is of little use in this context and should not be performed prior to transplant (EII).
3. Serological tests are of no interest for monitoring CMV infection in the post-transplant period and must not be used (EII). To monitor CMV infection, quantitative techniques must be used, given the known relationship between replication intensity, disease development and relapse risk (AII).
4. Systematic monitoring with urine or saliva cultures is not recommended for diagnosing CMV disease due to their limited value for predicting infection (EI). Blood cultures, including rapid shell viral cultures, are of no interest due to the time it takes to obtain results and their low sensitivity (EII).
5. It has been shown that both pp65 antigenemia and molecular tests based on PCR techniques are useful for diagnosis, starting

pre-emptive therapy and monitoring treatment response (AI). No recommendation can be made on the cut-off point for starting treatment. This cut-off point must be established at each center and may even be established individually for each patient according to his/her risk factors (CIII).

6. Since PCR-based molecular techniques have certain advantages over antigenemia (greater sensitivity, quantification of viral kinetics, speed, less risk of contamination), the consensus panel recommends the use of molecular methods, although antigenemia is still acceptable (BIII).
7. The viral load value may be determined from plasma or whole blood samples; individual patients must always be monitored using the same type of sample (AII).
8. Pre-emptive treatment must only be used in centers with viral load quantification methods (AII). For this purpose, at least one weekly test is recommended during hospitalization and during the period of greatest risk (BIII).
9. Quantitative tests must be carried out to monitor response to treatment, except in exceptional circumstances, for example in patients with central nervous system disease (BII). Molecular methods seem to be more appropriate than antigenemia in this context (BIII); viral load should be determined at least once per week during treatment (BIII).

#### **Study of resistance to antiviral drugs**

Viral resistance depends on the existence of mutations in the CMV genome. Although most studies mention resistance to ganciclovir, resistance has also been described to any antiviral drug used for prophylaxis or treatment of CMV disease.<sup>14</sup> The main risk factors favoring the appearance of resistance are the absence of pre-existing immunity to CMV (D+/R–), prolonged exposure to antiviral drugs, continued use of antiviral medication, especially at suboptimum concentrations, intermittent treatments, the presence of high viral loads and intense immunosuppression.

Although not all clinical findings during treatment may be attributed to viral resistance, the presence of resistant strains is often associated with invasive disease, progressive dysfunction, rejection of the transplanted organ and even a high mortality, that may affect up to 65% of patients.<sup>15–17</sup>

#### *Guidelines from the resistance study. Available methods*

During the first 2 weeks of treatment, antigenemia or DNAemia levels may increase in more than two thirds of patients, although this is not necessarily indicative of the presence of resistant strains. For this reason, resistance studies are not recommended in these cases.

Resistance to antiviral drugs must be suspected in the presence of progressive or stable viral loads (virological resistance) or if clinical symptoms persist 2 weeks after the start of appropriate antiviral treatment (clinical resistance). Clinical resistance is not necessarily accompanied by virological resistance since it may be due to the presence of factors related with the immune response of the recipient<sup>18</sup> or because adequate levels of the antiviral drug at plasma and/or tissue level are not reached. Therefore, in patients displaying slow response to treatment it would be advisable (in centers where this is possible) to determine the plasma levels of ganciclovir and to study CMV-specific immunity.

The presence of resistance to antiviral medication may be confirmed by phenotypic or genotypic methods. Phenotypic methods measure the concentration of antiviral medication necessary to inhibit 50% of viral growth (CI<sub>50</sub>). These methods require the virus to be isolated beforehand; hence, the time required to obtain results is a limitation. Furthermore, the lack of standardization of such

**Table 2**

Main mutations associated with the resistance of cytomegalovirus to antiviral agents.

Gene studied	Antiviral agent	Codons associated with resistance
UL54	FOS	495, 700, 715, 756, 838
	GCV, CDV	301, 408, 410, 412, 413, 501, 503, 513, 516, 521, 522, 545, 987
	GCV, FOS	776, 781, 787, 802, 809, 821
	GCV, FOS, CDV	588, 812, 813, 834, 841, 981
	CDV	805
UL97	GCV	405, 460, 466, 520, 590–607 <sup>a</sup>
	MARIBAVIR	353, 397, 409, 411

FOS: foscarnet, GCV: ganciclovir, CDV: cidofovir.

<sup>a</sup> Not all changes in the sequences between 590 and 607 are associated with resistance (1).

methods conditions the variability of results. These methods must only be used to study *in vitro* sensitivity to antiviral medication in order to characterize mutations not described previously, or to determine the combined effect of various antiviral drugs.

Genotypic methods are the most frequently used and consist of detecting genetic mutations associated with resistance. The selection method consists of amplifying specific regions of the viral genome, followed by sequencing. This technique may be performed using a CMV strain isolated in culture or, easier still, directly from clinical samples, since in this way results can be obtained in 2–3 days. To obtain results, viral load must be at least 1000 copies/ml. The main limitations of genotypic methods are that they do not provide quantitative results and that the results are difficult to interpret since irrelevant mutations may be detected that do not offer resistance to ganciclovir.<sup>19</sup>

CMV resistance to antiviral agents is mainly due to mutations in the gene encoding the viral protein kinase responsible for initial phosphorylation of ganciclovir (UL97 gene), and less frequently for mutations in the gene encoding the viral DNA polymerase (UL54 gene). If mutations only appear in the UL97 gene, viruses are not resistant to ganciclovir but they are the UL97 gene, cidofovir and foscarnet. If mutations appear in the UL54 gene, there may be resistance to one or all antiviral agents (ganciclovir, cidofovir, foscarnet). Tables 2 and 3 show the most frequent resistance mutations to ganciclovir, as well as their interpretation and the recommended approach for each type of mutation.

#### Consensus recommendations

1. Antigenemia or DNAemia levels often increase during the first 2 weeks of treatment. In such cases, in resistance studies are not recommended (CIII).
2. In patients who respond slowly to treatment, plasma ganciclovir levels should be determined (in centers where this is possible) and CMV-specific immunity studied (CIII).
3. Resistance to antiviral medication may be confirmed using phenotypic or genotypic methods. Phenotypic methods are recommended for studying sensitivity *in vitro*, for characterizing

mutations, or for determining the combined effect of various mutations. In other cases, genotypic methods are recommended (CIII).

## Immunological monitoring

### Immunological control of cytomegalovirus infection

Controlling CMV infection is a complex process that involves both innate and adaptive immune mechanisms.<sup>20,21</sup> Natural killer (NK) cells play an important role in controlling primary and recurrent CMV infections, increasing in number in response to viral replication.<sup>22–24</sup> However, T lymphocyte-mediated response plays a critical role in controlling CMV infection.<sup>20,21</sup> CD8<sup>+</sup> and CD4<sup>+</sup> T-lymphocytes intervene decisively in resolving replication episodes<sup>20,21</sup> through the recognition of a broad spectrum of viral proteins, including most notably the proteins pp65 and IE-1, which appear to generate dominant responses.<sup>25–27</sup> Therefore, the monitoring of T-lymphocyte immune response to these proteins may be useful for identifying patients with a greater risk of developing viral replication episodes.<sup>28–40</sup>

As regards humoral immunity, it has been suggested that glycoprotein B (gB) and H (gH) neutralizing antibodies may reduce the risk and severity of viral primary infection.<sup>20,21,41–43</sup> However, there is no consensus in this regard, since although hypogammaglobulinemia is associated with a greater risk of CMV infection in heart and lung transplant patients, the same does not occur in liver transplant patients.<sup>44,45</sup> Humoral response to CMV enables the identification of transplant patients at greater risk of primary CMV infection (D+/R–), although there is no unanimity on its usefulness for predicting the development of the disease.<sup>46,47</sup>

### Methods for quantifying and analyzing CMV-specific T cells

Various methods are currently available for functional and phenotyping and quantification *ex vivo* of CMV-specific T lymphocytes. Most of these methods are used for experimental purposes. The methods that employ HLA peptide multimers determine the number of T-lymphocytes that recognize a specific viral epitope but do not provide information on their functional capacity. In contrast, other methods provide information on the functionality of lymphocytes based on the quantification of cytokine production after stimulation of T cells with CMV peptides or viral lysate. These methods include intracellular staining, which provides functional and quantitative information on the population of CMV-specific T lymphocytes since it allows IFN $\gamma$  quantification to be combined with the expression of surface markers. The ELISPOT technique quantifies the number of individual T cells that release a specific cytokine (usually IFN $\gamma$  or TNF- $\alpha$ ) after stimulating them, although it does not distinguish between CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes. The QuantiFERON-CMV technique can be used to estimate the number of T lymphocytes compared with a limited number of immunogenic CMV epitopes presented by a broad spectrum of HLA specificities, through IFN $\gamma$  quantification. None of these tests are standardized,

**Table 3**

Different levels of resistance to GCV in mutations in the UL97 gene.

Mutations	IC <sub>50</sub> GCV <sup>a</sup> ratio	Interpretation	Response
M460V/I, H520Q, A594V, L595S, C603W	5–10	High grade resistance	Increase to FOS
C592G, A594T, L405P	2–3	Low grade resistance	Increase dose of GCV. Study mutations in UL54; if a mutation is detected that confers resistance to GCV, change to FOS
N597D, L600I	<2	Insignificant resistance	Continue with GCV
Q449K, H469Y, D605E	<1.5	No resistance to GCV	Continue with GCV

<sup>a</sup> IC<sub>50</sub> viral strain of patient/IC<sub>50</sub> wild-type viral strain of reference (number of times the IC<sub>50</sub> of the patient strain increases with respect to the IC<sub>50</sub> of the wild-type strain, necessary to inhibit viral growth); GCV: ganciclovir, FOS: foscarnet.

with the exception of the recently commercialized QuantiFERON-CMV technique.<sup>48</sup>

#### *T cell immunological monitoring strategies*

Immunological monitoring of CMV-specific T lymphocyte response and virological monitoring of CMV infection could be used to individualize and optimize antiviral treatment in SOT patients.<sup>49</sup> In high-risk (D+/R–) and intermediate risk (R+) patients, an inverse relationship has been reported between the peripheral levels of certain specificities of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes (productors of IFN $\gamma$ , TNF- $\alpha$  and IL-2 against CMV), with the consequent risk of developing CMV disease.<sup>28–40,48</sup> Phenotyping of CMV-specific T-lymphocytes can also provide information on the risk of CMV replication and disease. In this sense, the expression of the PD-1 (programmed death-1) marker on the surface of CMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes has been linked to a high risk of developing replication, viral syndrome and organ disease.<sup>50,51,52</sup>

#### *Consensus recommendations*

1. Various immunological markers are available for estimating, with variable degrees of precision, the risk of active CMV infection and disease within the scope of SOT. For now, none are universally accepted.
2. The ideal method for monitoring immune response to CMV must not only be quantitative but must also offer information on the functionality and surface phenotype of CMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes (CIII). It must also be easy to perform, fast, cost-effective and reproducible (CIII). Only the QuantiFERON-CMV method has been made commercially available, although its benefits are being evaluated.
3. Although the monitoring of T-cell response to CMV is potentially useful for therapeutic management of CMV infection in SOT recipients (CIII), there is no informed clinical experience in this respect. For now, therapeutic intervention strategies based on the immunological monitoring of patients are not recommended.

### **Risk factors of cytomegalovirus infection in solid organ transplant recipients**

#### *General aspects*

CMV infection appears in between 30% and 80% of SOT recipients, although its incidence and the presence of symptomatic disease vary depending on the type of transplant, the presence of associated risk factors (Table 4) and the prevention strategies used.<sup>53</sup>

The period of maximum risk is between the first and sixth month post-transplant. Maximum incidence occurs between the second and third month. However, some factors may alter this chronology, either causing infection to start earlier, such as in the case of treatment with OKT3 monoclonal antibodies, or delaying infection, for example in patients receiving universal prophylaxis or pre-emptive treatment.<sup>53</sup>

In primary infection, the lack of specific immunity of the recipient leads to increased viral replication (increasing by 1.82 units/day), normally associated with the development of CMV disease.<sup>54</sup> In reactivations, humoral immunity and cellular immunity of the recipient reduce the viral replication process (0.61 units per day)<sup>54</sup> with the subsequent decline in the incidence and severity of the disease, which develops in between 10% and 20% of patients. In re-infections, *in situ* reactivation of CMV in the transplanted organ, in addition to producing the disease in up to 30% of

**Table 4**

Risk factors of cytomegalovirus disease in solid-organ transplant patients.

<b>Primary infection in seronegative recipients (D+/R–) from</b>
<ul style="list-style-type: none"> <li>■ Transplanted organ</li> <li>■ Blood derivatives</li> </ul>
<b>Factors favoring the reactivation of cytomegalovirus in recipients</b>
<ul style="list-style-type: none"> <li>■ Stress</li> <li>■ Surgery</li> <li>■ Intraoperative hypothermia</li> <li>■ Sepsis or severe bacterial infections (<i>pneumococcus</i> or <i>gram-negative bacteria</i>)</li> <li>■ Co-infections caused by other viruses</li> <li>■ Herpes virus 6 (HHV6)</li> <li>■ Herpes virus 7 (HHV7)</li> </ul>
<b>Factors favoring progression to CMV disease</b>
<i>Immunosuppressants</i>
<ul style="list-style-type: none"> <li>■ OKT3, anti-thymocyte globulins, anti-lymphocyte globulins</li> <li>■ Mycophenolate, azathioprine</li> <li>■ Methylprednisolone</li> <li>■ Alemtuzumab</li> <li>■ Viral load</li> <li>■ Immunomodulation</li> <li>■ Herpes virus 6 (HHV6)</li> <li>■ Herpes virus 7 (HHV7)</li> </ul>
<i>Immunological factors favoring CMV infection</i>
<ul style="list-style-type: none"> <li>■ Mutations in TLR2 and TLR4 genes</li> <li>■ Deficiency of mannose-binding lectin or genotype associated with low production of same</li> </ul>
<i>Factors that reduce CMV disease</i>
<ul style="list-style-type: none"> <li>■ Recipient immunity against CMV prior to transplant</li> <li>■ Immunosuppressants: mTOR inhibitors (sirolimus and everolimus)</li> <li>■ Anti-viral prophylaxis or with immunoglobulin</li> <li>■ Pre-emptive antiviral treatments</li> </ul>

patients, may prompt the onset of terminal disease in the transplanted organ.

In SOT recipients, the risk of CMV disease is the result of the balance between the amount of virus present or viral load and the humoral and cellular immune response or viral load of the recipient. Factors such as rejection<sup>55</sup> or co-infections,<sup>56</sup> which are accompanied by the production and secretion of cytokines triggering the inflammatory cascade, may stimulate latent CMV replication.<sup>57</sup>

#### *Risk factors*

##### *Immunological status of the recipient and donor*

The transplantation of a seropositive organ to a seronegative recipient (D+/R–) has been shown to be the main risk factor for CMV disease in all types of transplant.<sup>58–62</sup> Seronegative recipients who receive seronegative transplanted organs (D–/R–) run a very low risk of developing infection unless they receive hemoderivative transfusions of unfiltered leukocytes from seropositive donors<sup>63,64</sup> or exhibit primary infection.

##### *Viral load and transplanted organ*

The degree of viral replication has been directly associated with the development of CMV disease,<sup>63,65</sup> with primary infections being the most symptomatic and severe as they are normally accompanied by higher viral loads. Other factors that influence viral load are type of transplant, prophylaxis strategy and the net state of immunosuppression of the recipient.

As regards the type of transplanted organ, the onset of CMV disease is more frequent, and normally more severe, in kidney transplants and lung transplants than in liver, heart and kidney transplants. This greater incidence of CMV disease in intestinal and pancreatic transplants is probably due to the fact that both allografts have abundant lymphoid or macrophage tissue with high loads of latent or replicating CMV.<sup>58–60</sup> For the same reason,

**Table 5**  
Indirect effects of CMV infection described in each type of transplant.

Lung	Kidney	Liver	Heart	General
Bronchiolitis obliterans	Acute rejection Chronic allograft nephropathy Cardiovascular events Diabetes mellitus	Chronic rejection Accelerated HCV recurrence Hepatic arterial thrombosis	Vasculopathy post-transplant	Bacterial infections Fungal infections PTLD Immunosenescence

HCV: hepatitis C virus; PTLD: post-transplant lymphoproliferative disease.

multiple transplants (renal-pancreatic, cardiopulmonary) are riskier than single organ transplants.<sup>60,63</sup>

#### Immunosuppressive treatment

Immunosuppressants are used to prevent rejection delay and/or mitigate specific humoral and cellular immunological responses, permitting the uncontrolled replication of the latent virus. Immunosuppressants displaying this activity include methylprednisolone at high doses,<sup>65</sup> anti-lymphocyte agents such as anti-lymphocyte (ALG) and anti-thymocyte (ATG) globulins, the monoclonal antibody OKT3 (currently not commercially available),<sup>66,67</sup> and mycophenolate mofetil.<sup>68,69</sup>

The use of anti-lymphocyte antibodies for induction therapy, or to treat rejection, increases the rate of CMV infection three-to-four-fold, especially in seropositive patients.<sup>70</sup> The mechanism may be related with fever and the release of tumor necrosis factor alpha, the depletion of helper T lymphocytes and the inversion of the CD4/CD8 coefficient.

The anti-CD25 monoclonal antibodies basiliximab and daclizumab (the latter is currently not commercially available) have not been associated with a higher risk of CMV infection or disease. However, it has been shown that alemtuzumab<sup>71,72</sup> is a risk factor when used to treat rejection in renal-transplant or heart transplant recipients. Cyclosporin, tacrolimus and prednisone at conventional doses do not normally reactivate latent CMV, although they do reactivate replicating CMV.<sup>73</sup> The use of mycophenolate in kidney transplant patients has significantly reduced the incidence of rejection, although it is accompanied by a greater risk of CMV disease, particularly in the intestinal tract.<sup>68,69</sup> Results in other types of transplants are less conclusive.

The mTOR (mammalian target of rapamycin) inhibitors sirolimus and everolimus<sup>74–76</sup> used in kidney and heart transplants have been associated with a lower incidence of CMV infection when compared with approaches that include cyclosporin or azathioprine.

#### Other factors

Some observational studies in liver and kidney transplant patients have shown that the reactivation and replication of other beta-herpes viruses, such as human herpes viruses type 6 and 7, are associated with CMV disease.<sup>77–79</sup>

Similarly, other factors such as donor age over 60 years,<sup>74</sup> kidney transplanted from cadaver, female recipients, advanced age of recipients, re-transplant, the need for multiple transplants and prophylaxis or short pre-emptive treatments, have been associated with a greater incidence of CMV infection.<sup>63</sup>

Moreover, in recipients of abdomen transplants, factors such as intraoperative hypothermia, stress associated with surgery or with critical situations and post-operative bacterial infections have also been associated with greater CMV replication.<sup>80,81</sup>

Finally, recent evidence shows that some defects in the immune system of the host may be associated with greater risk of CMV infection. These situations include certain polymorphisms of Toll-like receptors 2 and 4 and certain deficiencies in the complement, cytokines, chemokines or mannose-binding lectin.<sup>4,18,63,82,83</sup>

#### Consensus recommendations

The main risk factors of CMV disease depend on the serological condition of the donor and recipient, the type of organ transplanted and the degree of immunosuppression of the recipient once the organ has been transplanted.

1. Transplantation from a seropositive donor to a seronegative recipient (D+/R–) is one of the main risk factors (AI).
2. Intestine, pancreas and lung transplant recipients have a greater risk of CMV disease than other transplants (AI).
3. Some immunosuppressants such as steroids<sup>65</sup> and anti-lymphocyte antibodies used for induction treatment or to prevent rejection<sup>66,67</sup> are associated with a greater incidence of CMV disease (AI).

#### Indirect effects of cytomegalovirus infection

In addition to the direct effects produced by invasive organ infection, CMV produces a series of indirect effects that may be defined as those resulting from interaction of the virus with the host immune system and which are independent of the presence of high levels of viremia, and are probably related with the existence of low levels of viral load over prolonged periods. These indirect effects have been described in all types of SOT (Table 5) and include, among others, acute and chronic allograft rejection, opportunistic infections, post-transplant diabetes mellitus and increases in opportunistic infections. There is no consensus among the panel of experts regarding the demonstrated existence of a cause-effect relationship between CMV and indirect effects, since the evidence available until now only allows a relationship of greater or lesser association to be established between these indirect effects and CMV for each type of transplant.

#### Kidney transplant

In kidney transplant recipients, results from observational studies have associated both asymptomatic viremia and CMV disease with greater risk of acute allograft rejection<sup>84</sup> (BII). However, the results of studies that have evaluated the association between CMV and chronic allograft nephropathy are controversial,<sup>85–87</sup> hence no relationship of causality can be established between CMV and chronic allograft nephropathy. In these patients, CMV infection has been related with a greater risk of cardiovascular events. One observational and prospective study has reported a greater frequency of these events (myocardial infarction, cardiovascular disease and peripheral vasculopathy) in patients displaying CMV replication during the first year post-transplant.<sup>88</sup> Another later retrospective review described greater frequency of cardiovascular diseases during the first 4 years post-transplant in patients with CMV infection compared with patients without infection after adjustment for traditional cardiovascular risk factors.<sup>89</sup> Finally, some authors have suggested that CMV infection and disease are associated with a greater risk of post-transplant diabetes mellitus, although the design of these studies made it impossible to establish a direct causal relationship.<sup>90–92</sup>

### Lung transplant

The main indirect effect of CMV described in lung transplant recipients has been the development of bronchiolitis obliterans syndrome (BOS). However, studies evaluating the causal relationship between CMV and BOS present contradictory results, probably due to differences in the definition of CMV infection, immunosuppression methods and prophylaxis strategies used.<sup>93–99</sup> To conclude, based on data currently available, a definitive causal relationship cannot be established between CMV and the development of BOS. Similarly, the influence of different prophylaxis strategies to combat the development of CMV disease must be determined in future studies.

### Liver transplant

CMV infection has been related to chronic allograft rejection in liver transplant recipients, although the etiological role of the virus in rejection has yet to be determined.<sup>100–103</sup> Similarly, available evidence on the role of CMV in the accelerated recurrence post-transplant of hepatitis C virus is contradictory.<sup>104–106</sup> Finally, CMV donor-recipient serodiscordance has been related with a greater risk of liver artery thrombosis in such patients,<sup>107–110</sup> although the design of these studies made it impossible to establish a definitive causal relationship.

### Heart transplant

In heart transplant recipients, CMV has been related with a greater risk of allograft vasculopathy. Findings supporting this relationship have mainly been published in studies comparing different prophylaxis strategies, reporting that patients who did not receive prophylaxis or who received pre-emptive treatment were more at risk of developing CMV than those who received universal prophylaxis.<sup>111–113</sup>

### General indirect effects

CMV has been related with a greater risk of bacterial and fungal infections, with evidence to support that this risk can be reduced through the use of prophylaxis strategies to combat the virus.<sup>114–118</sup> CMV has also been related with a greater risk of lymphoproliferative disease post-transplant, although most existing evidence in this respect has been reported in retrospective studies.<sup>119</sup> Finally, the replication of CMV has been associated in a cross-sectional study with the appearance of immunosenescence through the proliferation of CD27/CD28 CMV-specific CD8<sup>+</sup> T cells,<sup>120</sup> although these results need to be confirmed in prospective studies.

### Consensus recommendations

1. CMV has been associated with the appearance of different indirect effects, including acute and chronic rejections (known as chronic allograft nephropathy in renal transplantation, allograft vasculopathy in cardiac transplantation and bronchiolitis obliterans syndrome in lung transplantation), increases in the number of opportunistic bacterial and fungal infections, lymphoproliferative disease post-transplant, cardiovascular disease and diabetes mellitus.
2. The panel of experts considers that an association relationship exists between CMV infection and acute rejection in kidney transplant recipients (BII), as well as an increase in opportunistic infections, lymphoproliferative disease post-transplant and CMV infection (CIII).

3. However, this panel did not reach a consensus on the role of CMV in the other indirect effects.

## Prevention of cytomegalovirus infection in solid organ transplants

### General considerations

The two main strategies for preventing CMV disease are universal prophylaxis and pre-emptive therapy. Universal prophylaxis consists of administering effective antiviral medication to all patients at risk, even in the absence of clinical suspicion and microbiological data of infection. Pre-emptive treatment consists of starting antiviral treatment in patients showing asymptomatic CMV replication, detected by regularly monitoring the amplification of nucleic acids or viral antigenemia in blood. Each of these strategies has advantages and disadvantages. Universal prophylaxis has the advantage of potentially preventing the reactivation of other herpes viruses, as well as preventing indirect effects, and the need to obtain repeated samples in order to quantify viral load or antigenemia. However, prolonged exposure to antiviral drugs may increase the risk of resistance and toxicity related with antiviral treatment. Universal prophylaxis has also been related with the late CMV disease, probably due to defective development of specific cellular immunity to the virus.<sup>121</sup> Pre-emptive therapy can reduce the cost and toxicity of antiviral medication, however this strategy depends on the availability of adequate logistics at each transplant center.

### Universal prophylaxis

The advantages of universal prophylaxis have been demonstrated in clinical trials comparing this strategy with non-prophylaxis or placebo. In these trials, the administration of prophylaxis has been associated with a 58–80% reduction in the incidence of CMV disease.<sup>122–124</sup> The drugs evaluated for this strategy were acyclovir, valganciclovir, oral and intravenous ganciclovir and valganciclovir. In the first studies, acyclovir was inferior to ganciclovir.<sup>125,126</sup> In another controlled trial performed with kidney transplant recipients, valganciclovir administered for 90 days was associated with a decrease in the incidence of CMV disease and a delay in the onset of this disease.<sup>127</sup> A later randomized, controlled and multicenter trial with kidney, liver, pancreas and heart transplant recipients compared oral ganciclovir and valganciclovir and reported comparable efficacy for the prevention of CMV disease in D+/R– patients.<sup>121</sup> Adverse effects were similar in both groups, although a higher incidence of invasive CMV disease in organs was observed in the subgroup of liver transplant patients receiving valganciclovir.<sup>121</sup>

Oral ganciclovir is currently not commercially available, in spite of achieving good outcomes and widespread use in recent years. Thus, although different studies have analyzed the role of oral ganciclovir, this drug is not included in any of the final recommendations of the consensus panel.

### Pre-emptive therapy

Different comparative studies have contrasted the efficacy of pre-emptive therapy with non-treatment or placebo. These approaches have been analyzed jointly in three meta-analyses.<sup>122–124</sup> In these studies, the incidence of CMV disease was reduced by an average of 70%; pre-emptive therapy was as effective as universal prophylaxis and its cost was similar.<sup>122</sup>

### Comparison between universal prophylaxis and pre-emptive therapy

No clinical trials have been carried out with SOT recipients to compare universal prophylaxis and pre-emptive treatment in the prevention of CMV disease. In non-randomized studies comparing prophylaxis and pre-emptive therapy, no differences in efficacy were observed. The authors of a meta-analysis of 17 randomized studies carried out previously in 2006 in high-risk kidney and liver transplant recipients concluded that compared with placebo, both strategies reduced the frequency of CMV disease by 80% and 72%, respectively, as well as the frequency of rejection. However, since only prophylaxis reduced the frequency of bacterial and fungal infections (51%) and mortality (38%), the aforementioned authors preferred prophylaxis,<sup>124</sup> even though most of the studies included in the meta-analysis were small and open and individually reported no benefit in mortality. In contrast, a greater frequency of leukopenia has been reported in patients receiving prophylaxis compared with those undergoing pre-emptive treatment.<sup>128</sup> Small et al. performed another meta-analysis, which included 17 prophylaxis studies and 9 pre-emptive treatment studies, but did not observe any differences in efficacy for the prevention of CMV disease.<sup>123</sup>

The most comprehensive comparative study of prophylaxis and pre-emptive treatment has been carried out in kidney transplant recipients.<sup>129</sup> In this study, no differences were observed in terms of the efficacy of both strategies, including the subgroup of high-risk patients (D+/R–), with no reported differences in cost between either form of treatment. Another comparative study, also performed in kidney transplant patients, compared prophylaxis with valganciclovir and pre-emptive treatments with intravenous ganciclovir in 148 kidney transplant recipients, and reported a greater frequency of CMV infection in the pre-emptive treatment group (51% vs. 18%). Allograft survival after 4 years was lower in the group of patients displaying CMV infection and receiving pre-emptive treatment, and no differences in mortality were observed.<sup>130</sup>

### General recommendations

- Both universal prophylaxis and pre-emptive therapy are useful strategies for preventing CMV disease (AI).
- In D+/R– patients, both universal prophylaxis and pre-emptive therapy can be used (Table 7), although universal prophylaxis is particularly recommended in high-risk transplants (lung, intestine, pancreas, pancreas-kidney transplantations). Most of the panel would also recommend universal prophylaxis in D+/R– patients receiving other organ transplants if rigorous compliance with the applicable virological monitoring protocol cannot be guaranteed. The recommended duration of prophylaxis is 3–6 months (AI) but this decision will depend on the degree of immunosuppression and the type of organ transplanted. In lung transplants, the duration of prophylaxis will be 6–12 months post-transplant. Due to the risk of late disease, pre-emptive therapy is recommended for a period of 3–6 months after the end of universal prophylaxis (CIII).
- In R+ patients, pre-emptive therapy (BII) is recommended except in high-risk transplants (lung, intestine) and at centers where it is difficult to monitor viral load or antigenemia; in the latter case, universal prophylaxis is recommended for 3 months (CIII).
- D–/R– patients are considered to be low risk, and therefore anti-CMV prophylaxis is not recommended in this population (DI). In these patients, leukocyte-depleted blood products from seronegative donors must be used and patients should be monitored using conventional virological monitoring techniques.

**Table 6**

Recommended doses in prophylaxis and treatment (including pre-emptive treatment) with intravenous ganciclovir and valganciclovir.

Creatinine clearance (ml/min)	i.v. ganciclovir <sup>a</sup>	Valganciclovir <sup>a</sup>
>70	5 mg/kg/day <sup>b</sup>	900 mg/day
60–69	2.5 mg/kg/day	900 mg/day
50–59	2.5 mg/kg/day	450 mg/day
40–49	1.25 mg/kg/day	450 mg/day
25–39	1.25 mg/kg/day	450 mg/48 h
10–24	0.625 mg/kg/day	450 mg/2 × week
<10	0.625 mg/kg 3 × week (after hemodialysis)	

i.v.: intravenous.

<sup>a</sup> During treatment, dose every 12 h.

<sup>b</sup> Or 6 mgr/Kg/day (from Monday to Friday).

- When a primary infection is detected, standard treatment must be applied (see section on treatment) (BII).
- If universal prophylaxis is selected, in both seropositive and seronegative patients, neither viral load nor pp65 antigenemia has to be monitored during prophylaxis because the risk of disease during this period is very low (BII). In these cases, viremia or antigenemia must only be determined if the patient develops symptoms indicative of CMV disease during this period. One unresolved aspect is the action to take once universal prophylaxis has ended. This panel considers that the patient progress must be monitored through monthly prospective evaluations of pp65 antigenemia or viral load (CIII).
- Whenever a pre-emptive therapy strategy is selected, each center is recommended to establish and validate its own practice protocols. Treatment should be started taking into account the characteristics of each patient, and antiviral therapy may begin after the first positive antigenemia or viral load test is obtained in accordance with the cut-off points established in each center (CIII).
- Pre-emptive therapy may be prolonged until a negative antigenemia or viral load result is obtained. However, in D+/R– or high-risk transplant patients it is advisable to obtain two consecutive negative results (CIII).
- For prophylaxis in patients receiving combined transplants (e.g. pancreas-kidney transplants), the instructions for higher-risk transplants should be followed (CIII).
- In patients with severe post-transplant kidney failure (creatinine clearance <10 ml/min) the use of ganciclovir or valganciclovir is not recommended because the kidney is the only elimination route. In these patients, the start of prophylaxis or pre-emptive treatment should be delayed until the creatinine clearance is greater than 10 ml/min or up to a maximum of 15 days post-transplant (CIII).
- Since exposure to valganciclovir and ganciclovir depends entirely on elimination in urine, the administered dose must be adjusted strictly according to creatinine clearance (Table 6).

### Prophylaxis during treatment with anti-lymphocyte and/or monoclonal antibodies

The use of anti-lymphocyte antibodies in induction treatment or as CMV-rejection prophylaxis is associated with an increase in the risk of CMV disease.<sup>66,70,72</sup> The effect of antiviral prophylaxis in patients receiving antiviral medication has been demonstrated in different trials with SOT recipients. Two of these trials, which compared the administration of intravenous ganciclovir with no treatment in

**Table 7**  
Summary of recommendations for the prevention of CMV in solid-organ transplants.

Type of transplant	D+/R– patients	Other patients
Liver	PPX: valganciclovir (900 mg/d) for 3–6 months (AIII), i.v. ganciclovir (5 mg/kg/d) (AI) followed by pre-emptive treatment up to 3 months after end of PPX (AII), or: PT: valganciclovir (900 mg/12 h) for 14 days, checking negativization of viremia and monitoring every 1–2 weeks, according to risk, during the first 4 months (AII).	In R+ patients, either PPX (valganciclovir, 900 mg/d 3 months (BII), or i.v. ganciclovir, 5 mg/kg/d (AI), followed by valganciclovir, 900 mg/d) or PT with valganciclovir, 900 mg/12 h (AII), or i.v. ganciclovir, 5 mg/kg/12 h (AI), for 14 days and subsequent monitoring. In D–/R– patients, leukocyte-depleted blood products and products from seronegative donors can be used. Treatment in case of primary infection.
Kidney	PPX: valganciclovir (900 mg/d) (AI) for 6 months post-transplant (AI). Alternatives: oral valaciclovir (2 g/6 h) (AI), or i.v. ganciclovir (5 mg/kg/d) (if not tolerated orally), for a maximum of 3 months post-transplant (AI).	In R+ patients, PT is recommended with valganciclovir (900 mg/12 h) or i.v. ganciclovir (5 mg/kg/12 h) for 14–21 days and monitoring (BI). Alternative: PPX with valganciclovir (900 mg/d), valaciclovir (2 g/6 h) or i.v. ganciclovir for 3 months (AII). In D–/R– patients, leukocyte-depleted blood products and products from seronegative donors can be used. Treatment in case of primary infection. In treatment with anti-lymphocyte antibodies: i.v. ganciclovir (5 mg/kg/d) for at least 14 days (BI) or valganciclovir for 3 months (BI).
Heart	PPX: i.v. ganciclovir (5 mg/kg/day) or valganciclovir (900 mg/day) for 3–6 months (AI).	Patients receiving induction with anti-lymphocyte antibodies (except basiliximab) or exhibiting steroid-resistant rejection must receive ganciclovir (5 mg/kg/day) for at least 14 days (BI) or valganciclovir (900 mg/d) for 3 months (CIII). In R+ patients: PPX or PT. Monitoring of CMV in non-prophylaxis patients. If monitoring results are positive, i.v. ganciclovir (5 mg/kg/12 h) or valganciclovir (900 mg/12 h) for 2–4 weeks (BII). In some situations, CMV-specific gammaglobulin may be considered (BII). Exclude hypogammaglobulinemia in patients with relapsing CMV disease (BIII).
Lung	PPX: i.v. ganciclovir (5 mg/kg/24 h) until tolerated orally and then valganciclovir (900 mg/d) for 6–12 months (AII). Anti-CMV gammaglobulin in association with i.v. ganciclovir may benefit high-risk patients (BII). At the end of PPX, monitor patients and start PT with valganciclovir (900 mg/12 h) or i.v. ganciclovir (5 mg/kg/12 h IV) (AII).	PPX: i.v. ganciclovir (5 mg/kg/12 h) until tolerated orally and then valganciclovir (900 mg/d) until month six (AII). Anti-CMV gammaglobulin in association with i.v. ganciclovir may benefit high-risk patients (BII). In the case of treatment with anti-lymphocyte antibodies or steroids at doses above 10 mg/kg/day, treatment with valganciclovir must be restarted at doses of 900 mg/day for a maximum of 3 months (BIII).
Pancreas and pancreas-kidney	PPX: valganciclovir (900 mg/12 h) for 3 months (C–II). In the presence of other associated risk factors (co-infections, anti-rejection treatment, co-morbidity) consider increasing prophylaxis to 6 months (CIII). Then PT with valganciclovir (900 mg/12 h) or ganciclovir (5 mg/kg/12 h) (CIII).	In R+ patients who have received anti-lymphocyte antibodies or high doses of steroids as rejection treatment, pre-emptive therapy is recommended (CIII) with valganciclovir (900 mg/12 h) or ganciclovir (5 mg/kg/12 h). In D–/R– patients, leukocyte-depleted blood products and products from seronegative donors can be used. Treatment in case of primary infection. In patients who have received anti-lymphocyte antibodies for more than 3 days in the induction phase, treatment with valganciclovir (900 mg/day) for 3 months is recommended (CIII). In patients with acute rejection and receiving anti-lymphocyte antibodies or steroids at high doses, valganciclovir (900 mg/day) for 1–3 months is recommended (CIII).
Intestine	PPX: i.v. ganciclovir (5 mg/kg/12 h) or valganciclovir (900 mg/d) for a minimum of 6 months; treatment may be prolonged until lymphocyte counts = CD4+ > 200 cells/ml (CIII). Anti-gammaglobulin may be considered (150 mg/kg) in weeks 0, 2, 4, 6 and 8, followed by 100 mg/kg in weeks 12 and 16 (CIII).	In R+ patients, PPX with i.v. ganciclovir (5 mg/kg/day) or valganciclovir (900 mg/day) for between 3 and 6 months post-transplant (CIII). In patients receiving anti-lymphocyte antibodies or presenting cortico-resistant rejection, start prophylaxis with i.v. ganciclovir or valganciclovir for between 1 and 3 months (CIII).

Recommended doses of antiviral agents for normal renal function (creatinine clearance >70 ml/min) and neutrophil counts >1000/μl.

D+/R–: positive donor/negative recipient; R+: positive recipient; PPX: universal prophylaxis; PT: pre-emptive treatment; i.v.: intravenous.

kidney transplant recipients receiving anti-lymphocyte antibodies, reported the protective effect of ganciclovir.<sup>131,132</sup>

### Recommendations

In patients receiving anti-lymphocyte or monoclonal antibodies such as OKT3 or alemtuzumab, prophylaxis must be used with antiviral drugs (AI). No conclusive data are available on the duration, optimum dose and drug that must be used, and may vary according to the characteristics of individual patients (e.g. CMV

serostatus) and type of transplanted organ. In this guideline, both intravenous and valganciclovir and valganciclovir were used.

This strategy may also be considered to treat rejection with high doses of steroids (CIII).

### Liver transplant

#### Universal prophylaxis

In liver transplant patients, universal prophylaxis with different drugs is effective for preventing CMV disease. In a randomized

double-blind trial with 55 liver transplant recipients, prophylaxis with oral acyclovir (800 mg/6 h) for 28 days compared with placebo reduced the frequency of CMV disease (25% vs. 52%,  $P = .05$ ).<sup>133</sup> In another randomized trial that included 304 liver transplant recipients, 46 of whom were high-risk patients (15%), prophylaxis with oral ganciclovir (1 g/8 h) for 90 days compared with placebo reduced the frequency of CMV disease during the first 6 months post-transplant (5% vs. 19%) and also in high-risk recipients (15% vs. 44%).<sup>134</sup> In another randomized trial with 64 high-risk liver transplant recipients (D+/R-), prophylaxis with oral ganciclovir (1 g/8 h) was as effective as intravenous ganciclovir (6 mg/kg/day, 5 days per week until day +100), and the frequency of CMV disease was 9% and 12.5%, respectively.<sup>135</sup> In 219 CMV-seropositive liver transplant recipients, oral ganciclovir (1 g/8 h) was more effective than acyclovir (800 mg/6 h) in reducing CMV disease (1% vs. 7%)<sup>126</sup> during the first 100 days post-transplant.

The incidence of CMV disease in high-risk liver transplant patients (D+/R) receiving prophylaxis with valganciclovir for 100 days was 9%.<sup>136</sup>

Finally, the efficacy and safety of valganciclovir (900 mg/day  $\times$  100 days) were compared with oral ganciclovir (1 g/8 h  $\times$  100 days) in a randomized double-blind study in 364 high-risk SOT recipients (D+/R-), of whom 177 were liver transplant recipients, and who represented the largest group of patients in the study (48%). In liver transplant recipients, the results were unfavorable for valganciclovir due to the greater frequency of CMV disease (19% vs. 12% in the first 6 months) and neutropenia (8% vs. 3%).<sup>121</sup> Similar results were described in a retrospective study comparing valganciclovir (900 mg/day) with oral ganciclovir (1 g/8 h) and intravenous valganciclovir (6 mg/kg/day) in 66 high-risk liver transplant recipients (D+/R-). CMV disease was four times more frequent in the group receiving valganciclovir (22%) than in the one receiving oral ganciclovir (6%) or intravenous ganciclovir (4.5%).<sup>137</sup> A recent meta-analysis evaluated the efficacy of valganciclovir for preventing CMV disease in solid-organ transplant recipients and concluded that it is no more effective than standard treatment, accompanied by a greater risk of neutropenia, late disease and invasive CMV disease, which, coupled with its higher cost, has prevented this drug from being recommended as first-line medication for prophylaxis or pre-emptive treatment in these patients.<sup>138</sup>

Due to these results, guidelines on the use of valganciclovir in prophylaxis established in technical data sheets do not include liver transplant recipients, or adult or pediatric patients. However, valganciclovir has been used in these patients since oral ganciclovir was withdrawn from the market.

#### Pre-emptive treatment

Different studies have shown that pre-emptive treatment effectively prevents CMV disease in liver transplant recipients. One meta-analysis consisting of 10 randomized trials (including four liver transplant trials) with 476 patients showed that pre-emptive treatment was effective compared with placebo or no prevention.<sup>128</sup> In another study carried out on 69 liver transplant recipients, pre-emptive treatment with oral ganciclovir (1 g/8 h for 8 weeks) monitored by PCR and compared with placebo reduced the overall frequency of CMV disease (12% vs. 3%,  $P < .05$ ) and in the subgroup of D+/R- recipients (36% vs. 0%,  $P < .05$ ).<sup>139</sup> Another trial in 72 liver transplant recipients compared the efficacy of pre-emptive treatment with oral ganciclovir (2 g/8 h  $\times$  2 weeks followed by 1 g/8 h  $\times$  4 weeks) vs. intravenous ganciclovir (5 mg/kg/12 h  $\times$  7 days) in 22 patients with positive antigenemia. The incidence of disease was similar in the group receiving oral ganciclovir vs. intravenous ganciclovir (0% vs. 11%,  $P = ns$ ) and zero in 50 patients with negative antigenemia.<sup>140</sup>

The efficacy of valganciclovir (900 mg/12 h) in pre-emptive treatment was evaluated in 36 high-risk liver transplant recipients, guided in the first phase by antigenemia (cut-off point  $\geq 1$  cell with characteristic immunofluorescence per  $2 \times 10^5$  leukocytes) and PCR in a second phase (DNA  $\geq 15$  copies/ml), and the frequency of CMV disease was null.<sup>141</sup> At the same center, a cohort of 216 liver transplant patients was studied to determine the frequency of CMV disease, re-plantation, bacterial infections, fungal infections, frequency of hepatitis C, re-transplant and short and long-term survival (6 months to 5 years), including patients who received pre-emptive treatment and those who did not (58 vs. 158), and no differences were reported.<sup>142</sup>

Viremia relapse is frequent after pre-emptive treatment and frequencies of between 8% and 40% have been described in high-risk patients.<sup>141,143</sup> This viremia is normally resolved after new treatment. Recently, BenMarzouk et al. studied 21 high-risk liver transplant patients (D+/R-) undergoing pre-emptive treatment and presenting episodes of viral replication. None of these patients displayed symptoms associated with CMV. All the patients also developed specific immunity to CMV.<sup>144</sup>

The duration of pre-emptive treatment has not been established. Most studies apply treatments for between 2 and 3 weeks. Diaz-Pedroche et al., in a study with high-risk SOT recipients, reported that pre-emptive treatment with valganciclovir (900 mg/12 h) reduced basal viral load by 78% and 98% after 7 and 14 days of treatment, respectively.<sup>143</sup> In seropositive recipients, shorter treatment periods may be effective. In one study of 58 CMV-seropositive liver transplant recipients, pre-emptive treatment was administered for 7 days, resulting in only one probable case of disease, and relapse of the infection in 20% of the patients.<sup>145</sup>

#### Consensus recommendations

In liver transplant patients, CMV disease prevention strategies must be designed according to serostatus.

1. In R+ recipients, prophylaxis and pre-emptive treatments are two recommendable strategies (AI). Most of the panel recommends pre-emptive treatment.
2. In high-risk recipients (D+/R-), both strategies are equally recommendable. Most of the panel recommends universal prophylaxis if rigorous compliance with the applicable virological monitoring protocol is not possible. If prophylaxis is selected, pre-emptive treatment is recommended for at least 3 months after prophylaxis (AII).
3. If prophylaxis is selected, and since oral ganciclovir has been withdrawn from the market, the best option available is valganciclovir at doses of 900 mg/24 h in adult patients with normal renal function and during the first 3 and 6 months post-transplant (AIII). The duration (i.e. 3 or 6 months) can be determined according to the degree of immunosuppression of the patient, including the use of anti-lymphocyte antibodies. It is recommended to start prophylaxis on day +10 post-transplant; if ganciclovir cannot be administered orally, it should be administered intravenously at doses of 5 mg/kg/day until the patient is able to tolerate oral medication (AI).
4. If pre-emptive treatment is chosen, valganciclovir is recommended at doses of 900 mg/12 h (in patients with normal renal function) for 2 weeks, checking for suppression of viremia (AII). During the first 4 months post-transplant, viremia should be monitored weekly in high-risk patients and every 2 weeks in other patients (AII).
5. The selection test for monitoring viremia is quantitative PCR; each center must establish the logistics required to perform the test and the viral load at which to start treatment. The alternative is pp65 antigenemia and each center must establish its own

cut-off point, although, as a guide, the level recommended by the panel is  $\geq 2$  cells  $\times 10^5$  leukocytes (BII).

## Kidney transplant

### Universal prophylaxis

Universal prophylaxis with acyclovir,<sup>146</sup> valaciclovir,<sup>127</sup> oral ganciclovir<sup>130</sup> or valganciclovir<sup>121</sup> in D+/R– kidney transplant patients can be used to effectively reduce the incidence of CMV disease, at least during the period it is administered (normally 100 days). All these antiviral drugs reduce the frequency of the disease to below 15% in this population. Valganciclovir is currently the most frequently used drug due to its better bioavailability and ease of administration.

In previous efficacy trials, more than 25% of D+/R– patients developed late CMV disease 12 months after the suspension of universal prophylaxis administered for 100 days.<sup>121</sup> Later, and in spite of the controversy surrounding its methodology,<sup>147,148</sup> the results of the IMPACT study have shown that prolonging anti-CMV prophylaxis for 200 days in D+/R– kidney transplant patients can reduce the incidence of late CMV disease by 16%.<sup>149</sup> This reduction corresponded mainly to a decrease in the incidence of viral syndrome (in this study, 83 of the 85 patients [96.7%] diagnosed with CMV disease corresponded to viral syndrome, the definition of which differs from that established in this consensus paper). Universal prophylaxis for 200 days was associated with a decrease in the incidence of opportunistic infection – but not rejection – in these patients.

In CMV-seropositive recipients there is much less evidence of the efficacy of universal prophylaxis, although some studies have reported significant reductions in CMV infection and disease with both oral ganciclovir and valganciclovir.<sup>130,150</sup> Moreover, it has been shown that in patients requiring the administration of anti-lymphocyte antibodies (ATG/ALG/OKT3), universal prophylaxis with both intravenous ganciclovir and valganciclovir significantly reduces the frequency of CMV disease in kidney transplant patients.<sup>132,143</sup>

### Pre-emptive treatment

It has been shown that antigenemia or PCR-monitored pre-emptive treatment with oral ganciclovir<sup>151</sup> or valganciclovir<sup>143,152</sup> is effective in kidney transplant recipients. However, it has been suggested that patients receiving pre-emptive treatment may be at greater risk of acute rejection 12 months after transplant.<sup>153</sup> This effect could be related to the higher degree of viremia observed in patients receiving pre-emptive treatment compared with those receiving universal prophylaxis.<sup>154</sup>

### Recommendations for kidney transplants

Different studies published in recent years have shown that both pre-emptive treatment and universal prophylaxis can reduce the incidence of CMV disease in kidney transplant recipients.<sup>130,150</sup> A number of meta-analyses have reported that universal prophylaxis can also reduce the incidence of rejection, opportunistic infection and death in this population.<sup>124</sup>

1. In D+/R– patients, universal prophylaxis is recommended with valganciclovir (900 mg/day) (AI) for a maximum of 6 months post-transplant (AI). Alternatively, intravenous ganciclovir (5 mg/kg/day) (AI) or oral valganciclovir (2 g/6 h) (AI) can be used, especially in patients with severe leukopenia, for 3 months (AI). In cases of oral intolerance, ganciclovir can be administered intravenously at doses of 5 mg/kg/day until the treatment is completed (AI). Pre-emptive therapy may be an alternative to

universal prophylaxis in centers with adequate infrastructure to guarantee the monitoring of patients. In recipients treated with anti-lymphocyte antibodies, valganciclovir is recommended for 3 months (BI) or intravenous ganciclovir (5 mg/kg/day) for at least 14 days (BI).

2. In CMV-seropositive recipients, pre-emptive treatment is recommended with valganciclovir (900 mg/12 h) or intravenous ganciclovir (5 mg/kg/12 h), in case of oral intolerance, for 14–21 days or until antigenemia or viral load of CMV are negative or undetectable (BII). Another alternative is universal prophylaxis with oral valganciclovir (450–900 mg/day), valaciclovir (2 g/6 h) or intravenous ganciclovir (5 mg/kg/day) (if it cannot be administered orally) for a maximum of 3 months (AII).

## Lung transplants

### Prophylaxis

In lung transplant patients, it is difficult to make recommendations because most studies are cohort studies and very few controlled randomized studies have been carried out in these patients. In general, these studies have shown that prophylaxis, either with intravenous or oral ganciclovir, or with valganciclovir, delays the onset of CMV disease. However, late CMV disease has been reported even after prophylaxis maintained during the first 6 months post-transplant.<sup>155–158</sup>

In lung transplants, the only studies that have reported a decrease in the incidence of CMV disease to levels close to the annual level of 5% based their strategies on universal prophylaxis for 6 months followed by pre-emptive treatment for 1 year. Zamora et al.<sup>159</sup> determined the safety and efficacy of valganciclovir in 90 lung transplant recipients who survived for more than 30 days and who also received anti-CMV gammaglobulin. In these patients, prophylaxis was used in combination with valganciclovir (450 mg/12 h) for 180, 270 or 365 days, followed by pre-emptive treatment. The results of this group were compared with a historical cohort of 140 patients who received high doses of acyclovir after intravenous ganciclovir plus specific gammaglobulin. CMV disease was significantly lower in the group that received valganciclovir (2.2% compared with 20%;  $P < .01$ ), and no differences were observed when the duration of prophylaxis was increased to more than 180 days, although in 32% of patients the drug had to be withdrawn due to the appearance of leukopenia.

Later, a multicenter study carried out in Spain<sup>160</sup> included 66 lung transplant recipients who received universal prophylaxis with valganciclovir (900 mg/day for 120 days) followed by pre-emptive treatment in patients displaying significant infection. These patients were compared with a historical group of patients who received prophylaxis with oral ganciclovir using the same strategy, where the incidence of CMV disease in the group receiving valganciclovir was 7.9% compared with 16% in the group receiving oral ganciclovir. However, the use of valganciclovir was associated with a greater risk of leukopenia and withdrawal due to adverse effects.

Jaksch et al.<sup>161</sup> compared a group of 15 high-risk patients (D+/R–) who received valganciclovir (900 mg/day) for 3 months, compared with another group receiving valganciclovir for 1 year. Results for the latter group showed a decrease in the incidence of viremia (75% vs. 33%), CMV disease (44% vs. 13%) and acute rejection (26% vs. 5%). However, and in spite of these results, the publication of the study by Zamora et al.<sup>159</sup> has prompted most guidelines to recommend prophylaxis for 6 months.

Valentine et al.<sup>98</sup> published a study that included 90 lung transplant recipients who received valganciclovir at 900 mg/day in an unspecified manner, and reported an incidence of CMV-induced

pneumonitis of 2% over an average treatment period of 4 years. However, these authors did not observe any decrease in the incidence of bronchiolitis obliterans after 5 years, which was 50% and similar to that reported in most published series.

Recently, Palmer et al.<sup>162</sup> performed a multicenter, randomized, double-blind and controlled trial with placebo to compare the efficacy of prolonged prophylaxis with valganciclovir (900 mg/day) for 12 months compared with 3 months. These authors reported a significant reduction in the incidence of CMV disease (4% vs. 32%;  $P < .001$ ), CMV infection (10% vs. 64%;  $P < .001$ ) and viral load upon diagnosis of CMV disease (3200 copies/ml vs. 110,000 copies/ml;  $P = .009$ ). The incidence of acute rejection, opportunistic infections, adverse effects and ganciclovir resistance mutations was similar in both groups. However, in spite of the randomized and double-blind nature of this study, these results must be interpreted with caution, since in the comparison group valganciclovir was administered for 3 months, while normal practice in most transplant groups is 6 months. More importantly, the group treated for 1 year was only monitored for a period of 30 days.

Although the role of hyperimmune gammaglobulin has been examined in different studies with contradictory results,<sup>163,164</sup> recently published studies seem to demonstrate that a combination of specific gammaglobulin with intravenous ganciclovir reduces the incidence of CMV disease. Valantine et al.<sup>165</sup> reported a lower incidence of CMV disease, acute rejection and chronic evolution after 12 and 24 months in patients who received a combination of these drugs compared with those who only received ganciclovir. Similarly, other studies with historical controls have also reported favorable results with combined treatment.<sup>166</sup>

#### Pre-emptive treatment

In lung transplant recipients, the efficacy and safety of pre-emptive treatment have not been studied and therefore, cannot be recommended (DII). Although various studies carried out in this population seem to demonstrate that this strategy could be valid if used sequentially with universal prophylaxis (BII),<sup>159,160</sup> the use of pre-emptive treatment entails a risk for the patient because the first infection diagnosed could be a serious infection, such as pneumonitis.<sup>160</sup>

#### Recommendations for lung transplant

1. Universal prophylaxis is the best strategy for preventing CMV disease in lung transplant recipients (AII). Intravenous ganciclovir is recommended in doses of 5 mg/kg/24 h until it can be tolerated orally, followed by valganciclovir at a dose of 900 mg/day for 6 months post-transplant (AII). In D+/R– recipients, it can be increased to 12 months in patients who are difficult to monitor (AII).
2. Evidence exists to suggest benefits in the use of specific gammaglobulin against CMV. This panel considers that this gammaglobulin can be used according to the characteristics of the patient (e.g. clinical evolution or immunosuppression status) (BII).
3. Antigenemia or viral load should be monitored by PCR every 2 weeks or otherwise during every medical examination in order to exclude breakthrough viremia which could imply a greater risk of later appearance of ganciclovir-resistant CMV. If a breakthrough viremia is diagnosed, the dose of valganciclovir must be increased to 900 mg/12 h and viral load must be monitored weekly to reduce the dose to 900 mg/day after negativization (AII). After prophylaxis has been completed, pre-emptive treatment should be started in the event of a significant infection with valganciclovir (900 mg/12 h) provided that there is no high-grade viremia, in which case treatment must be started with

intravenous ganciclovir (5 mg/kg/12 h IV) (AII). In D+/R– patients or in patients with higher levels of immunosuppression, pre-emptive treatment could also be considered provided that there is no evidence of viral replication. Patients must be monitored in each medical examination until the second year post-transplant, except D+/R– patients who must be monitored at least every 2 weeks until the ninth month post-transplant. If these patients cannot be monitored, prophylaxis may be extended until 1 year post-transplant.

4. Whenever immunosuppression has to be increased, viral load should be monitored by quantitative PCR. The frequency of monitoring must be determined according to the characteristics of the patient, and must be at least once per month (CIII).
5. In the case of treatment with anti-lymphocyte antibodies or with steroids at doses above 10 mg/kg/day, treatment with valganciclovir should be restarted at a dose of 900 mg/day for a maximum of 3 months (BIII).

### Intestine transplants

#### Universal prophylaxis

Intestine transplants are associated with a high risk of CMV infection, probably as a consequence of the abundance of lymphoid tissue and the high level of immunosuppression to which these patients are subjected. Unlike other types of transplants, little information is available on anti-CMV prophylaxis in intestine transplant recipients. No randomized studies on antiviral prophylaxis have been carried out in these patients. Different management protocols exist that all employ universal prophylaxis with both ganciclovir and valganciclovir for between 3 and 6 months. Specific gammaglobulin is also used by some centers, for various treatment durations.<sup>167,168</sup> Despite the lack of information in this respect, valganciclovir seems to be well tolerated orally by intestine transplant recipients.<sup>169</sup>

In a Spanish group administered alemtuzumab for induction therapy, CD4<sup>+</sup> T-lymphocyte counts were monitored; this monitoring strategy was used as a criterion for increasing anti-CMV prophylaxis to more than 6 months post-transplant in patients with lymphocyte counts below 100 cells/mm<sup>3</sup>.<sup>170</sup>

#### Pre-emptive treatment

This prophylaxis strategy has not been studied in depth in intestine transplant recipients. In this group of patients, due to their high risk and the high morbidity and mortality associated with CMV disease, pre-emptive therapy is currently not recommended (DIII).

#### Use of specific anti-cytomegalovirus gammaglobulin

In a recent meta-analysis that reviewed the efficacy of gammaglobulin for preventing CMV disease in solid-organ transplant recipients, studies with intestine transplant patients were not included.<sup>171</sup>

Specific anti-CMV gammaglobulin is widely used in many intestine transplant groups in the USA, at a dose of 150 mg/kg in weeks 0, 2, 4, 6 and 8, followed by 100 mg/kg in weeks 12 and 16 (C-III).

#### Recommendations for intestine transplant

1. In D+/R– recipients, the use of universal prophylaxis with intravenous ganciclovir (5 mg/kg/day) or valganciclovir (900 mg/day) is recommended for a minimum of 6 months (especially if alemtuzumab is used for induction) and may be prolonged until CD4<sup>+</sup> T-lymphocyte counts are higher than 100 cells/mm<sup>3</sup>. The committee of experts considers that the use of specific anti-CMV

gammaglobulin (150 mg/kg in weeks 0, 2, 4, 6 and 8 followed by 100 mg/kg in weeks 12 and 16) may be considered, taking into account the characteristics of the patient and his/her clinical evolution or immunosuppression status (CIII).

2. In seropositive recipients, universal prophylaxis is recommended with intravenous ganciclovir (5 mg/kg/day) or valganciclovir (900 mg/day) for a duration of between 3 and 6 months post-transplant (CIII).
3. In patients receiving induction treatments or anti-rejection treatment with anti-lymphocyte antibodies, or who display corticosteroid-resistant rejection, prophylaxis should be started with intravenous ganciclovir or valganciclovir for between 1 and 3 months (CIII).

## Pancreas-kidney transplant

### Prophylaxis

From a practical standpoint, most pancreas transplant recipients will be treated as high-risk patients due to the high percentage of treatments that employ induction therapy with anti-lymphocyte antibodies in these patients. In pancreas-kidney transplants, prophylaxis with ganciclovir has been shown to reduce the incidence of CMV infection by 50%, as well as the rate of acute rejection by 20%, compared with non-therapy or acyclovir.<sup>172</sup> Although the required duration of prophylaxis has not been established, the results of different studies propose durations of between 3 and 6 months. Becker et al. showed that prophylaxis for 3 months with acyclovir or oral ganciclovir was followed by CMV disease in 13.4% of cases, and was significantly associated with acute rejection and the existence of co-infections.<sup>173</sup> Moreover, 6-month prophylaxis has been shown to be more cost-effective than 3-month prophylaxis in a cohort of kidney and pancreas-liver transplant recipients, reducing the incidence of CMV disease from 24.4% to 12%.<sup>174</sup> In this sense, López-Medrano et al. have reported an incidence of CMV disease of 33% in seronegative recipients who received 3-month prophylaxis with ganciclovir.<sup>175</sup>

### Pre-emptive treatment

In one study in which only pre-emptive treatment was used, the incidence of CMV infection was 75%<sup>176</sup> with a tendency for a shorter survival of pancreas transplants. In another retrospective study, universal 3-month prophylaxis was shown to be more effective than pre-emptive therapy for reducing the rate of CMV disease (6.9% versus 23%,  $P < .05$ ).<sup>175</sup>

### Recommendations for pancreas-kidney transplants

1. In D+/R– patients, valganciclovir is recommended (900 mg/day) for 3 months (CII). If other associated risk factors (co-infection, anti-rejection treatment, comorbidity) are observed in these patients, the duration of prophylaxis may be increased to 6 months (CIII).
2. In patients who have received anti-lymphocyte antibodies for more than 3 days in the induction phase, the use of valganciclovir is recommended at 900 mg/day for 3 months (CIII).
3. In patients with acute rejection and receiving anti-lymphocyte antibodies or steroids at high doses, the use of valganciclovir is recommended at 900 mg/day for 1–3 months (CIII).
4. In patients who have received universal prophylaxis, the presence of CMV infection must be monitored by antigenemia or PCR on a weekly basis for the following 3 months, and monthly until 1 year post-transplant. In the case of active replication, valganciclovir is recommended at 900 mg/12 h or ganciclovir at 5 mg/kg/12 h for at least 2 weeks and until one or two negative

antigenemia or PCR results are obtained once or twice a week (CIII).

5. In R+ patients not receiving anti-lymphocyte antibody treatment or high doses of steroids to treat rejection, pre-emptive therapy is recommended (CIII).

## Heart transplants

### Universal prophylaxis

Few studies have compared different prophylaxis strategies in heart transplant recipients. The use of intravenous ganciclovir has been compared with placebo in D+/R– patients, showing the benefits of prophylaxis administered for 6 weeks and also for 14 days after each rejection episode.<sup>177</sup> However, when prophylaxis was received by D+/R– patients for 4 weeks, outcomes were not superior to placebo.<sup>178</sup>

In addition to intravenous strategies, prophylaxis may be effective if administered orally in high-risk patients (D+/R–). In this sense, good outcomes have been reported with valganciclovir (900 mg/day) compared with oral ganciclovir (3 g/day) during the first 100 days post-transplant.<sup>121</sup> This study included 56 heart transplant recipients and the proportion of patients with CMV disease was 6% vs. 10%. These good outcomes have also been reported by other authors, with some groups maintaining prophylaxis with valganciclovir (450–900 mg/day) for 6 months.<sup>179,180</sup> However, the benefits of universal prophylaxis have been undermined by the appearance of late CMV disease in both D+/R– and D+/R+ patients<sup>181,182</sup> without any clinical risk factors having been identified for the appearance of this complication.

Universal prophylaxis is also recommended in patients receiving anti-lymphocyte antibodies. Aguado et al. carried out a prospective randomized open study with 31 seropositive patients who received induction with OKT3 for 14 days.<sup>183</sup> Administration of full doses of intravenous ganciclovir for 14 days was more effective than anti-CMV gammaglobulin in the prevention of disease, although no differences were observed in the incidence of infection. Similarly, in a retrospective study which included 115 patients treated with OKT3 and receiving oral acyclovir and anti-CMV gammaglobulin, 14 days of treatment with intravenous ganciclovir reduced the incidence of CMV disease in D+/R– patients, albeit without statistical significance.<sup>184</sup>

Two (2) retrospective studies have been carried out with heart-lung transplant recipients, reporting good outcomes by combining the administration of anti-CMV gammaglobulin and intravenous ganciclovir for the first 21–28 days post-transplant,<sup>165,185</sup> with a significant reduction in the disease, associated death and vascular allograft disease.

In R+ patients, some transplant groups recommend universal prophylaxis with intravenous ganciclovir for 4 weeks (5 mg/kg/12 h for 2 weeks followed by 6 mg/kg/day for another 2 weeks) based on the results of a prospective study.<sup>178</sup> However, in another prospective study universal prophylaxis (5 mg/kg 3 times per week for 6 weeks) did not reduce the incidence of CMV disease in such patients. Finally, a recent study in seropositive patients compared retrospectively<sup>177</sup> the use of high doses of ganciclovir (10 mg/kg/day for 2 weeks, followed by 5 mg/kg/day for another 2 weeks) with low doses (5 mg/kg/day for 4 weeks), without recording significant differences in the incidence of infection, disease and acute rejection.<sup>186</sup>

### Pre-emptive treatment

In CMV-seropositive patients who do not receive anti-lymphocyte antibodies, preventive measures are normally based

on pre-emptive treatment, which some authors have even successfully applied in high-risk patients.<sup>187</sup> A recently published study included 37 heart transplant recipients with positive antigenemia who received intravenous ganciclovir or anti-CMV gammaglobulin without developing the disease.<sup>188</sup> Other authors have also published favorable outcomes when administering intravenous ganciclovir in such patients.<sup>189–191</sup>

Favorable outcomes have also been reported with valganciclovir in pre-emptive treatment, although the studies published have only included small numbers of heart transplant recipients.<sup>152,192–194</sup>

### Recommendations for heart transplants

1. D+/R– patients must receive universal prophylaxis (AI). For this purpose, intravenous ganciclovir (5 mg/kg/day) or valganciclovir (900 mg/day) may be administered for 3–6 months (AI). Pre-emptive therapy may be an alternative to universal prophylaxis in centers with an adequate infrastructure to guarantee patient monitoring.
2. Patients treated with anti-lymphocyte antibodies (excluding basiliximab) or showing steroid-resistant rejection must receive ganciclovir (5 mg/kg/day) for at least 14 days (BI) or valganciclovir (900 mg/day) for 3 months (CIII).
3. There is no ideal preventive treatment for R+ patients. Quantitative sequential monitoring of CMV is recommended in non-prophylaxis patients. If monitoring is positive, intravenous ganciclovir (5 mg/kg/12 h) or valganciclovir (900 mg/12 h) should be administered for 2–4 weeks (BII).
4. In patients with CMV disease relapse, the absence of hypogammaglobulinemia must be confirmed (BIII).
5. In some situations (e.g. clinical evolution or immunosuppression) the use of specific anti-CMV gammaglobulin may be considered (BII).

### Treatment of CMV disease

Ganciclovir has been the standard recommended treatment for CMV disease in SOT recipients for more than 15 years. More recently, valganciclovir has been shown to have an efficacy similar to intravenous ganciclovir.<sup>195</sup> Valganciclovir is a esterified derivative of ganciclovir that is rapidly hydrolyzed after absorption in the intestine, forming ganciclovir as an active metabolite, with a bioavailability of 60% after oral administration.<sup>196,197</sup> Recently, the VICTOR study, an international, multicenter, randomized trial, showed that valganciclovir was as effective and safe as intravenous ganciclovir in the treatment of CMV disease in a population of 321 SOT recipients.<sup>198</sup> Although this study has some limitations (most of the patients included in the study were kidney transplant recipients and it did not include patients with serious CMV disease or pediatric patients), its results support the recommendation of valganciclovir for the treatment of CMV disease, at least in selected patients, since during long-term monitoring no significant differences were observed in clinical recurrence and virological recurrence between the valganciclovir group and the intravenous ganciclovir group.<sup>199</sup>

Oral ganciclovir cannot be used to treat patients with CMV disease because it is no longer commercially available. Other antiviral drugs that can be taken orally, such as acyclovir or valaciclovir, must also not be used to treat CMV disease.<sup>200</sup>

In mild or moderate CMV disease, the recommended drug for first-line treatment is valganciclovir (oral doses of 900 mg every 12 h) or intravenous ganciclovir (doses of 5 mg/kg every 12 h).<sup>198</sup> Intravenous ganciclovir must be used in patients with severe or potentially fatal CMV disease and when valganciclovir is poorly

tolerated or inadequately absorbed since current information on the efficacy of oral treatment in such patients is limited.<sup>198</sup>

Another potential therapeutic strategy is sequential therapy, i.e. treatment with intravenous ganciclovir followed by valganciclovir once the patients condition starts to improve. In a Spanish pilot study, this strategy provided effective therapy with adequate exposure to drugs, reducing treatment costs and avoiding prolonged hospitalization, thus resulting in greater comfort for the patients.<sup>201</sup>

Adequate doses of both valganciclovir and ganciclovir must be administered as doses below therapeutic levels may lead to therapeutic failure and promote the development of resistance,<sup>202</sup> while doses above the therapeutic levels may lead to the appearance of toxic effects.<sup>16,203</sup> During treatment, renal function must be monitored with frequent tests of the glomerular filtration rate by either direct quantification or estimation.<sup>204</sup> Doses and dose intervals must be adjusted according to creatinine clearance values, as shown in Table 6.

Reducing ganciclovir or valganciclovir doses based on secondary effects, such as leukopenia must be avoided whenever possible. Before reducing these doses, consideration must be given to reducing the doses of other drugs such as mycophenolic acid derivatives (mycophenolate mofetil and sodium mycophenolate), mTOR inhibitors (sirolimus and everolimus), azathioprine and trimethoprim/sulfamethoxazole. In severe leukopenia, particularly when absolute neutrophil counts are lower than  $1000 \mu\text{L}^{-1}$ , the use of a granulocyte colony-stimulating factor (G-CSF) may be considered.<sup>200</sup>

The optimum duration of treatment of CMV disease must be determined on an individual basis and guided by clinical monitoring and virological monitoring. Antigenemia or quantitative PCR must be determined weekly in order to monitor response and the development of any resistance to ganciclovir.<sup>198,205</sup> Treatment must be maintained until a negative viral load or antigenemia results are obtained. Nevertheless, in high-risk patients it would be recommendable to obtain two consecutive negative results at 1-week intervals to ensure elimination of the virus. In any case, the minimum duration of treatment must not be less than 2 weeks.<sup>198,199,205</sup> This therapeutic strategy minimizes the risks of developing resistance and recurrence of CMV disease.<sup>199,206,207</sup>

Sometimes, certain forms of invasive tissue disease are not accompanied by detectable viremia. These are so-called forms of “compartmented disease” in which serial PCR as a tool for guiding treatment is of limited use.<sup>208</sup> This is especially evident in forms of local reactivation in intestinal lymphoid tissue or in different forms of central nervous system disease.

In some nervous system diseases, secondary prophylaxis is used with valganciclovir at doses of 900 mg/day after treatment has been completed, for between one and three months.<sup>199,205</sup> according to the presence of CMV infection recurrence risk factors (primary CMV infection, high basal viral load, persistence of viremia at the beginning of secondary prophylaxis, multiorgan disease, high-risk organs and increases in immunosuppression due to rejection<sup>63,199,207</sup>). During secondary prophylaxis, viral load must also be monitored at unspecified intervals, although tests must clearly be performed more frequently in patients with a higher risk of relapse.

In cases of severe and compartmented disease, longer treatment periods are recommended with clinical monitoring focusing on the detection of specific expressions of the disease. In patients suffering from a recurrence of CMV disease, secondary prophylaxis must be prolonged.<sup>200</sup>

### Consensus recommendations

1. In mild or moderate CMV disease, the recommended first-line treatment drugs are valganciclovir (900 mg/12 h) or

- intravenous ganciclovir (5 mg/kg every 12 h) (AI). In clinical situations that require the patient to be hospitalized, intravenous ganciclovir is recommended (AII).
2. In patients with severe, potentially fatal disease, and when valganciclovir is poorly tolerated or cannot be adequately absorbed, intravenous ganciclovir must be used (AII).
  3. An alternative therapeutic strategy is sequential therapy, i.e. starting treatment with intravenous ganciclovir and then changing to valganciclovir once the patient starts to improve (BII). This strategy may be considered in patients displaying clinical improvement, oral tolerance and a decrease in viral load or antigenemia in control tests.
  4. Oral ganciclovir, acyclovir or valaciclovir must not be used to treat CMV disease (DIII).
  5. During treatment, renal function must be monitored and the glomerular filtration rate must be determined frequently through direct quantification or calculated. Doses and dose intervals must be adjusted according to creatinine clearance values (BII).
  6. Reducing doses of ganciclovir or valganciclovir, based on secondary effects such as leukopenia, must be avoided whenever possible. Consideration must be given at first to the possibility of reducing doses of other myelotoxic drugs such as, mycophenolic acid derivatives, mTOR inhibitors, azathioprine and trimethoprim/sulfamethoxazole (BIII).
  7. In severe leukopenia (neutrophil counts < 1000/ $\mu$ L), the use of granulocyte colony-stimulating factor (G-CSF) may be considered (BIII).
  8. The optimum duration of treatment must be determined on an individual basis and guided by clinical monitoring and virological monitoring. Antigenemia or quantitative PCR must be determined on a weekly basis in order to monitor treatment response and the development of any resistance (AII).
  9. Treatment must be maintained until the detected virus has been eliminated, confirmed by negative antigenemia or quantitative PCR results for CMV. However, in high-risk patients it is recommended that two consecutive negative results at one-week intervals should be confirmed to ensure elimination of the virus. In any case, the minimum duration of treatment must never be less than two weeks in viral syndrome cases or less than 4 weeks in patients with organ disease (BIII).
  10. In some cases (primary CMV infection, high basal viral load, persistence of viremia at the start of secondary prophylaxis, multiorgan disease, high-risk organ transplant and increases in immunosuppression due to rejection), secondary prophylaxis with valganciclovir at 900 mg/day may be considered, although the risk/benefit ratio of this treatment has not been entirely clarified (CII). If used, the virus must be monitored in blood, at unspecified intervals, in order to detect any resistance (BIII). In this sense, treatment with valganciclovir may be preferable (BIII).
  11. The duration of secondary prophylaxis will be one to three months, according to the existence of risk factors for the recurrence of CMV infection (BIII).
  12. In forms of "compartmentalized disease", which compromise organs without detectable viremia, the response to treatment will be guided by clinical response and histological response (BIII).
  13. In patients with severe disease and compartmentalized disease, longer treatment periods are recommended (BIII).
  14. In cases of recurrence of CMV disease, secondary prophylaxis, after re-treatment, must be prolonged (BIII).
  15. For the treatment of patients receiving combined transplants (e.g. pancreas-kidney), the instructions for the treatment of higher-risk transplants must be followed (CIII).

16. In deferred transplant patients (e.g. kidney transplants in heart transplant recipients) the treatment instructions for the last transplant performed should be followed (CIII).

#### *Alternative treatments of ganciclovir-resistant cytomegalovirus*

There are currently no controlled clinical trials that indicate the best alternative treatment in the event of evidence of ganciclovir-resistant CMV. Therapeutic decisions must be based on the genotype analysis of the UL97 and UL54 genes, patient immune state and disease severity. Reducing immunosuppressive therapy may be effective in some patients, particularly in those not subject to prolonged exposure to antiviral agents. However, depending on the severity of CMV disease, empirical treatment may be necessary until the results of the genotype analysis are available. These alternative empirical treatments include increasing the dose of ganciclovir (to more than 10 mg/kg twice per day for normal renal function) in patients with mild disease and ganciclovir and foscarnet<sup>209</sup> or administering foscarnet separately to patients with severe CMV disease.

If genotype analysis reveals the presence of a mutation in the UL97 gene related to a high degree of resistance (M460V/I, A594V, H520Q, L595S, C603W), the most appropriate approach is to change to foscarnet. However, if this is related to a low degree of resistance, treatment can be continued with high doses of ganciclovir, provided that renal function is stable.

Patients exhibiting a mutation in the UL54 gene, generally associated with resistance to ganciclovir and cidofovir, should change to foscarnet since crossed resistance to this drug is rare.<sup>210</sup> The mutations responsible for resistance to foscarnet usually only appear in patients taking foscarnet.

#### *Consensus recommendations*

1. Mutations in the UL97 kinase gene and the *pol* UL54 polymerase gene may confer resistance to ganciclovir (BII).
2. Mutations in the UL54 polymerase gene may be isolated or occur in combination with mutations in the UL97 kinase gene and may confer resistance to cidofovir and/or foscarnet or crossed resistance to ganciclovir, foscarnet and cidofovir (BIII).
3. Risk factors for the development of resistance are: (a) seronegative recipients receiving organs from CMV-seropositive donors; (b) pancreas and lung transplant recipients; (c) high-level viral replication; (d) intense concomitant immunosuppressive treatment; and (e) prolonged exposure or suboptimum levels of ganciclovir (BII).
4. Resistance to antiviral medication must be suspected when viral load or clinical progression of CMV disease increases despite adequate exposure to treatment two weeks after persistence is detected (BIII).
5. When resistance to ganciclovir is demonstrated, immunosuppressive treatment should be reduced as much as possible (BIII).
6. In the presence of severe disease or resistance risk factors, empirical treatment must be started until the results of the genotype resistance study are obtained (BIII).
7. Alternative antiviral treatment, in the presence of severe disease and ganciclovir resistance risk factors, will consist of replacing ganciclovir with foscarnet (60 mg/kg every 8 h or 90 mg/kg every 12 h). As an alternative, foscarnet can be added to prior treatment with ganciclovir, either at standard or reduced doses (BIII). In patients exhibiting less severe disease and in the absence of risk factors, the dose of ganciclovir may be increased to above the standard dose, up to 10 mg/kg every 12 h, provided that renal function is maintained within normal limits (BIII).
8. If genotype tests reveal a high-resistance mutation in the UL97 kinase gene (M460V/I, A594V, L595S, C603W, H520Q),

ganciclovir must be replaced with foscarnet (60 mg/kg every 8 h or 90 mg/kg every 12 h) provided that this has not been done empirically beforehand (BIII). In the case of mutations that confer lower resistance, treatment can continue with ganciclovir at doses of up to 10 mg/kg every 12 h, provided that renal function is maintained within normal limits (BIII). The scope of the genetic study should be broadened in the case of mutations in the *pol* UL54 gene. If mutation in this gene is confirmed, treatment must be changed to foscarnet (BIII).

9. The use of cidofovir as an alternative to ganciclovir is not recommended unless the absence of mutations in the *pol* UL54 gene is confirmed and the disease is not severe from a clinical standpoint (BIII).

#### *Alternative therapies*

There is currently insufficient evidence regarding the role of alternative treatments for CMV disease. A series of drugs have been used as potentially effective agents for treating CMV. These include leflunomide<sup>211</sup> and artesunate,<sup>212</sup> which have been shown to have anti-CMV effects, probably associated with an alteration in the physiology of host cells that hinder viral replication. However, data justifying the use of these drugs are still scarce. Different studies with sirolimus<sup>213</sup> and everolimus have reported a lower incidence of CMV disease,<sup>74,76,214</sup> prompting proposals for the use of these drugs to treat patients with ganciclovir-resistant CMV infection. The recent anti-viral drug maribavir is a good alternative against ganciclovir-resistant strains. This drug has good oral bioavailability and does not show hematological, renal or hepatic toxicity. Since it inhibits UL97 (viral kinase), it may interfere in the phosphorylation of ganciclovir, and must therefore not be used in combination with this drug. However, it does not affect the activity of foscarnet or cidofovir and may be administered in combination with these drugs. A phase II trial performed on patients subjected to hematopoietic progenitor transplant demonstrated the efficacy of maribavir in anti-CMV prophylaxis. However, phase II trials carried out to confirm this effect were interrupted after it was determined that the results with maribavir were similar to those obtained with placebo.<sup>200</sup> As a result, at the time of publishing this consensus document its clinical development had been suspended. In spite of these results, maribavir has been used in isolated cases as rescue therapy in patients with multi-resistant CMV infection. Although the existence of crossed resistance between maribavir and currently available medication has not been reported,<sup>215</sup> resistance with this drug has been described elsewhere.<sup>216</sup>

In addition to these commercially available drugs, other antiviral medication is currently in research phase, such as CMX-001, an esterified derivative of cidofovir that shares a similar action mechanism to that of cidofovir, albeit with lower toxicity<sup>208</sup>; hence, it may be a therapeutic alternative in the future.

#### *Immunologically based therapies*

Passive immunotherapy, with administration of specific anti-CMV immunoglobulin,<sup>217</sup> has been used in an attempt to improve the immune status of the host against the virus. Similarly, the adoptive transfer of CMV-specific T-cells, either directly from the donor or after their activation and expansion *ex vivo*, has been evaluated in both the prevention of viral replication and in the treatment of CMV disease, with encouraging results.<sup>218,219</sup> Consequently, this therapy is currently being evaluated in clinical trials for both the prevention and treatment of resistant CMV infection.<sup>220,221–226</sup> The efficacy of the adoptive transfer of T-lymphocytes is not only influenced not only by the functionality of infused cells, but also by their state of differentiation, proliferative capacity and longevity. Although 'HPT' ('hematopoietic progenitor transplant') has shown

that lower differentiation of transferred cells is associated with greater protection against CMV, data available in this respect in patients undergoing SOT are still scarce.<sup>227,228</sup>

#### *Prophylactic vaccination*

In recent decades, various vaccines have been developed against CMV. These vaccines have been shown to be safe and immunogenic in phase I preclinical or clinical trial, although none have yet been evaluated in phase II clinical trials.<sup>21,229</sup> The attenuated live virus vaccine, the "Towne strain", has been shown to minimize the severity of CMV disease in CMV-seronegative patients receiving kidney transplants from CMV-seropositive donors, although it does not prevent primary infection.<sup>230,231</sup> Prototype vaccines that use replicating recombinant viruses or replicating vectors as immunogens generate B and T responses of variable magnitude,<sup>232–239</sup> although data are currently not available on their efficacy for preventing CMV infection. The recombinant subunit vaccine gB, administered with the adjuvant MF59, generates a neutralizing antibody response comparable to that observed after natural infection. It has been shown that this vaccine provides protection against primary infection in 50% of vaccinated subjects.<sup>235,236</sup> A bivalent DNA vaccine (VCL-CB01) has been developed recently containing two gB and pp65-encoding plasmids, respectively; its innocuity and immunogenicity have been demonstrated in a phase I clinical trial in both healthy CMV-seropositive subjects and CMV-seronegative patients.

#### *Consensus recommendations*

1. There is insufficient evidence on the role of adjuvant treatments such as leflunomide, artesunate, sirolimus and everolimus in the treatment of ganciclovir-resistant CMV infection (BIII).
2. Passive immunotherapy with anti-CMV immunoglobulin and the adoptive transfer of T cells could be effective alternatives for treating active infection or CMV disease in organs, although there is insufficient evidence of their role in SOT patients (BIII).
3. The usefulness of maribavir for the management of ganciclovir-resistant CMV is unknown (CIII).
4. The adoptive transfer of T cells in HPT seems to be an effective alternative for treating active CMV infection or disease in organs and refractory to antiviral treatment in HPT. There is no informed experience in SOT.
5. There are no anti-CMV vaccines licensed for clinical use. The priority objective of the use (prophylactic or therapeutic) of a vaccine against CMV in SOT is to minimize the risk of viremia and organ disease. Until new data become available, no recommendations can be made on the type and time of vaccination.

#### **Specific aspects of CMV infection in pediatric sot patients**

The importance of CMV infection has decreased in children receiving solid-organ transplants, mainly due to the availability of sensitive techniques for the diagnosis of this disease, the development of prevention strategies and the possibility of introducing effective antiviral treatments. However, in some types of transplants, such as lung transplants, CMV infection remains an important risk factor of mortality or re-transplant in D+/R– patients.<sup>240</sup> The specific peculiarities of CMV infection in children are explained below, although this panel recommends reading the whole document in order to obtain full information on each section.

#### *CMV disease risk factors*

As in the adult population, the main risk factor for the development of CMV disease post-transplant in children is the absence

of specific immunity against the virus in the pre-transplant period. This scenario is more likely in children than in adults, and may occur in up to 60–70% of cases. Furthermore, this risk is greater in the patients receiving organs from seropositive donors (D+/R). Other possible donor-recipient combinations, such as D–/R+ or D+/R+, are considered to be medium risk and, in the case of D–/R–, low risk.<sup>241</sup>

When interpreting the serostatus of pediatric donors and recipients aged under 18 months, it must be remembered that positive results may indicate the presence of maternal IgG-CMV transferred passively during pregnancy. Therefore, in order not to underestimate the risk for these patients, these shall be considered to be positive in the case of donors and negative in the case of recipients.

#### Diagnostic methods

CMV infection and disease in children are managed in a similar way to adults. Since the amount of blood obtained by venipuncture in children may be limited, the use of PCR techniques is normally preferred over antigenemia. However, a cut-off value has not yet been established for the start of antiviral treatment.<sup>242</sup>

#### Prevention of CMV disease in pediatric patients

No randomized studies have yet been carried out to compare the efficacy of prophylaxis over pre-emptive treatment in children.<sup>243</sup> Most transplant centers normally use universal prophylaxis strategies with ganciclovir at doses of 5 mg/kg/day, although some transplant programs start with ganciclovir doses of 5 mg/kg/12 h during the first two weeks. The use of prolonged 12-week prophylaxis treatments with ganciclovir has also been used safely in some patients.<sup>244</sup> Although some studies have shown that valganciclovir is an effective and safe drug, information on its use for prophylaxis in pediatric patients is very scarce.<sup>245,246</sup> The high variability of ganciclovir in plasma levels reported in children may justify the need to monitor these levels, especially in high-risk patients.<sup>247,248</sup>

The duration of prophylaxis has not been clearly defined, although this is normally between 3 and 6 months; prophylaxis tends to be applied for longer periods in high-risk patients, e.g. lung or intestine transplant recipients.<sup>244</sup>

The role of immunoglobulin used on its own or in combination with antiviral medication has not been defined. Different trials in children receiving liver<sup>249,250</sup> or lung<sup>251</sup> transplants have not reported any benefits in its use when compared with antiviral drugs.

#### Treatment of CMV disease in pediatric patients

Limited evidence is currently available to make firm recommendations on the treatment of CMV disease in children.<sup>200</sup> In these patients, the proposed treatment is intravenous ganciclovir at a dose of 5 mg/kg/12 h until negative PCR or antigenemia results are achieved, the duration of treatment being equivalent to that in adults in cases of both viral syndrome and organ disease. The strategy of initial treatment followed by secondary prophylaxis is recommended by some experts.<sup>252</sup> Since it has not been validated by studies with pediatric patients, no recommendations can be made for this population group regarding the use of valganciclovir to treat CMV disease. In patients with CMV-induced pneumonitis and enteritis, as well as in cases of hypogammaglobulinemia, the use of specific anti-CMV immunoglobulin is recommended.

The onset of ganciclovir-resistant CMV disease has been described anecdotally.<sup>253</sup> As in adults, different agents are available for treatment of this condition, including foscarnet and cidofovir,<sup>200</sup> although their use is limited due to their potential

nephrotoxicity. Other agents such as maribavir, leflunomide or artesunate are currently being researched.

#### Consensus recommendations

1. Due to the difficulty in characterizing the serostatus of donors and recipients aged under 18 months, it must be assumed that children in this age group present a greater risk. Thus, if serological tests for CMV in donors aged under 18 months are positive, these patients must be treated as seropositive, even if these antibodies originate from the mother. Similarly, seronegative recipients included in this age group will be treated as seronegative since anti-CMV antibodies may be of maternal origin (AIII).
2. Both PCR and pp65 antigenemia techniques have been shown to be effective for diagnosing and monitoring CMV infection in children (AII).
3. Prophylaxis is recommended rather than early treatment (AIII). Most experts recommend the use of intravenous ganciclovir (5 mg/kg/12 h) for between 2 and 4 weeks, followed by valganciclovir (dose =  $7 \times$  body surface area in  $m^2 \times$  creatinine clearance) until the end of 3–6 months' prophylaxis (AII). Creatinine clearance is recommended using the modified Schwartz formula [ $k \times$  height (cm)/serum creatinine (mg/dl)], where  $k$  is 0.33 for newborns, 0.45 for children aged between 4 months and 2 years, 0.55 for male patients aged between 2 and 13 and female patients aged between 2 and 16, and 0.7 for male patients aged between 13 and 16. The maximum creatinine clearance value applicable to this formula is 150 ml/min/1.73  $m^2$ ; hence, even if the clearance value exceeds this level, the aforementioned maximum value will be used to calculate the dose.
4. In the treatment of CMV disease, ganciclovir is recommended (5 mg/kg/12 h) until a negative PCR or pp65 antigenemia result is obtained on a weekly basis (AII). The total duration of treatment in both viral syndrome and organ disease will be the same as that established in cases of pneumonitis or enteritis, as well as in patients with hypogammaglobulinemia (BIII). The efficacy of valganciclovir has not been established in this population. Some experts consider that the treatment period can be completed by replacing intravenous ganciclovir with oral treatment in some older children and adolescents (BIII).

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