Posttransplant CMV infection and the role of immunosuppression

Immunosuppression permits graft survival after transplantation and consequently a longer and better life. On the other hand, it increases the risk of infection, for instance with cytomegalovirus (CMV). However, the various available immunosuppressive therapies differ in this regard. One of the first clinical trials using de novo everolimus after kidney transplantation [1] already revealed a considerably lower incidence of CMV infection in the everolimus arms than in the mycophenolate mofetil (MMF) arm. This result was repeatedly confirmed in later studies [2–4]. Everolimus is now considered a substance with antiviral properties. This article is based on the expert meeting "Posttransplant CMV infection and the role of immunosuppression". The expert panel called for a paradigm shift: In a CMV prevention strategy the targeted selection of the immunosuppressive therapy is also a key element. For patients with elevated risk of CMV, mTOR inhibitor-based immunosuppression is advantageous as it is associated with a significantly lower incidence of CMV events.

There are good reasons to examine the role of immunosuppression with regard to posttransplant CMV infection:

- Patients with CMV infection have a poorer prognosis: CMV infections increase the risk of graft loss and patient mortality [5–8], and they remain clinically relevant in the long-term [9, 10].
- Everolimus-based immunosuppression is associated with a significantly reduced incidence of CMV events after kidney transplantation [1–4, 11, 12]. Review articles comprehensively present the evidence and confirm the antiviral effect of mTOR inhibitors [13–15].
- Experimental research describes the mechanisms of action through which mTOR inhibition interferes with CMV infection and suppresses viral replication [16–19].

Prevalence of CMV and risk factors for CMV infection

The prevalence of CMV is estimated to be 30–40 % in the healthy population and 70–80 % in dialysis patients. Without CMV prophylaxis or preemptive therapy, 50–80 % of patients experience posttransplant reactivation of latent CMV infection [20, 21].

The main risk factor for CMV infection in immunosuppressed patients is the serostatus constellation: The constellation of a CMV seropositive donor (D+) and a CMV seronegative recipient (R−) leads to a high rate of primary CMV infection in kidney transplant recipients (68 % according to [5]), with a high risk for organ-invasive CMV disease [22]. If the recipient is CMV-positive as well (D+/R+), reactivation or infection with a new CMV strain still occurs frequently despite the recipient's CMV-specific immunity (in 33 % according to [5]). CMV infections are less common in the D−/R+ (13 %) and D−/R− groups (4 %) [5]. The ratios are also similar for liver [23] and heart transplantation [24, 25].

Patients with a serostatus constellation of D+/R+ may have a lower CMV infection rate than the D+/R− group, but in this group mortality is particularly high in case of active CMV infection [8].

Another risk factor for CMV infection after kidney transplantation is the use of T-cell-depleting antibodies, particularly for the prevention or treatment of rejections [5].

The type and intensity of immunosuppression also is a risk factor for CMV infection and disease. In organ transplant recipients there is a higher incidence of CMV events under mTOR inhibitor-free immunosuppression than under mTOR inhibitor-based immunosuppression: A meta-analysis showed a relative risk (RR) of 2.45 for CMV events when comparing a combination of mTOR inhibitor + calcineurin inhibitor (CNI) vs. mTOR inhibitor-free immunosuppression [13]. A center-based analysis also confirmed that the type of

EXPERT MEETING

"Posttransplant CMV infection and the role of immunosuppression" on August 18, 2015 in Frankfurt am Main, Germany

Prof. Dr. Duska Dragun, Berlin
Prof. Dr. Christine S. Falk, Hanover
Prof. Dr. Thorsten Feldkamp, Kiel
Prof. Dr. Ingeborg Hauser, Frankfurt
Priv.-Doz. Dr. Nils Heyne, Tübingen
Prof. Dr. Björn Nashan, Hamburg
Prof. Dr. Barbara Suwelack, Münster

▶ **Table 1** Stages of CMV infection; mod. acc. to [27].

	CMV IgG	CMV DNA, pp65 antigen	nonspecific symptoms	organ manifestation
latent CMV infection	X	–	–	–
active CMV infection	X	X	–	–
CMV syndrome	X	X	X	–
invasive CMV disease	X	X	X	X

immunosuppression is a risk factor for CMV in kidney transplant patients: the rate of CMV infection and disease was 12.5 % with mycophenolate and 3.3 % with everolimus (p = 0.013, odds ratio [OR] 4.8) [11].

Clinical symptoms, diagnostics, and therapy of CMV infection

After every CMV infection, a life-long latent infection with detectable CMV-specific immunoglobulin G (CMV-IgG) remains, which is characteristic for herpes viruses.

CLINICAL RELEVANCE OF CMV INFECTION

Prophylaxis and treatment of CMV infection are extremely important because the clinical effects of this infection are not limited to organ-invasive CMV disease. Indirect and secondary effects are also problematic.

CMV infection

- increases the risk of acute rejection, partly due to the reduction of immunosuppression which may be required and partly due to immunological secondary effects
- promotes the development of opportunistic infections, e. g. with Epstein-Barr virus (EBV), and thereby increases the incidence of posttransplant lymphoproliferative disorder (PTLD) [29]
- adversely affects long-term graft function [5] and
- increases the incidence of cardiovascular disease and mortality [5–8]

Hence, CMV presents a risk not only due to the CMV infection itself, but also through secondary effects such as acute rejections, opportunistic infections, cardiovascular disease, chronic graft loss, and increased mortality.

An active CMV infection is present if the virus starts to replicate again, which can be detected by CMV DNA in whole blood (PCR) or a pp65 antigen assay. Active CMV infection associated with fever, general weakness, typically normal to slightly elevated CRP, occasionally leukopenia and thrombocytopenia as well as CD4+ T-cells < 150/ml is defined as CMV syndrome. Among organ-invasive CMV diseases currently the most frequent form is CMV colitis (detected by mucosal PCR), whereas other manifestations have become rare (▶ **Table 1**) [26].

Treatment is (preemptively) initiated when active CMV infection is diagnosed, typically with ganciclovir (intravenous) or valganciclovir (oral). The guidelines [28] recommend continuing therapy until the CMV PCR is negative in two consecutive weekly assays. For some patients with severe CMV disease and/or drug-resistant CMV a reduction of immunosuppression and/or treatment with CMV hyperimmunoglobulin is required; however, this is a balancing act with regard to the prevention of rejections.

CMV prophylaxis and preemptive therapy

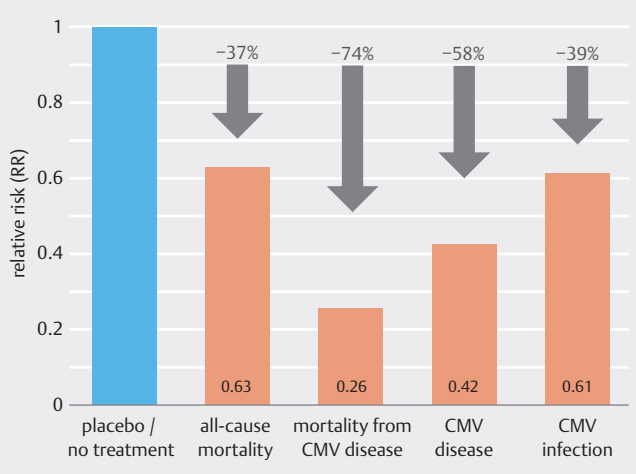
All measures preventing CMV infection have a positive effect on the long-term outcomes of transplant recipients. The specific selection of the immunosuppression is of relevance here as well as the prophylaxis. Currently the common CMV prophylaxis is:

- The high-risk group D+/R− typically receives valganciclovir in the first 200 days after transplantation.
- In the serostatus constellations (D+/R−) and (D−/R+), patients receive either prophylaxis in the first 100 days or preemptive therapy with weekly monitoring by CMV PCR and treatment in case of CMV replication.

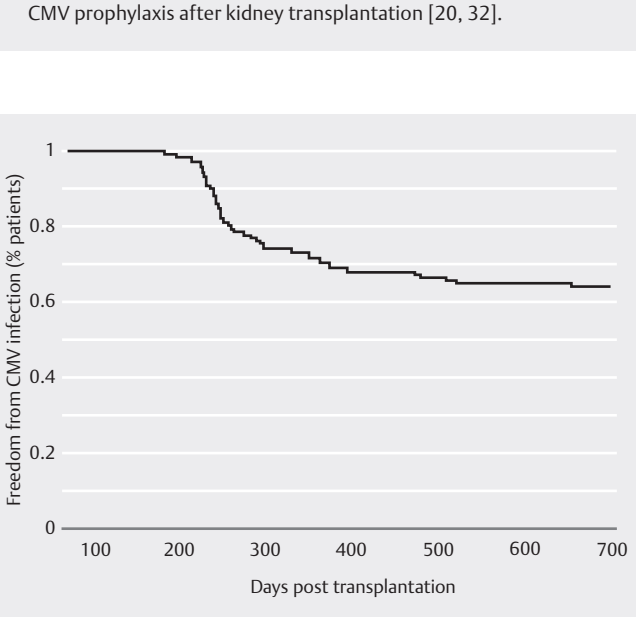
- In the low-risk group (D−/R−) generally neither prophylaxis nor preemptive therapy is used.

Prophylaxis in high-risk patients is very successful ([30–33], etc.). According to a meta-analysis [20, 32], it reduces the rate of CMV infections by 39 %, of CMV disease by 58 %, and of mortality from CMV disease by 74 % (▶ **Fig. 1**).

However, the use of prophylaxis is problematic and often associated with side effects (including leukopenia) and higher costs. Patients with impaired renal function require monitoring of renal function and appropriate dose adjustments. There might be a cumulative effect of its toxicity and the concurrent use of immunosuppressants. Preemptive therapy with regular monitoring can to some extent avoid this toxicity.



▶ **Fig. 1** This meta-analysis demonstrates the positive effect of CMV prophylaxis after kidney transplantation [20, 32].



▶ **Fig. 2** Late-onset CMV infection after six months of valganciclovir prophylaxis in the high-risk group (D+/R−) [9].

Late-onset CMV infection and CMV reinfection

Late-onset CMV infection is defined as CMV infection occuring after cessation of CMV prophylactic therapy. CMV reinfection is a de novo infection with a different strain than the one of the donor. Reactivation refers to a renewed activation of the persistent strain.

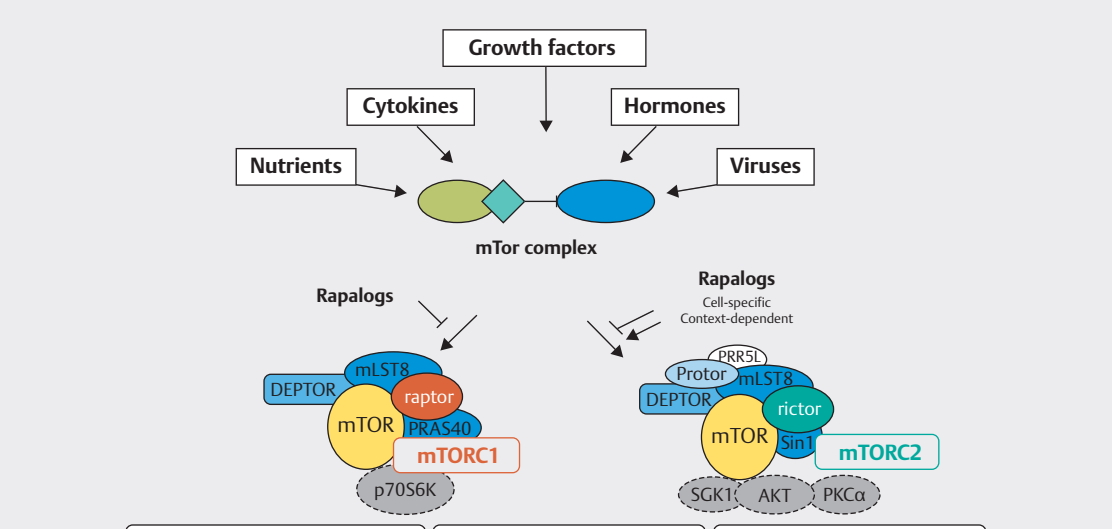
Following a 100-day or even 200-day CMV prophylaxis, a certain percentage of patients develops late-onset CMV infection. In a Finnish study patients received 180 days of valganciclovir prophylaxis after kidney transplantation. In 37 % of the patients in the high-risk group (D+/R−) CMV infection or disease occurred after median 244 days (150–655 days). Approximately 100 days after cessation of prophylaxis, the number of late-onset CMV infections dropped again [9] (▶ **Fig. 2**).

The main risk factor for late-onset infection is the serostatus constellation: In the first year after discontinuing prophylaxis, 30 % of the kidney transplant patients in the high risk group D+/R−, 7.7 % in the D+/R+ group, and 3.7 % in the D−/R+ group developed CMV infection [10]. Late-onset CMV infection is hence a problem predominantly in the first year after discontinuation of prophylaxis.

Renal function represents another risk factor for late-onset CMV infection; patients with an eGFR < 45 ml/min/1.73 m^2 were at twice the risk of late-onset CMV infection compared to patients with better renal function [10]. A long-term observational study also found chronic graft dysfunction to be a significant risk factor for later CMV reactivation. According to this analysis, patients with mTOR inhibitor-based immunosuppression developed fewer late-onset CMV infections (hazard ratio [HR] 0.3; 95 % confidence interval 0.1–1; p = 0.051 in multivariate analysis) [34].

CMV and the mTOR cascade

For replication and the expression of viral proteins, CMV requires the intrinsic mTOR-signaling pathway of the infected cell, which plays an important role in cell proliferation and survival. By blocking this signaling pathway, mTOR inhibitors slow viral replication [14, 16, 17]. Understanding this context requires a better comprehension of the mTOR cascade. While several



▶ **Fig. 3** mTOR inhibitors form a complex with FKBP12, which then binds to the kinase mTOR. The mTOR complexes 1 and 2 (mTORC1 and mTORC2) serve as central interfaces in cell metabolism; mod. acc. to D. Dragun.

years ago, the mTOR cascade was assumed to be relatively linear, it is now considered a complex system and a central interface of cellular metabolism. The mTOR complex (mTORC) is composed of mTORC1 and mTORC2. The input – growth factors, cytokines, hormones, and viruses such as CMV – is similar for both complexes, but within the cell, it passes either through the mTORC1 or the mTORC2 interface.

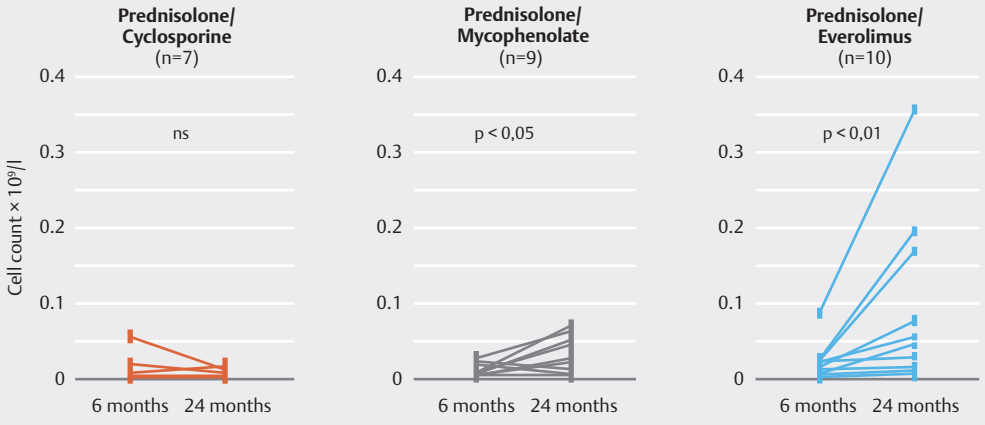
In terminally differentiated cells, such as cardiomyocytes, mTORC1 regulates cell growth or hypertrophy, while in non-terminally differentiated cells, such as lymphocytes, it regulates proliferation and survival signals. In addition, the mTOR cascade regulates cell survival through autophagy (nutrient release in case of cellular stress) and programmed cell death, so-called apoptosis, or other cell death signaling pathways (▶ **Fig. 3**). mTOR inhibitors block not only mTORC1, but depending on the context, cell, and duration of exposure, also mTORC2, which in turn affects the balance between cell survival and cell death.

Within the cell, mTORC2, which is also important for the cytoskeleton organization or cell microarchitecture, is located near the cell membrane. mTORC1, in contrast, is found closer to the nucleus and endoplasmic reticulum. The latter complex regulates the anabolic signal transduction or "growth management" of the cell, while mTORC2 tends to regulate "survival management".

Viruses such as CMV utilize the mTOR-signaling pathway, among others, for their own replication [19]. The viruses reprogram the cell by binding to mTORC2, which results in cytoskeletal reorganization and an anti-apoptotic effect. In addition, mTORC1 is prompted to trigger the translation and synthesis of viral proteins. mTOR inhibitors interfere with the translation of these viral proteins at mTORC1 and can also have a proapoptotic effect at mTORC2, which drives the cell into programmed cell death and interferes with viral persistence. The entire scenario depends not only on the metabolism, but also on the cell type [19]. As a result, various cell types respond differently to treatment with an mTOR inhibitor.

CMV-specific immunomodulation and the role of mTOR inhibitors

Dirks et al. [35] have shown that following antigen-specific stimulation, T-cells that carry the receptor PD-1 (programmed death 1) as well as the costimulatory receptor CD28 and ideally have lost CD27 form the T-cell compartment with a high percentage of CMV-specific T-cells. By monitoring this compartment under immunosuppression, their influence on the development of virus-specific T-cells can be assessed. Natural killer (NK) cells can be assessed in a similar manner. In case of nonspecific activation by PMA/ionomycin, they formed IFNγ and down-regulated



▶ **Fig. 4** CMV-specific CD8+ T-helper-cells under various immunosuppressive regimens in the long term [18].

their CD16 expression. If the activated NK cells were treated with mTOR inhibitors, the activation remained largely intact, while it was neutralized under calcineurin inhibitors [36].

The effect that mTOR inhibitors increase the quantity and quality of virus-specific CD8+ T-memory-cells was first shown in animal models [37]. Later the impact of immunosuppressants on the number of CMV-specific CD8+ T-cells was also demonstrated in clinical practice [18]. After 24 months, the prednisolone/everolimus group exhibited the most pronounced increase in CMV-specific CD8+ T-cells (▶ **Fig. 4**).

Clinical data: Lower rate of CMV infections under everolimus

An immunosuppressive drug which simultaneously can prevent CMV reactivation or new infection may offer patients a decisive advantage. Clinical trials with de novo everolimus have shown that everolimus-based immunosuppression is associated with fewer CMV events than MPA-based immunosuppression (▶ **Table 2**).

In the A2309 trial, the incidence of CMV infection after 24 months was significantly lower in the low-dose everolimus group than in the mycophenolate group, both in the overall population of the trial and in the various serostatus constellations [4, 40] (▶ **Fig. 5**).

In a recently published randomized, prospective, comparative study [12] with CMV as the primary endpoint, de novo everolimus treatment resulted in significantly lower CMV incidence than treatment with MPA (10.8 vs. 37.6 %; p < 0.001). Even the high-risk group of patients did not receive CMV prophylaxis but preemptive therapy.

> The available evidence demonstrates that everolimus significantly reduces the risk of CMV infection, particularly if it is started de novo after kidney transplantation.

Immunosuppression and CMV – expert consensus on recommendations for clinical practice

According to the expert panel, patients at high risk of CMV include not only those with the serostatus constellation D+/R− (very high risk) but also patients with the serostatus constellation D+/R+ (high risk) since the course of CMV infection is frequently severe. Based on the available evidence, which confirms the antiviral effect of everolimus and a significantly lower incidence of CMV infections under everolimus, the expert consensus recommends that these patients receive an everolimus-based immunosuppression. In the moderate-risk group (D−/R+), everolimus should be used preferentially. In patients at low risk of CMV (D−/R−), CMV infection is generally so rare that in terms of CMV prevention there is no preference for a specific immunosuppressant (▶ **Table 3**). In general, choice of immunosuppressive treatment must always take into account the various individual factors as well as the patient's overall situation.

The expert panel called for a paradigm shift: In the CMV prevention strategy, not only the type and dura-

▶ **Table 2** CMV infections in studies with de novo everolimus after kidney transplantation; mod. acc. to [14].

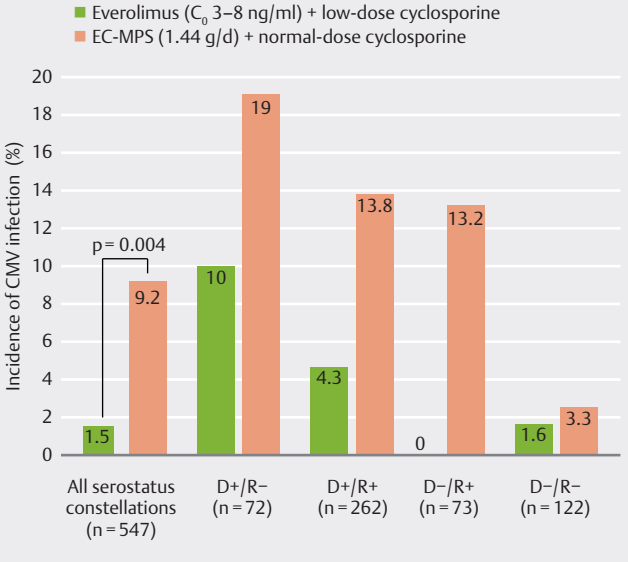
Study	n	Immunosuppression	CMV manifestation*
B156 [38]	111	• everolimus (3 mg/d) + low-dose cyclosporine • everolimus (3 mg/d) + normal-dose cyclosporine (all with steroids)	CMV infection after 36 months: 0 vs. 1.9 %
B201 [1]	588	• everolimus (1.5 mg/d) + low-dose cyclosporine • everolimus (3 mg/d) + low-dose cyclosporine • MMF (2 g/d) + normal-dose cyclosporine (all with steroids)	CMV infection after 36 months: 5.7 vs. 8.1 vs. 19.9 % Significantly lower rates of CMV infection under everolimus (p = 0.001 vs. MMF for both arms).
B251 [39]	583	• everolimus (1.5 mg/d) + low-dose cyclosporine • everolimus (3 mg/d) + low-dose cyclosporine • MMF (2 g/d) + normal-dose cyclosporine (all with steroids)	CMV infection after 36 months: 5.2 vs. 4.1 vs. 6.1 % No significant difference.
A2309 [3, 4]	833	• everolimus (C_0 3–8 ng/ml) + low-dose cyclosporine • everolimus (C_0 6–12 ng/ml) + low-dose cyclosporine • EC-MPS (1.44 g/d) + normal-dose cyclosporine (steroids according to center protocol)	CMV events after 12 months: CMV infection: 0.7 vs. 0.0 vs. 5.9 % CMV syndrome: 1.5 vs. 1.4 vs. 4.4 % CMV disease: 0.7 vs. 0.7 vs. 2.2 % CMV events after 24 months: CMV infection: 1.5 (everolimus C_0 3–8 ng/ml) vs. 9.2 % (EC-MPS) (p = 0.004) Significantly lower incidence of CMV events under everolimus C_0 3–8 ng/ml, including in patient groups with and without prophylaxis and in all (D/R) subgroups (▶ **Fig. 6**).
A2420 CALLISTO [2]	139	• everolimus de novo + low-dose cyclosporine • MPA (EC-MPS or MMF) de novo + everolimus after 4 weeks	CMV infection after 12 months: 1.5 vs. 6.8 % Significantly lower incidence of CMV infections with de novo use of everolimus.

* Results shown in the sequence of the trial treatment arms listed on the left (immunosuppression)
CMV = cytomegalovirus, EC-MPS = enteric-coated mycophenolate sodium, MMF = mycophenolate mofetil, C_0 = trough level, MPA = mycophenolic acid

tion of CMV prophylaxis should be considered, as is the case at present, but risks should also be reduced through the deliberate selection of an immunosuppressive agent with antiviral properties.

According to the expert consensus, CMV prevention strategy under everolimus-based or MPA-based immunosuppression thus may differ. Patients with D+/R− serostatus, who are at very high risk of CMV, should always receive prophylaxis for 100–200 days. For the serostatus constellations D+/R+ and D−/R+, up to 100-day-prophylaxis is also recommended if using MPA. Immunosuppression with everolimus permits a preemptive strategy in seropositive recipients (▶ **Table 4**).

This is supported by the positive clinical data for everolimus, which show a reduced CMV incidence for the various serostatus constellations. Based on these results, a decision in favor of a preemptive strategy under everolimus-based immunosuppression seems feasible and preferable for a large percentage of patients (including D+/R+ and D−/R+). The preemptive



▶ **Fig. 5** Incidence of CMV infection under everolimus or mycophenolate at 24 months, safety population (for 18 patients, no information was available on the serostatus constellation) [4, 40].

▶ **Table 3** Baseline immunosuppression by CMV risk constellation (acc. to expert panel recommendations).

D+/R− (very high risk)	D+/R+ (high risk)	D−/R+ (moderate risk)	D−/R− (low risk)
everolimus*	everolimus*	everolimus preferred*	no preference

* in combination with CNI

▶ **Table 4** CMV prophylaxis by baseline immunosuppression by risk constellation.

D+/R−		D+/R+	D−/R+	D−/R−
with everolimus	prophy-laxis	preemptive therapy alternatively: Prophylaxis	preemptive therapy	none
with MPA / MMF	prophy-laxis	prophylaxis alternatively: preemptive therapy	prophylaxis alternatively: preemptive therapy	none

strategy has the advantage of sparing the patients the side effects associated with prophylaxis, particularly bone marrow toxicity. For a preemptive strategy, close monitoring and adherence to the recommended (weekly) intervals for CMV DNA testing are essential.

Conclusion

CMV infections are a serious concern after organ transplantation and may lead to graft loss and increased mortality. De novo use of everolimus-based immunosuppression significantly reduces the risk of CMV events when compared to MPA-based immunosuppression. Everolimus has a positive effect on the cellular immune response to CMV. The pathophysiology, mechanism of action, and clinical data favor (de novo) immunosuppression with everolimus in patients with elevated risk of CMV.

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