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Title

A split strategy to prevent cytomegalovirus after kidney transplantation using prophylaxis in serological high risk patients and a pre-emptive strategy in intermediate risk patients: combining the best of two options?

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Authorship page

Authorship

Rachel Hellemans participated in the development of the split CMV protocol, designed and coordinated the study, analyzed the data and wrote the report.

Veerle Wijtvliet collected the data and participated in the analysis of the data and the writing of the report.

Veerle Matheeussen and Kristof Bergs collected the microbiological data and participated in the writing of the report.

Ester Philipse, Rowena Vleut, Annick Massart and Marie-Madeleine Couttenye contributed to the interpretation of the results and the writing of the report.

Daniel Abramowicz participated in the development of the split protocol and in the design and coordination of the study. He participated in the interpretation of the results and the writing of the report.

All authors reviewed and approved the manuscript before submission.

Disclosure

The authors declare no conflicts of interest

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Key words

Kidney transplantation – cytomegalovirus - valganciclovir – prophylaxis – pre-emptive - leucopenia

Abbreviations

ATG, antithymocyte globulin

Cdc-PRA, complement-dependent cytotoxicity-panel reactive antibodies

CIT, cold ischemia time

CMV, cytomegalovirus

CNI, calcineurin-inhibitor

CsA, cyclosporin A

DCD, donation after circulatory death

D/R, donor/recipient

DGF, delayed graft function

DSA, donor-specific antibodies

EDTA, ethylenediamine tetraacetic acid

EVL, everolimus

GCSF, granulocyte colony stimulating factor

HR, hazard ratio

MPA, mycophenolic acid

qPCR, quantitative polymerase chain reaction

rATG, rabbit antithymocyte globulin

Tac, tacrolimus

VGC, valganciclovir

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Abstract

Background

Cytomegalovirus (CMV) remains an important challenge after kidney transplantation. Current Transplantation Society International Consensus Guidelines recommend antiviral prophylaxis or pre-emptive therapy for high-risk CMV-seronegative recipients with a CMV-seropositive donor (D+/R-) and moderate-risk CMV-seropositive recipients (R+). However, a split strategy according to CMV serostatus is not specifically mentioned.

Methods

We evaluated a split strategy to prevent CMV infection after kidney transplantation in which D+/R- patients received valganciclovir (VGC) prophylaxis for 200 days, and R+ patients were treated pre-emptively according to CMV DNAemia. Patients were followed until 1 year post-transplant.

Results

Between April 2014 and March 2018, 40 D+/R- and 92 R+ patients underwent kidney transplantation. Forty-six percent received antithymocyte globulin (ATG) induction, and 98% were treated with calcineurin inhibitors, mycophenolic acid (MPA), and steroids. No D+/R- patient developed CMV disease during prophylaxis (median 200 days), but 15% developed post-prophylaxis or late-onset disease. Fifty-three percent developed neutropenia during prophylaxis, including 16/40 (40%) grade 3 or 4 neutropenia requiring reduction/discontinuation of MPA (30%) and/or VGC (35%), and an occasional need for granulocyte colony stimulating factor (5%). In the R+ group, 40% received antiviral therapy for a median duration of 21 days; 5% developed early-onset CMV disease. Only 5% developed neutropenia. D+/R+ status (hazard ratio (HR) 2.09, P=0.004) and ATG use (HR 2.81, $P < 0.0001$) were risk factors for CMV reactivation.

Conclusions

Prophylaxis leads to acceptable CMV control in high-risk patients but comes with a high risk of neutropenia. Pre-emptive therapy is effective and limits drug exposure in those at lower risk of CMV.

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Introduction

Cytomegalovirus (CMV) infection is the most common viral complication in the early post-transplant period, but its management remains a challenge. Untreated, the virus can cause disease ranging from mild flu-like symptoms to life-threatening organ damage, such as pneumonitis or colitis. CMV-seronegative recipients with a CMV-seropositive donor (D+/R-) are at highest risk of developing CMV-related complications by primo-infection through the infected graft, whereas CMV-seropositive recipients (R+) are at moderate risk based on CMV-reactivation or supra-infection. Currently, two strategies are widely used to prevent symptomatic CMV disease after transplantation: antiviral prophylaxis and pre-emptive therapy. Although both strategies have been shown to be effective in preventing symptomatic CMV disease, they have their own advantages and disadvantages[1–3] In the case of prophylaxis, all at-risk patients receive an antiviral drug, usually valganciclovir (VGC), during the first 3 to 6 months after transplantation. This leads to excellent control of the virus during treatment[4]. However, it may also hamper the development of a CMV-specific immune response, and, consequently, some patients develop late-onset CMV disease after stopping treatment with the antiviral drug[2,4,5]. In addition, long-term drug exposure leads to high drug costs and increases the risk of side effects, such as leucopenia[2,4,6]. The pre-emptive strategy could be an attractive alternative. In this case, patients are regularly (often weekly) monitored for CMV viremia and antiviral therapy started only when a predefined viral load threshold is reached, in order to prevent evolution to symptomatic disease, and continued until viremia disappears. This strategy has the advantage of limiting drug exposure, leading to lower drug toxicity and drug costs. The drawbacks of the pre-emptive strategy, however, include high monitoring costs and more difficult logistics. In addition, as the optimal threshold for treatment has not been established, evolution to symptomatic CMV disease may not always be prevented[2,6].

In a previous study, we audited the pre-emptive approach used at our center[7]. We found suboptimal results in the high-risk (D+/R-) patient subgroup, as 57% developed symptomatic CMV disease despite pre-emptive monitoring. In the moderate-risk groups (R+), the risk of symptomatic CMV disease was approximately 20%, and symptoms were milder, which are results that are comparable to reports in R+ patients from other centers[8–10]. Furthermore, our pre-emptive strategy was associated with high treatment costs, especially in the D+/R- subgroup. A subsequent cost simulation suggested that a split preventive strategy, in which D+/R- patients receive VGC prophylaxis and R+ patients are managed with a pre-emptive strategy would result in the lowest costs[7]. Consequently, we adopted this split approach at our center in 2014. In the present study, we critically evaluate the clinical outcomes of this split strategy

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using VGC prophylaxis in D+/R- patients and a pre-emptive strategy in R+ patients for preventing CMV disease after kidney transplantation.

Materials and methods

Data collection

The study was approved by the hospital ethical committee (UZA EC 20/32/423). All adult kidney transplant patients transplanted between 01/01/2014 and 10/03/2018 were included consecutively. Patients were followed up during the first year posttransplant. All patient charts were reviewed individually and the following data collected: demographics (date of birth, gender, age at transplantation, transplantation date, number of previous transplantations), donor-related data (deceased vs. living), biopsy-proven acute rejection during the first 3 months after transplantation (yes or no), immunosuppressive treatment-related data (type of induction and maintenance therapy), CMV strategy (pre-emptive vs. prophylaxis), CMV DNAemia, duration of CMV treatment, and CMV clinical disease data, including symptoms, hospitalization (yes or no), and neutropenia (yes or no and grade).

Population

A total of 184 adults underwent kidney transplantation at the University Hospital of Antwerp in Belgium between 01/01/2014 and 10/03/2018. Three patients were excluded: two patients refused to have their data collected, and one patient died at day 20 post-transplant because of gastrointestinal bleeding and bacterial sepsis (death not related to CMV, CMV DNAemia remained negative on days 2, 9 and 16 postoperatively). Therefore, our final study population consisted of 181 patients. All patients received induction immunosuppression. In patients at high immunologic risk or high-risk of delayed graft function (DGF), rabbit antithymocyte globulin (rATG; Thymoglobulin®) was used; all others were treated with the anti-interleukin-2 receptor antagonist basiliximab (Simulect®). Patients were considered to be at high immunological risk if they had two or more previous transplants, a peak complement-dependent cytotoxicity panel reactive antibodies (cdc-PRA) test > 50% or last cdc-PRA > 30%, loss of a previous renal graft within 1 year due to rejection, > 4 HLA mismatches, or donor-specific antibodies (DSA). Patients were considered to be at high risk of DGF in case of transplantation with a donation after cardiac death (DCD), a donor aged > 65 years, or cold ischemia time (CIT) > 24 hours. Standard maintenance immunosuppression consisted of triple therapy with cyclosporine (CsA) or tacrolimus (Tac),

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mycophenolate acid (MPA), and prednisolone. rATG was administered at 1.0-1.25 mg/kg/day from day 0 to day 6.

All patients received 20 mg basiliximab on day 0 and day 4 after transplantation. Target peak (C2) levels of CsA were 1000-1400 ng/mL during the first month and 800-1200 ng/mL in the second and third months. Target trough levels of Tac were 8-10 ng/mL during the first 3 months. The MPA dose was 2500 mg/d when combined with cyclosporine, and 2000 mg/d when combined with Tac. Steroids consisted of 250 mg methylprednisolone preoperatively and 125 mg methylprednisolone on day 1. In standard risk settings, prednisolone was administered as 15 mg/d from day 2 to 30, 10 mg/d in the second month, and 5 mg/d thereafter. In settings with high immunological risk or high risk of delayed graft function, prednisolone was administered as 20 mg/d from day 2 to 15, 15 mg/d from day 16 to 30, 10 mg/d in the second month, and 5 mg/d thereafter. Four patients (2%) were treated off-protocol with everolimus (EVL; target trough levels 3- 7 ng/mL) and low-dose calcineurin inhibitors (CNIs)[11]. All patients received *Pneumocystis jirovecii* prophylaxis with 80/400 mg sulfamethoxazole/trimethoprim once daily during the first 3 months. Treatment of biopsy-proven acute rejection consisted of intravenous corticosteroid boluses, with additional intravenous immunoglobulins and plasma exchange being reserved for antibody-mediated rejections.

Split CMV strategy

High-risk group

All D+/R- patients received valganciclovir prophylaxis starting within the first week after transplantation. The dosage for prophylactic VGC was adjusted for renal function according to the manufacturer's manual: 900 mg/d when creatinine clearance ≥ 60 mL/min based on the Cockcroft-Gault formula. VGC prophylaxis was administered until day 200 after transplantation unless it had to be stopped earlier because of side effects. CMV DNAemia was not routinely monitored (both during prophylaxis and after cessation of prophylaxis) and only tested under clinical suspicion of CMV disease.

Moderate-risk group

R+ patients were monitored weekly for CMV infection by the quantitative polymerase chain reaction (qPCR) method with EDTA plasma during the first 3 months after transplantation as part of a pre-emptive protocol using in-house real-time PCR targeting the phosphorylated matrix protein pp65 gene[12]. Nucleic

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acids were extracted using the NucliSENS® EasyMAG® (bioMérieux) semi-automated extractor, and amplification was carried out on the LightCycler 480® (Roche).

The threshold for initiation of pre-emptive therapy was plasma CMV DNAemia > 3.5 Log IU/mL and/or an increase in CMV DNAemia ≥ 1 log IU/mL within 1 week. Pre-emptive treatment or treatment of CMV disease consisted of high-dose valganciclovir according to the manufacturer's manual: 2 x 900 mg/d when creatinine clearance ≥ 60 mL/min based on the Cockcroft-Gault formula. Treatment was stopped after 21 days, regardless of CMV DNAemia, unless the patient still had symptoms. Occasionally, patients were treated longer than 21 days at the physician's discretion. A reduction in immunosuppression was left up to the discretion of the treating physician if a patient developed CMV DNAemia, usually starting by reducing the MPA dose. After 3 months posttransplant, CMV DNAemia was only tested under clinical suspicion of CMV disease.

Neutropenia was recorded for all patients during their exposure to VGC prophylaxis, and for the first 3 months in those managed with a pre-emptive strategy.

Definitions

CMV infection was defined as detectable CMV DNAemia based on a positive PCR test (even if below the lower limit of quantification). CMV disease was defined as CMV infection with attributable symptoms, classified as either a viral syndrome (fever, myalgia), or CMV disease with respiratory symptoms (possible pneumonitis) or other symptoms (e.g., gastrointestinal complaints). Furthermore three different types of CMV-disease were distinguished as defined by Razonable & Blumberg[13]:

1. Early-onset CMV-disease, which occurs during the first 4 months after transplantation
2. Post-prophylaxis CMV-disease, which occurs within 6 months after cessation of prophylaxis
3. Late-onset CMV-disease, which occurs > 4 months after transplantation among patients who did not receive antiviral prophylaxis; or which occurs > 6 months after cessation of prophylaxis.

D+/R- patients were defined as being at 'high risk' for CMV complications. R+ patients were defined as being at 'moderate risk' for CMV complications.

Neutropenia was graded as proposed in Common Terminology Criteria for Adverse Events, version 5.0, drawn up by the National Cancer Institute (U.S.)[14]. Following a blood neutrophil count, neutropenia was scored as: grade 1, < lower limit of normal to 1500/mm^3; grade 2, 1000-1500/mm^3; grade 3, 500-1000/mm^3; grade 4, < 500/mm^3.

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Statistical analysis

All variables are presented as frequencies and percentages, means and standard deviations, or medians and 25th and 75th percentiles unless otherwise specified. Data were compared by Pearson chi-squared, Fisher exact test, Student's t test, or non-parametric testing (Wilcoxon rank-sum test) where appropriate. A multivariable Cox proportional hazard model was constructed for the R+ subgroup, which was managed with a pre-emptive strategy, to assess the impact of rATG induction on the incidence of CMV-infection using CMV D/R serostatus as a covariable. Statistical analyses were performed using SAS software version 9.4. A two-sided p-value <0.05 was considered significant.

Results

Baseline characteristics

One-hundred and eighty-one patients were divided into four CMV D/R categories. Their baseline characteristics are provided in table 1. The age of the D+/R- patients was slightly higher than in other D/R groups (high-risk patients *versus* moderate-risk patients: p=0.006, high-risk patients versus non-high-risk patients: p=0.02). We found no significant differences between the groups in other baseline characteristics. Forty-six percent received induction therapy with rATG, whereas the others received IL2Ra. Sixty-three percent received maintenance therapy with Tac, MPA, and steroids, 35% with CsA, MPA, and steroids, and 2% (n=4) were treated with EVL, low-dose CNI, and steroids.

Outcomes in the high-risk (D+/R-) group managed with VGC prophylaxis

Outcomes in the D+/R- group are presented in Table 2a. Patients received VGC prophylaxis for a median 200 days (range 66 - 200). No patient developed CMV disease during prophylaxis, but 6/40 (15%) developed post-prophylaxis (5/40) or late-onset CMV disease (1/40) afterwards; one patient developed a viral syndrome, another patient developed respiratory symptoms, and four patients developed other symptoms (e.g., gastro-enteritis). Of these six patients, only one was hospitalized, for a period of 3 days. The median time between stopping prophylaxis and post-prophylaxis CMV disease was 66 days (37-103). The one patient with late-onset CMV-disease, developed symptoms 190 days after cessation of prophylaxis. In addition, one patient first treated with prophylaxis later received an additional course of VGC in a pre-emptive setting because of CMV DNAemia = 3.0 Log IU/mL documented during hospitalization for a varicella zoster infection. Neutropenia during VGC prophylaxis was a very frequent

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complication (53%), which was often severe. Sixteen patients (40%) developed grade 3 or 4 neutropenia. This led to reduction in VGC dose in two patients (5%), premature discontinuation of VGC in twelve patients (30%), a reduction in MPA in eight patients (20%), and cessation of MPA in four patients (10%). Two patients (5%) required additional therapy with granulocyte colony stimulating factor (GCSF). Biopsy-proven acute rejection occurred in 2/40 patients (5%).

Outcomes in the moderate-risk (R+) group managed with a pre-emptive strategy

Compliance with the weekly CMV-PCR monitoring was excellent: 77/92 (84%) patients did not miss any test during the first 3 months. For those with at least one missing test, median number of missed tests was 2 (range 2-5). Outcomes in the R+ group are presented in Table 2b. In the moderate-risk (R+) group managed with a pre-emptive strategy, CMV infection developed in 68/92 patients (74%), with CMV DNAemia usually appearing in the first month after transplantation (median 21 days (14 – 28)). One-third of the patients (n=31) only developed low-grade CMV DNAemia (< 3.5 log IU/mL), which disappeared without requiring antiviral treatment. For those developing CMV DNAemia, the maximum CMV DNAemia values reached a median of 3.33 log IU/mL (2.49 – 4.05 log IU/mL). Forty percent (37/92) of patients received at least one treatment course with VGC based on the pre-emptive protocol, most without having clinical signs of CMV disease (32/37). Five patients (5%) of the R+ group developed early-onset CMV disease despite adhering to the pre-emptive strategy; two presented with mild flu-like symptoms, one of whom had a cough and small lobar infiltrate on chest radiography with PCR-positive throat swab for influenza A. Two of the patients presented with gastro-enteritis, one of whom also had urosepsis; both were hospitalized, one for 8 days and the other for 10 days. CMV infection requiring treatment was more frequent in D+/R+ patients than in D-/R+ patients (52% versus 26%, p=0.018). Furthermore, there was a trend towards repeated treatment in the D+/R+ group (9/50 versus 1/42, p= 0.12). In a multivariable Cox proportional hazards model, D+/R+ status (hazard ratio (HR) 2.09, p=0.004) and ATG use (HR 2.81, p < 0.0001) were identified as risk factors for CMV reactivation. The median number of days of VGC exposure in those requiring treatment was 21 days (21-28) in the D-/R+ group and 21 days (21-42) in the D+/R+ group. This corresponds to a mean duration of VGC exposure of 12 days in the entire pre-emptively treated group *versus* 180 days in the group receiving prophylaxis. Accordingly, neutropenia was much less frequent in the R+ cohort (5/92, 5%). Four patients (4%) developed grade 1 neutropenia, which was managed by reducing MPA. One patient developed grade 3 neutropenia, requiring premature

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discontinuation of VGC and GCSF. Biopsy-proven acute rejection occurred in 15/92 patients (16%), which was not significantly different from the risk of rejection in the high-risk cohort (16% versus 5%, p=0.09).

Discussion

We critically evaluated the results of a split approach to preventing CMV after kidney transplantation at our center in which high-risk (D+/R-) patients received VGC prophylaxis and moderate-risk (R+) patients were managed by a pre-emptive strategy. We found that prophylaxis led to acceptable CMV control in high-risk patients but came with a very high risk of neutropenia. Pre-emptive therapy was effective and limited drug exposure in those at lower risk of CMV.

To the best of our knowledge, this is the first report detailing the outcomes and side effects of a split approach for preventing CMV disease using prophylaxis in high-risk patients (D+/R-) and a pre-emptive strategy for those at moderate risk (R+) based on the current standards of care. It is remarkable that so little attention has been given to this split prophylaxis/pre-emptive risk-stratified approach, as it is estimated to be used in approximately 1 out of 3 transplant centers throughout the world[15,16]. Current consensus guidelines on the management of CMV after solid organ transplantation endorsed by the Transplantation Society recommend using either prophylaxis or pre-emptive therapy in D+/R- and R+ patients (strong statements, high quality evidence)[2]. The option of a split approach, however, is not specifically mentioned. Nonetheless, we think that such a split approach deserves more attention, and may even be superior to a universal strategy in both D+/R- and R+ patients. A split approach is particularly attractive because it limits long-term exposure to antiviral drugs to those who would benefit most, and limits drug toxicity and costs for those who are at lower risk of developing CMV complications. It also represents a risk-stratified approach towards more personalized medicine, which is a desired goal.

A recent survey estimated that 79% of transplant centers worldwide prefer prophylaxis in high-risk patients because it is generally perceived to be easier and safer in this setting[15]. Although some centers successfully use the pre-emptive strategy in D+/R- patients[2,6], others have had suboptimal results with this strategy, leading to excess CMV disease, recurrent need for treatment, and high costs[7]. However, there is also a price to pay with prophylaxis. Our study showed a 15 % risk of post-prophylaxis or late-onset CMV disease with 6 months of VGC prophylaxis, which is comparable to what was shown in the large IMPACT trial, where kidney transplant recipients receiving 6 *versus* 3 months of VGC prophylaxis developed CMV disease in 16% *versus* 37% in the first year[4]. In addition, we confirmed a high incidence of severe

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neutropenia requiring premature discontinuation of VGC and/or MPA, and occasionally requiring GCSF[4,10,17]. The IMPACT trial reported a 26% to 38% risk of leucopenia, depending on prophylaxis duration, including grade 3–4 leucopenia in 14% of patients [4]. Forteen % of patients in each treatment arm developed neutropenia. Overall, 13–14% of patients required treatment with GCSF. Other studies have reported incidences of neutropenia during VGC prophylaxis ranging from 4% to 35 %[1,3,8,18]. The large variation in neutropenia may be explained by differences in neutropenia definition, VGC dosage, and in the concomitant use of drugs with haematotoxic side-effects, e.g. ATG or mycophenolic acid (MPA). Neutropenia, in turn, may increase the risk of other complications such as late-onset CMV disease, biopsy-proven acute rejection, and other infections[19].

CMV-seropositive recipients (R+) are clearly at lower risk of developing CMV-related complications. Data from the 1990s, before antiviral therapy was available, estimated a 20% risk of CMV disease in R+ patients[20]. A recent analysis from Brazil in which R+ patients receiving a standard immunosuppressive regimen with IL2Ra, Tac, MPA, and steroids were only treated with antiviral drugs in the case of CMV disease, also reported a CMV disease incidence of 16% to 20%[21]. Given this lower risk of CMV disease, VGC prophylaxis may have a suboptimal risk/benefit ratio, making pre-emptive therapy more attractive. Using our current pre-emptive protocol in R+ patients, VGC treatment could be limited to 40% of the patients, with a median treatment duration of 21 days. Accordingly, there was a much lower incidence of neutropenia. Five percent developed early-onset CMV disease despite monitoring, mostly limited to mild symptoms. These results are comparable to the CMV disease rates in R+ patients reported at other centers, usually ranging between 0% and 13%[8–10]. With a lower threshold for pre-emptive therapy, we may have been able to further reduce the risk of CMV disease, but this apparent benefit should be balanced against the fact that, overall, more patients would be treated, some without really needing it. Indeed, approximately one-third of our cohort only developed low-grade viremia (< 3.5 log/IU), which disappeared again without antiviral therapy.

Our study confirms that the risk of CMV disease is higher for D+/R+ patients than for D-/R+ patients, and that rATG induction is a strong independent risk factor for CMV reactivation. Among D+/R+ patients receiving rATG, 59% required antiviral therapy, and 22% required more than one treatment course. It may be argued that, in the case of rATG induction, especially in D+/R+ patients, it may have been better to continue pre-emptive therapy until CMV DNAemia disappeared instead of the standard treatment of 21 days. An alternative option may be to use prophylaxis instead of a pre-emptive strategy in the case of ATG use, as has been suggested by several authors[2,22]. The advantages and disadvantages of either strategy in this particular setting, however, have not yet been systematically investigated in a clinical trial.

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We acknowledge that this study has limitations. First, the study is based on the experience of a single center, making the composition of the study population and therapeutic protocols potential limiting factors to the generalizability of our results. Furthermore, although we managed to adhere strictly to the split protocol, some centers may find it logistically challenging to apply a different CMV strategy according to D/R status. Further studies need to be carried out in order to validate our results.

We conclude that our current split strategy may be the first step towards personalized management for CMV. In a next step, we should optimize our strategy for patients in the grey zone between high and moderate risk, such as in D+/R+ patients or R+ patients receiving lymphocyte-depleting therapy. Currently, several clinical trials are investigating the role of CMV-specific T-cell monitoring in guiding therapeutic decisions [23,24]. In addition, the recent TRANSFORM and ATHENA clinical trials showed that the risk of CMV infection and disease is approximately 3-times lower with an immunosuppressive regimen based on low-dose CNIs plus mTOR inhibitors (mTORi) *versus* a classical regimen containing standard dose CNIs with MPA[11,25]. Such an MPA-free regimen has the additional advantage of reducing the risk of neutropenia[11,25]. A regimen with CNI and mTORi may be particularly interesting for patients at high risk of CMV, either as standard maintenance therapy starting from transplantation, or as alternative treatment in the case CMV infection develops. The latter is the topic of an ongoing randomized clinical trial (*NCT02671318*). Finally, newer drugs under development aim to be as effective, but less toxic than valganciclovir. A large phase III trial comparing prophylaxis with letermovir to valganciclovir in D+/R- kidney transplant recipients is currently ongoing[26] *(NCT03443869)*. The active research in the field of CMV demonstrates the persistent need for better management of this infection and gives hope for an easier and/or more individualized approach to fighting post-transplant CMV complications in the near future.

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Table 1: Baseline characteristics of the study population

	TOTAL	Low risk	Moderate risk		High risk
		D-/R-	D-/R+	D+/R+	D+/R-
n (%)	181	49 (27)	42 (23)	50 (28)	40 (22)
Mean age, years (SD)	50 (13)	51 (12)	49 (13)	47 (14)	55* (12)
Sex, male	109 (60)	32 (65)	23 (55)	28 (56)	26 (65)
First transplant	156 (86)	42 (86)	38 (90)	41 (82)	35 (88)
Induction					
IL2-Ra	97 (54)	25 (51)	25 (60)	23 (46)	24 (60)
ATG	84 (46)	24 (49)	17 (40)	27 (54)	16 (40)
Maintenance therapy					
Tac – MPA – steroids	113 (63)	32 (65)	25 (60)	30 (60)	26 (65)
CsA – MPA – steroids	64 (35)	17 (35)	15 (35)	19 (38)	13 (32)
CNI – mTORi - steroids	4 (2)	0 (0)	2 (5)	1 (2)	1 (3)

Data are given as n (%) unless otherwise noted.

*Significantly higher than other risk groups (p=0.02).

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Table 2a: Outcomes in the high risk (D+/R-) group managed with VGC prophylaxis

	High risk (D+/R-) n=40
Duration of prophylaxis, days Range, days Median (25^{th} - 75^{th} percentile) Mean (SD)	 66 – 200 200 (200 – 200) 180 (43)
CMV disease during prophylaxis	0 (0)
CMV disease after cessation of prophylaxis - Post-prophylaxis CMV disease Viral syndrome Respiratory symptoms Other symptoms - Late-onset CMV disease	6 (15) 5 (12.5) 1 1 3 1
Time between post-prophylaxis CMV and stop of prophylaxis, days, median (25 – 75 percentile)	66 (37 – 103)
Max. plasma DNAemia, log IU/mL, median (25 – 75 percentile)	4.01 (3.48 – 4.65)
Premature discontinuation of prophylaxis Due to neutropenia	12 (30) 12 (100)
Neutropenia during VGC prophylaxis - Grade 1 - Grade 2 - Grade 3 - Grade 4	21 (53) 4 (10) 1 (2) 8 (20) 8 (20)
Action for neutropenia[a] - MPA reduction - MPA stop - VGC reduction - VGC stop - GCSF	 8 4 2 12 2
Biopsy-proven acute rejection	2 (5)

Data are given as n (%) unless otherwise noted. [a] More than one option is possible per patient.

Table 2b. Outcomes in the moderate-risk groups (R+) managed with a pre-emptive strategy

Moderate risk (R+) Pre-emptive strategy	Total R+ n= 92	D-/R+ n= 42	D+/R+ n= 50	P-value
CMV infection	68 (74)	25 (60)	43 (86)	0.005
CMV infection according to induction - IL2Ra - rATG	 27 (29) 41 (45) }p <0.0001	 11 (44) 14 (82) }p 0.024	 16 (70) 27 (100) }p 0.002	
Days to CMV infection from transplantation, median (25 – 75 percentile)	21 (14 – 28)	22 (17-34)	20 (13-25)	0.29
Maximum plasma DNAemia, log IU/mL, median (25 – 75 percentile)	3.33 (2.49 – 4.05)	2.88 (<2.30-3.55)	3.64 (2.90-4.29)	0.018
Patients requiring antiviral therapy	37 (40)	11 (26)	26 (52)	0.018
Patients requiring antiviral therapy, according to induction - ILR2a - rATG	 12 (25) 25 (57) }p 0.028	 2 (8) 9 (53) }p 0.029	 10 (44) 16 (59) }p 0.39	
Number of antiviral therapy courses per patient - 1 - 2 - 3	 26 (28) 9 (10) 1 (1)	 10 (24) 1 (2) 0 (0)	 16 (32) 8 (16) 1 (2)	 0.13 0.22 1.00
Total days of antiviral therapy in total group[a], mean (SD)	12 (20)	6 (11)	17 (25)	0.01
Total days of antiviral therapy in those requiring treatment Median (25 – 75 percentile)	 21 (21-42)	 21 (21-28)	 21 (21-42)	 0.13
CMV disease - Viral syndrome - Respiratory symptoms - Other symptoms	5 (5) 2 1 2	2 0 1 1	3 2 0 1	
Hospitalization due to CMV disease	2 (2)	1	1	
Neutropenia during first 3 months - Grade 1 - Grade 2 - Grade 3 - Grade 4	5 (5) 4 (4) 0 (0) 1 (1) 0 (0)	1 1 0 0 0	4 3 0 1 0	

Action for neutropenia[b] - MPA reduction - MPA stop - VGC reduction - VGC stop - GCSF	 4 0 0 1 1	 1 0 0 0 0	 3 0 0 1 1	
Biopsy-proven acute rejection	15 (16)	6 (14)	9 (18)	0.78

Data are given as n (%) unless otherwise noted. [a]including patients that did not require antiviral therapy. [b] More than one option is possible per patient.