Everolimus-based immunosuppressive regimens in lung transplant recipients: impact on CMV infection.

Everolimus-based immunosuppressive regimens in lung transplant recipients: impact on CMV infection

Massimo Rittà[a, b*], Cristina Costa[a, b*], Paolo Solidoro[c], Francesca Sidoti[a, b], Daniela Libertucci[c], Massimo Boffini[d], Mauro Rinaldi[d], Sergio Baldi[c], Rossana Cavallo[a, b].

[a] Microbiology and Virology Unit, Laboratory of Virology, University Hospital "Città della Salute e della Scienza di Torino", Via Santena 9, 10126 Torino, Italy.

[b] Department of Public Health and Pediatrics, University of Turin, Via Santena 9 - 10126 Torino

[c] Pneumology Division, University Hospital "Città della Salute e della Scienza di Torino", Corso Bramante 88, 10126 Torino, Italy.

[d] Cardiac Surgery Division, Surgical Sciences Department, University of Turin, University Hospital Città della Salute e della Scienza di Torino, Torino, Italy.

[*] M.R. and C.C. equally contributed to this study and share first authorship.

Massimo Rittà, massimo.ritta@unito.it; Cristina Costa, cristina.costa@unito.it; Paolo Solidoro, psolidoro@cittadellasalute.to.it; Francesca Sidoti, francesca.sidoti@unito.it; Daniela Libertucci, dlibertucci@cittadellasalute.to.it; Massimo Boffini, massimo.boffini@ unito.it; Mauro Rinaldi, mauro.rinaldi@unito.it; Sergio Baldi, sbaldi@molinette.piemonte.it; Rossana Cavallo, rossana.cavallo@unito.it

Corresponding author: Massimo Rittà, massimo.ritta@unito.it +39 011 6705640

ABSTRACT

Cytomegalovirus (CMV) is one of the most important viral pathogen in solid organ transplant (SOT) recipients, with heart and lung transplant patients being at considerably high risk for CMV direct and indirect effects. Prevention strategies have resulted in significant reduction in disease and CMV related morbidity and mortality. Few studies reported a lower incidence of CMV infections in solid organ transplant recipients treated with immunosuppressive protocols including the mTOR inhibitor everolimus (EVR).

Purpose. The aim of the current study was to evaluate the impact of EVR-based immunosuppressive regimens on the occurrence and kinetics of CMV infection in a population of lung transplant recipients, at both systemic and pulmonary level. Thirty-two lung transplants (LT) were investigated; eighteen were on EVR-based immunosuppressive regimens. CMV events occurring in the first two years post-transplantation at both systemic and pulmonary levels were reported.

Principal results. No differences were reported in CMV viraemiae occurrence at both one- and two-year follow up between patients undergoing EVR-based and EVR-free immunosuppressive regimens. Considering CMV episodes at pulmonary levels, as determined by routinely performed broncho-alveolar lavages (BAL), during EVR-administration the patients experienced significantly fewer episodes of high-load CMV (as defined by viral loads ≥ 10^5 copies/mL) than during EVR-free immunosuppressive regimens.

Major conclusion. EVR-based immunosuppressive regimens in lung transplantation settings appear to be associated to lower incidence of clinically relevant CMV episodes at pulmonary levels, striking the possibility of extending the use of EVR to such a group of transplant recipients.

KEYWORDS

Everolimus (EVR), lung transplantation (LT), cytomegalovirus (CMV), immunosuppression, opportunistic infections.

1. INTRODUCTION

Cytomegalovirus (CMV) is a ubiquitous β-herpesvirus that establishes lifelong latency in host tissues following primary infection. The occurrence of CMV primary infection in seronegative individuals or the capability of viral reactivation in immunocompromised conditions makes CMV one of the most important viral pathogens in solid organ transplantation (SOT), with incidence of infection/disease ranging from 8% up to 50% depending on the transplanted organ. Heart and lung transplant (LT) recipients are at particularly high risk for CMV direct and indirect effects and evidence indicates that related morbidity and mortality are greater in LT recipients than in other SOT, as lung is a major site for CMV latency and recurrence. . Direct effects of CMV infection can manifest as systemic or organ-specific disease, whereas indirect effects reflect altered immune responses associated with infection, resulting in increased incidence of graft dysfunction, acute and chronic rejection, and opportunistic infections. (Fishman et al. 2007). Acute and chronic graft rejection are relevant determinants of morbidity and mortality, particularly in the first two years post-transplantation and several studies have reported an association between CMV infections and organ rejection, with the CMV donor/recipient (D/R) serological matching D+/R- being at the highest risk, although the underlying mechanisms are still unclear. .

Prevention strategies may result in significant reduction in CMV-related morbidity and mortality in SOT recipients. Two main prevention strategies are commonly used: universal prophylaxis with administration of antiviral agents in all the patients and pre-emptive therapy based on virological monitoring (usually by evaluation of CMV-DNA on whole blood) and antiviral administration in the presence of laboratory evidence of infection, with relevant variations in clinical practice in different transplant centers. .

Considering immunosuppressive protocols, few studies on heart and kidney transplantation reported a lower incidence of CMV infections in patients treated with regimens including everolimus (EVR), a proliferation signal inhibitor (PSI)/mammalian target of rapamycin (mTOR). mTOR inhibitors act by leading to inhibition of translational processes depending on mTORC1 activity, preventing cell-cycle progression from G1 to S-phase in T-cells; moreover, a potential antiviral effect through interruption of certain mTORC pathways or immune deviation has been evidenced. (Boffini et al. 2009) . The antiproliferative effect of EVR may represent a therapeutic option in immunosuppressive protocols of LT by reducing both the risk of acute rejection and the process of progressive fibrosis that determines chronic graft rejection. However, few data on EVR-based immunosuppression in LT are available and the effectiveness in conferring protection towards CMV infection, along with the specific indications and the most adequate time for its introduction or dosing, are still controversial.

The aim of this study was to prospectively evaluate the impact of EVR-based immunosuppressive regimens on the occurrence and kinetics of CMV infection and CMV-related events at systemic and pulmonary level, in a population of LT recipients.

2. MATERIALS AND METHODS

Thirty-two consecutive patients undergoing LT between 2007 and 2012 (mean age at transplantation ± SD, 49.7 ± 16.2 years; range 17-68.7), with at least one-year follow up, were prospectively studied. The main demographic and clinical features of the study population are summarized in Table 1. Informed consent was obtained from all the patients. The study population was divided in two groups: 1) 18 patients treated with EVR-based immunosuppressive regimens at different times post-transplantation (EVR-group); 2) 14 patients treated with EVR-free immunosuppressive protocols for all the study period (no-EVR-group). The EVR-group included two patients receiving EVR *de novo* within one

month post-transplantation (one for gastric intolerance to mycophenolate mofetil, in association with severe relapsing neutropenia flares following introduction of azathioprine; one due to history of breast cancer), four within 6 months, and five within 12 months; four and three patients switched to EVR-based protocols in the second and third year post-transplantation, respectively (mean time at EVR introduction, 14.5 ± 10.9 months). In 17 out of 18 patients EVR was administered for at least six months. EVR was administered twice daily, with a goal trough level of 3 to 8 ng/dL. The main indications leading to switching to EVR maintenance immunosuppressant are listed in Table 2. In the EVR group, five patients were at high risk for CMV infections (as identified by serological matching D+/R-) and began EVR-administration at 9, 12, 15, 21 and 33 months post transplantation, respectively.

According to our Centre's practice, LT recipients were submitted to surveillance visits, including bronchoscopy with bronchoalveolar lavage (BAL), transbronchial biopsy and whole blood draws, at 1, 3, 6, 9, and 12 months post-transplantation; further visits were performed at 18 and 24 months post-transplantation, and then annually, as well as in the presence of clinical signs and/or symptoms and/or rejection. Further whole blood specimens were collected for CMV-DNA quantitation with trimestral periodicity.

All LT recipients received a universal, prolonged and combined anti-viral prophylaxis for CMV consisting in the administration of ganciclovir or valganciclovir (450 mg bid) from day 21 for 3 weeks associated to CMV-immunoglobulins (Cytotect Biotest™) at days 1, 4, 8, 15, and 30 (1.5 ml/kg body weight) and monthly up to 1 year post-transplantation (1 ml/kg body weight), irrespective of CMV serostatus. . Furthermore, all patients received long-term general antiviral prophylaxis with acyclovir 200 mg bid. Ganciclovir or valganciclovir were further administered based on clinical judgements and/or in case of CMV-DNA viral loads on whole blood and/or BAL greater than 10^4 copies/mL. Long-term

immunosuppression was with cyclosporine A or tacrolimus (in patients with cystic fibrosis as underlying disease), mycophenolate mofetil, and prednisone (to be tapered or discontinued). In three patients with cystic fibrosis, also azathioprine was administered (one from the EVR-group, two from no-EVR). Allograft rejection was histopathologically diagnosed and graded on trans-bronchial biopsy specimens, according to the International Society for Heart and Lung transplantation Working Formulation .

For CMV-DNA quantitation on whole blood and BAL samples, a real time PCR assay was used. Total DNA was extracted on the automated QIAsymphony® system (Qiagen, Hilden, Germany), according to the manufacturer's instruction. A commercially available real time PCR assay amplifying a region of the exon 4 of MIEA (major immediate early antigen) of CMV (Q-CMV Real Time Complete Kit, Nanogen Advanced Diagnostic, Italy) was performed on a 7500 Real-Time thermo-cycler system (Applied Biosystems, USA). Briefly, 20 µL of DNA extracted from 200 µL of whole blood or 1 mL of BAL fluid (obtained from the firstly-recovered 10-mL aliquot from a volume of 150 mL saline solution injected for BAL diagnostic procedure) were added to 20 µL of mastermix, and amplified following manufacturer's instruction. The occurrence of CMV-DNA in whole blood and BAL specimens during the first two years post-transplantation was compared in EVR- versus no-EVR-treated LT recipients. The occurrence of CMV events was also stratified according to donor/recipient CMV serostatus.

Statistical analysis was performed using Student's t-test, χ^2-test and Fisher's exact test, as appropriate, on GraphPad Prism version 4.00 software. A p-value <0.05 was considered statistically significant.

3. RESULTS

The EVR group included 13 patients at low risk for CMV reactivations at baseline (i.e. serological matching status D+R+, D-R+, D-R-: 13/18, 72.2%), and five patients at high risk (D+/R-, 27.8%); all the patients in the no-EVR-group turned to be at low risk (14/14, 100%), p>0.05. Neither differences in age (mean age at transplantation, 54.2 vs 43.9 yrs, p>0.05), nor gender distribution (male to female ratio, 13/5, and 9/5, p>0.05) were reported between the two groups. The immunosuppressive regimens adopted are reported in table 2. Only two patients received EVR within one month post transplantation, with 3-year follow up data available. Given the small number of patients undergoing very early administration of EVR, and to missing at follow up, data for CMV replication were analyzed referring to events globally occurring in the first and second year post transplantation. In Figure 1 is graphically reported the distribution of CMV events in blood (panel A) and at pulmonary levels (panel B, global incidence; panel C, CMV-DNA viral loads $\geq 10^5$ copies/mL BAL) per single patient, in function of time (x-axis) and of concurrent EVR-including immunosuppressive regimens (fixed lines, EVR-based regimens; dot-lines, EVR-free regimens). Occurrence of CMV episode is depicted as black square/circle, and the absence as white. CMV events were analyzed by Fisher's exact test.

3.1 CMV events at pulmonary level

Globally, 29 out of 32 patients (90.6%) experienced at least one episode of pulmonary CMV infection during the first year post transplantation (as defined by occurrence of CMV-DNA on BAL), with a cumulative incidence of pulmonary CMV episodes of 48.7% (the first episode occurring at a median of 3 months post transplantation). Three patients (9.4%) persisted free of any CMV reactivation at two years follow up, when a global rate of CMV episodes of 47.8% was observed. These latter patients were on EVR-free regimens for the entire observation period. The distribution of CMV episodes in BAL specimens is reported

in Table 3, at both 1- and 2-year follow up, according to EVR inclusion in the immunosuppressive regimens.

Overall, 13 patients experienced high load CMV positive BAL (as defined by CMV loads ≥ 10^5 copies/mL), with 18 episodes in the first year and 2 episodes in the second year. Detailed analyses of pulmonary events showed no occurrence of high-load CMV-positive BAL specimens during treatment with EVR in the first year post transplantation (Fisher's exact test, p=0.0247). The strength of the association was confirmed at two-year follow up, when 20 out of 209 BAL determinations available for analysis (9.6%) reported high-load CMV results, with persistent absence of high-load determinations in the EVR-group (Fisher's exact test, p=0.0049). For EVR therapy data analyses, a minimum of 4-week administration course was necessarily required for an episode to be addressed to as occurring during EVR treatment itself. However, when excluding the high risk group (i.e. 5 patients with pre-transplant CMV D+R- serostatus), the strength of the correlation weakened in significance, at both one (p=0.1216) and two-year follow up (p=0.0715) (Data reported in Table 3). It has to be pointed that even during the second year post transplantation, no patient experienced high-load CMV episodes in BAL concomitant to EVR-based immunosuppression (the highest being 12700 copies/mL in the first year and 11646 copies/mL in the second year). In the no-EVR group the highest CMV load was 1641550 copies/mL at 3-month determination in a D+/R- subject experiencing a primary CMV infection. When comparing mean CMV viral loads during EVR administration versus no-EVR regimens, no difference was found between the two groups at both 1- and 2-year follow up (Table 5). The time course of CMV episodes in BAL in function of EVR-including immunosuppressive regimens is reported in Figure 1.

3.2 CMV episodes at systemic level

Twenty-three patients (71.9%) experienced at least one episode of CMV viraemia within the first year post transplantation, 25 (78.1%) within the first two years, with a cumulative incidence of viraemic episodes of 30.6% and 24.6% at 1- and 2-year follow up, respectively (median time for first occurrence, 3 months). No difference was found when comparing the cumulative incidence rates of viraemic episodes during EVR-based immunosuppressive regimens administration versus non-EVR, at both 1- and 2-year follow up ($p>0.05$). In Table 4 is reported the distribution of the CMV episodes in blood on the basis of EVR administration at both 1- and 2-year follow up. The time course of EVR administration together with CMV determinations in blood are depicted in Figure 1. Neither in the first nor in the second year post transplantation did the CMV viral loads significantly differ during EVR-based immunosuppression versus non-EVR (Table 5).

All five patients at greater risk for CMV infection (D+R- at pre-transplant) exhibited CMV-related events within six months post transplantation, with positive detections at both systemic and pulmonary levels (reported peak levels: 633155 copies/mL in blood and 1641550 copies/mL in BAL). Among them, only two patients received EVR within the first year (both starting at 9 months post transplantation), hampering the possibility to investigate the potential of EVR on CMV replication at a very early stage.

3.3 Graft rejection

Graft rejection was histopathologically monitored on trans-bronchial biopsies routinely performed at months 1, 3, 6, 9, 12, 18, 24 post-transplantation, and on clinical suspicion. Twenty-four (75%) patients out of 32 experienced at least one episode of acute rejection within the first year (19 at grades 1-2, 5 at grades 3-4), and 6 (24%) out of the 25 patients with available determinations in the second year (5 patients grade 1-2, 1 patient at grade 3). Data are summarized in Table 6. Focusing on the first year post-transplantation, out of 8 patients who underwent EVR-including regimens for a minimum of three months, no

case of A3 and A4 rejection was reported, in contrast to 4 and one patients experiencing the reported rejection intensities during non-EVR courses, without reaching statistical significance.

4. DISCUSSION

CMV infection, with its direct and indirect effects, is responsible for significant morbidity and mortality in lung post-transplantation period, thus justifying investigations on effective prevention strategies. The development of both novel laboratory and clinical markers to identify patients at greatest risk for CMV disease and therapeutic strategies to prevent CMV burden are rising as matters of great concern in clinical setting. Some studies suggested how the introduction of EVR in the immunosuppressive regimens could lower the incidence of CMV infection (specifically, in heart and kidney transplant recipients) , even though the benefit of mTOR inhibitors observed in the *de novo* immunosuppressive settings may not be apparent in patients switching to mTOR inhibitors following initial different immunosuppressive protocols.

The present study aimed to seek the benefit of using EVR-including immunosuppressive regimens on the course of CMV infections in lung transplant patients. At our knowledge, no study currently investigated the issue of EVR and CMV risk assessment in LT, whereas data are available as regards kidney and heart transplantation, showing the potential benefit in the prevention of CMV-related events. . A recent metanalysis of 11 randomized trials comparing immunosuppressive regimens containing either EVR or sirolimus versus non-mTOR-regimens pointed out that the incidence of acute rejection and CMV infection were reduced when the two drugs were used instead of either azathioprine or mycophenolate mofetil . Notably, few studies demonstrated significantly fewer CMV infections at both 12 and 24 months post cardiac transplantation, a difference that was also present in the high-risk D+/R- subgroup. . These observations have been confirmed

in 2012 by Gurk-Turner *et al.*, who reported higher rates of CMV infections in heart and kidney transplants receiving EVR-free immunomodulations than those EVR-based. .

In the present study no difference was found as regards the cumulative incidence rates of CMV viraemic episodes in EVR-based immunosuppressive regimens versus non-EVR, at both 1- and 2-year-follow up. Interestingly, patients receiving EVR-based regimens had a significantly lower cumulative incidence of overall high-load CMV pulmonary infections (as defined by CMV viral loads in BAL $\geq 10^5$ copies/mL), as indicated by the absence of high-viral load episodes at the end of both the first and the second year post-transplantation, in comparison to EVR-free recipients ($p<0.05$ and $p<0.005$, respectively). Since no difference was observed in the incidence rates of low-level CMV viral loads in the EVR-based group, it's intriguing to speculate that the anti-viral activity of the drug only potentially manifest in a background of highly-active viral milieu, likely sustained by immunosuppressive strategies. This is pertinent with the notion reporting the lung as a site of major latency for CMV. Even though only two out of five D+/R- patients at high-risk for CMV infection received EVR in the first year (both starting at 9 months post transplantation) - precluding the possibility to draw conclusions on everolimus implications during the most challenging period for CMV primary infections - it has to be pointed that none of them showed high-viral load infections at both pulmonary or systemic level concomitant to EVR administration at two-year follow up. This observation is consistent with previous reports on lower proportions of D+R- heart-transplant patients with CMV infections when receiving everolimus as immunosuppressant (Hill et al. 2007).

The findings reported in the present observational study are consistent with previously published results showing reductions in the incidence of CMV infection in EVR-treated kidney and heart transplants . The biological mechanisms behind the association of EVR administration and lower CMV manifestations are far from clear. Nonetheless, some data

suggest a compelling role of mTOR inhibitors in the modulation of CMV replication process, likely due to the negative interference with CMV translation mechanisms operated by EVR-mediated blockade of mTOR-dependent “proliferation” pathways, as well as by its influence on the immune-mediated responses, as mTOR inhibitors have recently been shown to enhance memory T-cell formation and effectiveness of virus-specific CD8+ T-cells, while inhibiting immune activity in response to graft transplantation. . The lower incidence of CMV episodes during EVR-regimens does not appear to be related to potential bias due to relatively less potent immunosuppression in the EVR-group, since no case of high intensity acute rejection (A3 and A4 stage) was reported in this group, whereas 5 patients reported A3 and A4 acute rejection during non-EVR immunosuppression in the first year post transplantation. Even though suffering from small sample size, it is likely that the reduced CMV infection rates in the lung are linked to improved long-term organ survival.

In conclusion, data from the present study seem to confirm the decrease in CMV events in transplant patients undergoing EVR-based immunosuppressive regimens, also including LT recipients. These preliminary data could justify additional studies and long-term follow up of EVR-based immunosuppression that are specifically designed to evaluate critical CMV endpoints.

GLOSSARY

EVR, everolimus; SOT, solid organ transplant; CMV, cytomegalovirus; LT, lung transplant.

LEGENDS TO FIGURE AND TABLES

Table 1. General characteristics of the study population.

[a] Including one patient with combined liver-lung transplantation belonging to the no-EVR group.

Table 2. Main results.

[a] Only episodes occurring in the first two years post transplantation during the very administration of everolimus were considered.

[b] Excluding one patient whose immunosuppressive regimen included everolimus ab initio.

[c] Including one patient undergoing EVR-based immunosuppression since the transplantation, uninterruptedly for the entire observation period. In the upper part of the cell are reported the EVR-free immunosuppressive regimens adopted (both in the EVR- and no-EVR group); in the lower part, the EVR-based regimens.

Table 3.

Distribution of CMV episodes in BAL specimens for the indicated observation periods and on the basis of concomitant EVR administration. CMV occurrences are compared by Fisher's exact test. For each time and viral load group, CMV occurrences in patients at low risk for CMV reactivation (i.e. excluding pretransplant CMV D+R- serostatus) are reported in Italics. The definition "high viral loads" refers to $\geq 10^5$/mL, as reported in the text. VL, viral load; IS, immunosuppression.

Table 4.

Distribution of CMV episodes in blood for the indicated observation periods and on the basis of EVR administration, irrespective of viral load values. For each period and viral load group, CMV occurrences in patients at low risk for CMV reactivation (i.e. excluding those presenting a pre-transplant CMV D+R- serostatus) are reported in Italics. CMV occurrences are compared by Fisher's exact test. IS, immunosuppression.

Table 5.

Distribution of CMV episodes and viral load means in BAL and in blood for the observation periods indicated in the text. Student's t-test was used for comparison of means. IS, immunosuppression.

Table 6.

Distribution of patients on the basis of histopathologically confirmed acute rejection on TBB. In right columns are reported the numbers of patients with the indicated maximum intensity-level AR episodes, stratified by concomitant EVR administration; when different intensity-level episodes were detected in the same time period post transplant, only the highest was reported. Data were analyzed by Fisher's exact test.

[a] In the first year post transplant, two patients experienced graft acute rejection during both EVR-free and EVR-based regimens (A3 and A2 maximum intensity during EVR-free regimens, respectively, followed by A1-rejection during EVR-based); three patients, considering two-year follow up.

[b] The reported n=31 is due to one patient undergoing EVR-based immunosuppression uninterruptedly during the 24-month follow up.

AR, acute rejection.

Figure 1. Incidence of CMV events over time in blood (panel A). Incidence of CMV episodes over time in BAL: global incidence (panel B), and high-values (viral loads ≥ 10^5 copies/mL, panel C). Fixed lines, EVR-based regimens; dot-lines, EVR-free regimens. The identification codes of the patients are reported on the y-axes. Red dots: CMV positive events; white dots, CMV negative results (in peripheral blood or BAL).

ACKNOWLEDGEMENTS

The study was supported by Fondazione Carlo Denegri – Turin (research grant for F. S.)

*Massimo Rittà acknowledges a grant for the Lagrange Project – CRT Foundation/ISI Foundation, Italy.

REFERENCES

Boffini, M., F. Sansone, et al. (2009). "Does everolimus associated with a low dose of cyclosporine in long-term cardiac transplant recipients improve renal function? Initial experience." Transplant Proc 41(4): 1349-52.

Brennan, D. C., C. Legendre, et al. (2011). "Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: pooled analysis of three clinical trials." Am J Transplant 11(11): 2453-2462.

Costa, C., A. Curtoni, et al. (2013). "Detection of human cytomegalovirus in transbronchial biopsies from lung transplant recipients." Arch Virol 158(7): 1461-1465.

Costa, C., D. Libertucci, et al. (2007). "Rapid shell vial culture for the detection of respiratory viruses from bronchoalveolar lavage in immunocompromised patients." Panminerva Med 49(1): 1-6.

Costa, C., A. Saldan, et al. (2012). "The lack of cytomegalovirus-specific cellular immune response may contribute to the onset of organ infection and disease in lung transplant recipients." Int J Immunopathol Pharmacol 25(4): 1003-1009.

de Pablo, A., F. Santos, et al. (2013). "Recommendations on the use of everolimus in lung transplantation." Transplant Rev (Orlando) 27(1): 9-16.

Eid, A. J. and R. R. Razonable (2010). "New developments in the management of cytomegalovirus infection after solid organ transplantation." Drugs 70(8): 965-981.

Eisen, H. J., E. M. Tuzcu, et al. (2003). "Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients." N Engl J Med 349(9): 847-858.

Ferrer, I. R., M. E. Wagener, et al. (2010). "Cutting edge: Rapamycin augments pathogen-specific but not graft-reactive CD8+ T cell responses." J Immunol 185(4): 2004-2008.

Fishman, J. A., V. Emery, et al. (2007). "Cytomegalovirus in transplantation - challenging the status quo." Clin Transplant 21(2): 149-158.

Gurk-Turner, C., W. Manitpisitkul, et al. (2012). "A comprehensive review of everolimus clinical reports: a new mammalian target of rapamycin inhibitor." Transplantation 94(7): 659-668.

Hill, J. A., M. Hummel, et al. (2007). "A lower incidence of cytomegalovirus infection in de novo heart transplant recipients randomized to everolimus." Transplantation 84(11): 1436-1442.

Imamura, T., T. Shiga, et al. (2012). "Successful conversion to everolimus after cytomegalovirus infection in a heart transplant recipient." Int Heart J 53(3): 199-201.

Kobashigawa, J., H. Ross, et al. (2013). "Everolimus is associated with a reduced incidence of cytomegalovirus infection following de novo cardiac transplantation." Transpl Infect Dis 15(2): 150-162.

Nashan, B., R. Gaston, et al. (2012). "Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients." Transplantation 93(11): 1075-1085.

Roux, A., G. Mourin, et al. (2013). "CMV driven CD8(+) T-cell activation is associated with acute rejection in lung transplantation." Clin Immunol 148(1): 16-26.

Snydman, D. R., A. P. Limaye, et al. (2011). "Update and review: state-of-the-art management of cytomegalovirus infection and disease following thoracic organ transplantation." Transplant Proc 43(3 Suppl): S1-S17.

Stewart, S., M. C. Fishbein, et al. (2007). "Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection." J Heart Lung Transplant 26(12): 1229-1242.

Valantine, H. and A. Zuckermann (2005). "From clinical trials to clinical practice: an overview of Certican (everolimus) in heart transplantation." J Heart Lung Transplant 24(4 Suppl): S185-190; discussion S210-181.

Vigano, M., T. Dengler, et al. (2010). "Lower incidence of cytomegalovirus infection with everolimus versus mycophenolate mofetil in de novo cardiac transplant recipients: a randomized, multicenter study." Transpl Infect Dis 12(1): 23-30.

Vitko, S., R. Margreiter, et al. (2005). "Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in de novo renal transplant patients." Am J Transplant 5(10): 2521-2530.

Webster, A. C., V. W. Lee, et al. (2006). "Target of rapamycin inhibitors (sirolimus and everolimus) for primary immunosuppression of kidney transplant recipients: a systematic review and meta-analysis of randomized trials." Transplantation 81(9): 1234-1248.

Zamora, M. R., R. D. Davis, et al. (2005). "Management of cytomegalovirus infection in lung transplant recipients: evidence-based recommendations." Transplantation 80(2): 157-163.

Features	Study population	EVR group	no-EVR group
	Total N = 32	N = 18	N = 14
Age at transplantation mean ± SD (range), years	49.7 ± 16.2 (17-68.7)	52.8 ± 14.8 (24.2-68.3)	47.4 ± 17.0 (17-68.7)
M/F	22/10	13/5	9/5
Type of transplant			
Bilateral [a]	17 (53.1%)	11	6
Monolateral	15 (46.9%)	7	8
Donor/Recipient CMV matching, N (%)			
Low risk (D+/R+ and D- /R+)	26 (81.3%)	12 (66.7)	14 (100)
(D-/R-)	1 (3.1%)	1 (5.6)	0
High risk (D+/R-)	5 (15.6%)	5 (27.7)	0

Table 1

General characteristics of the study population.

[a] Including one patient with combined liver-lung transplantation belonging to the no-EVR group.

Features	Study population	EVR group	No-EVR group
Number of patients (%)	Total N = 32	N = 18	N = 14
CMV positive DNAemia		10/17 (58.8)[a]	3 (21.4%)
CMV infected BALs		16/17 (94.1%)[a]	11 (78.6%)
Indications to everolimus switch[b]		NA	NA
renal failure	2 (6.3)		
appearance or progression of chronic rejection or BOS	4 (12.5)		
chronic lung graft dysfunction (CLGD, including bronchial stenosis)	7 (21.9)		
repeated CMV or other Herpesviridae infections	2 (6.3)		
intolerance of other immunosuppressors (neurotoxicity, leukopenia, gastro-intestinal disorders, dermatosis)	2 (6.3)		
immunosuppressive regimens[c]			
CSA+MMF	9 (28.1)	7 (38.9)	2 (14.3)
CSA+MMF → TAC+MMF	9 (28.1)	6 (33.3)	3 (21.4)
TAC+MMF	10 (31.3)	3 (16.7)	7 (50)
TAC+AZA	1 (3.1)	1 (5.6)	0
TAC+AZA → TAC+MMF	1 (3.1)	0	1 (7.1)
TAC+MMF → TAC+AZA	1 (3.1)	0	1 (7.1)
CSA+EVR	6 (18.8)	6 (33.3)	NA
TAC+EVR	12 (37.5)	12 (66.7)	NA
Transplant-related mortality (years post tx)			
1	2 (6.3%)	2 (11.1)	0
2	0		
3	2 (6.3%)	2 (11.1)	0
4	0		
5	1 (3.1%)	1 (5.6)	0
6	3 (9.4%)	0	3 (21.4)

Table 2

Main results.

[a] Only episodes occurring in the first two years post transplantation during the very administration of everolimus were considered.

[b] Excluding one patient whose immunosuppressive regimen included everolimus ab initio.

[c] Including one patient undergoing EVR-based immunosuppression since the transplantation, uninterruptedly for the entire observation period. In the upper part of the cell are reported the EVR-free immunosuppressive regimens adopted (both in the EVR- and no-EVR group); in the lower part, the EVR-based regimens.

BOS, bronchiolitis obliterans syndrome; MMF, mycophenolate mofetil; TAC, tacrolimus; AZA, azathioprine; EVR, everolimus; CSA, cyclosporin A.

Time post transplant	Study population (N=32)	Patients with at least one CMV positive BAL	Pulmonary CMV infections				
			Evaluable BAL determinations	Number of CMV positive BAL samples (irrespective of IS regimens)	Concomitant with EVR-based regimens	During EVR-free regimens	*p*
1st year, any VL	32	29 (90.6%)	156	76 (48.7%)	18 (11.5%)	58 (37.2%)	0.3160
excluding CMV D+R-	*27*	*24 (88.9%)*	*131*	*58 (44.3%)*	*15 (11.5%)*	*43 (32.8%)*	*0.1995*
1st year, high VLs only	32	13 (40.6%)	156	18 (11.5%)	0	18 (11.5%)	0.0247
excluding CMV D+R-	*27*	*9 (33.3%)*	*131*	*10 (7.6%)*	*0*	*10 (7.6%)*	*0.1216*
1st + 2nd year, any VL	32	29 (90.6%)	209	100 (47.8%)	25 (11.9%)	75 (35.9%)	0.6277
excluding CMV D+R-	*27*	*24 (88.9%)*	*175*	*76 (43.4%)*	*18 (10.3%)*	*58 (33.1%)*	*0.5850*
1st + 2nd year, high VLs only	32	13 (40.6%)	209	20 (9.6%)	0	20 (9.6%)	0.0049
excluding CMV D+R-	*27*	*9 (33.3%)*	*175*	*12 (6.9%)*	*0*	*12 (6.9%)*	*0.0715*

Table 3

Distribution of CMV episodes in BAL specimens for the indicated observation periods and on the basis of concomitant EVR administration. CMV occurrences are compared by Fisher's exact test. For each time and viral load group, CMV occurrences in patients at low risk for CMV reactivation (i.e. excluding pretransplant CMV D+R- serostatus) are reported in Italics. The definition "high viral loads" refers to $\geq 10^5$/mL, as reported in the text. VL, viral load; IS, immunosuppression.

Time post transplant	Study population (n=32)	Patients with at least one CMV blood episode	CMV infections in blood				
			evaluable blood determinations	Number of CMV episodes, irrespective of IS type	concomitant with EVR-based regimens	during EVR-free regimens	*p*
1st year	32	23 (71.9%)	157	48 (30.6%)	11 (7%)	37 (23.6%)	0.5196
excluding CMV D+R- recipients	*27*	*18 (66.7%)*	*132*	*35 (26.5%)*	*9 (6.8%)*	*26 (19.7%)*	*0.4634*
1st + 2nd year excluding CMV D+R- recipients	32	25 (78.1%)	260	64 (24.6%)	21 (8.1%)	43 (16.5%)	0.1959
	27	20 (74.1%)	*217*	*48 (22.1%)*	*16 (7.4%)*	*32 (14.7%)*	*0.0829*

Table 4

Distribution of CMV episodes in blood for the indicated observation periods and on the basis of EVR administration, irrespective of viral load values. For each period and viral load group, CMV occurrences in patients at low risk for CMV reactivation (i.e. excluding those presenting a pre-transplant CMV D+R- serostatus) are reported in Italics. CMV occurrences are compared by Fisher's exact test. IS, immunosuppression.

	Study population	Number of CMV episodes in BAL samples, irrespective of IS type	**BAL CMV viral loads** mean ± SEM (range)			Number of CMV episodes in blood samples, irrespective of IS type	**blood CMV viral loads** mean ± SEM (range)		
Months post transplant	N of patients		concomitant with EVR-based regimens	during EVR-free regimens	p-value		concomitant with EVR-based regimens	during EVR-free regimens	p-value
1st year	32	76	5071 ± 953.5 (1400-12700)	156036 ± 47223 (1500-1641550)	0.0804	48/157 (30.6%)	2973 ± 896.3 (1500-11400)	190000 ± 160500 (600-5935100)	0.5315
excluding CMV D+R- recipients	*27*	*58*	*4999 ± 1069 (1400-12700)*	*68293 ± 22794 (1500-658904)*	*0.1086*	*35/132 (26.5%)*	*3100 ± 1093 (1500-11400)*	*237400 ± 227900 (600-5935100)*	*0.5533*
1st + 2nd year	32	100	5205 ± 812.1 (1400-12700)	126830 ± 37302 (1500-1641550)	0.0636	64/260 (24.6%)	2271 ± 487.3 (1500-11400)	163700 ± 138200 (600-5935100)	0.4194
excluding CMV D+R- recipients	*27*	*76*	*5129 ± 984.7 (1400-12700*	*58386 ± 18119(1500-658904)*	*0.1074*	*48/217 (22.1%)*	*2400 ± 632.5 (1500-11400)*	*193200 ± 185200 (600-5935100)*	*0.4725*

Table 5

Distribution of CMV episodes and viral load means in BAL and in blood for the observation periods indicated in the text. Student's t-test was used for comparison of means. IS, immunosuppression.

Time post transplant	Patients with available data	Patients with histopathologically confirmed AR episodes			Acute rejection intensity											
			concomitant with EVR-based regimens	during EVR free regimens	A1			A2			A3			A4		
					EVR	no-EVR	*p*	EVR	no-EVR	*p*	EVR	no-EVR	*p*	EVR	no-EVR	*p*
1st year	32	24[a] (75%)	4	22	3/8	6/31[b]	0.3548	1/8	11/31	0.3938	0/8	4/31	0.5628	0/8	1/31	1.0
1st + 2nd year	32	25[a] (78.1)	6	22	4/12	6/31[b]	0.4267	2/12	10/31	0.4563	0/12	5/31	0.2996	0/12	1/31	1.0

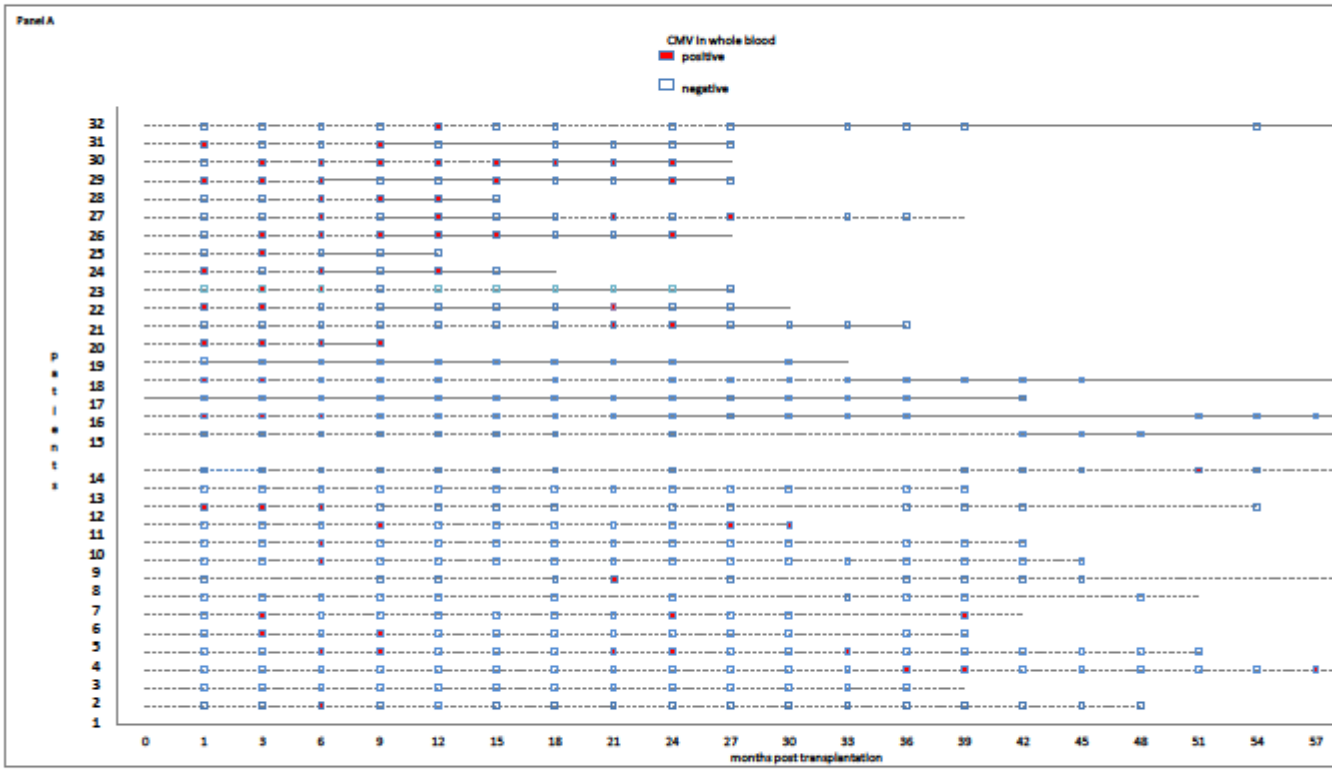
Table 6

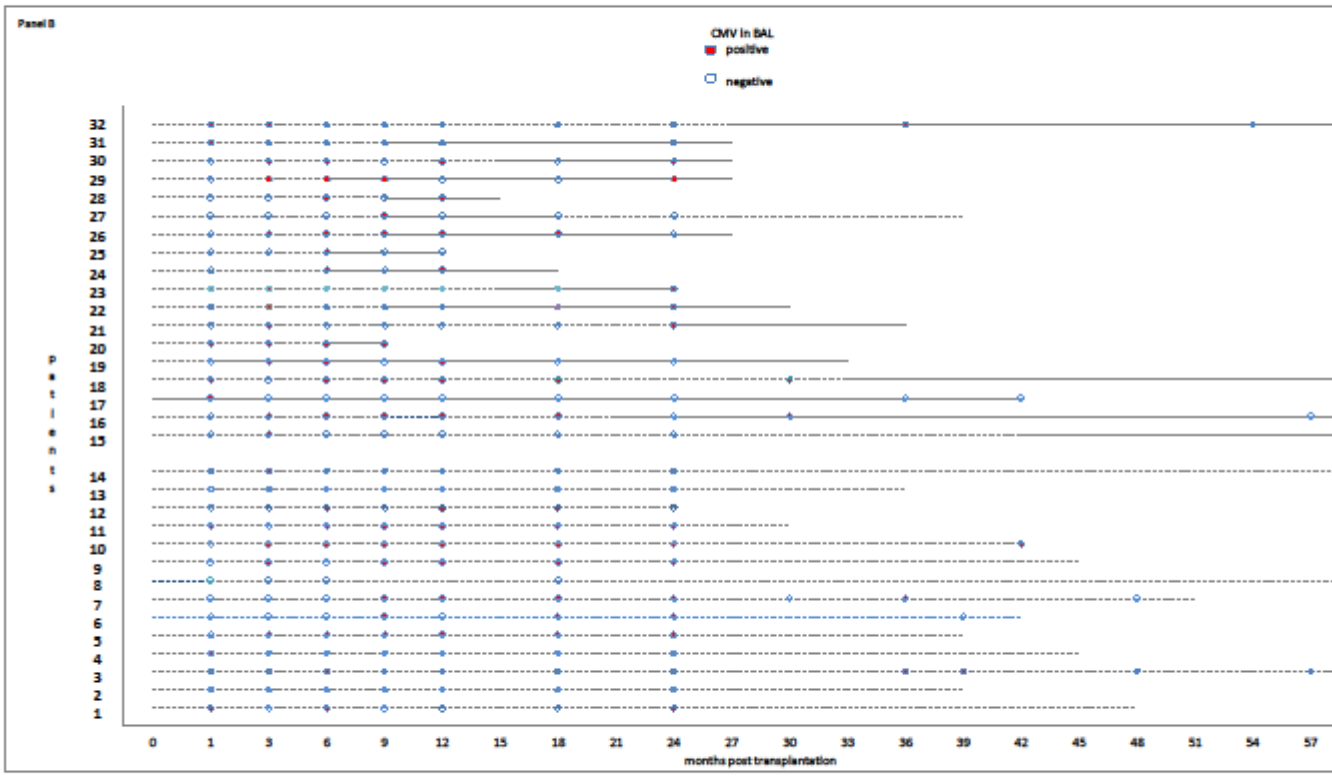
Distribution of patients on the basis of histopathologically confirmed acute rejection on TBB. In right columns are reported the numbers of patients with the indicated maximum intensity-level AR episodes, stratified by concomitant EVR-administration; when different intensity-level episodes were detected in the same time period post transplant, only the highest was reported. Data are analyzed by Fisher's exact test.

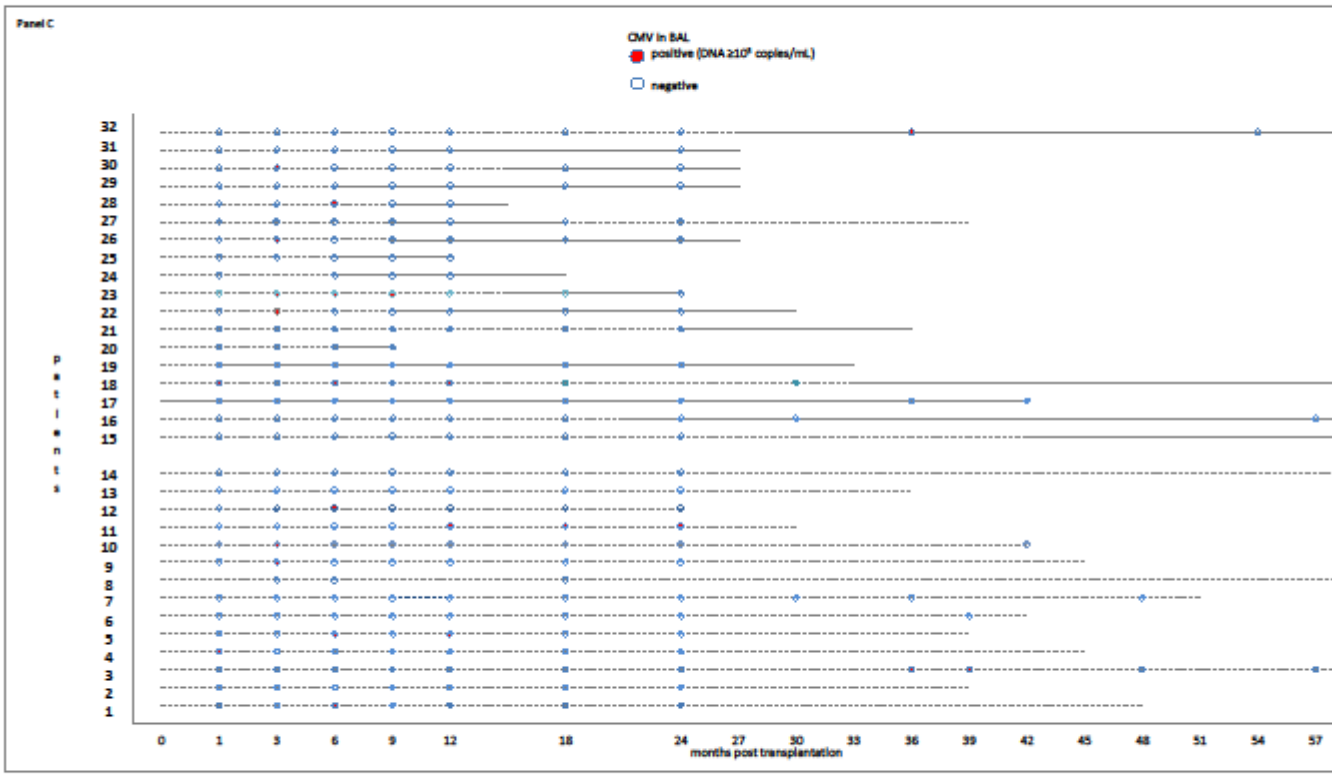
[a] In the first year post transplant, two patients experienced graft acute rejection during both EVR-free and EVR-based regimens (A3 and A2 maximum intensity during EVR-free regimens, respectively, followed by A1-rejection during EVR-based); three patients, considering two-year follow up.

[b] The reported n=31 is due to one patient undergoing EVR-based immunosuppression uninterruptedly during the 24-month follow up.

Figure 1


Panel A
CMV in whole blood
positive
negative
Patients
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
months post transplantation


Panel B
CMV in BAL
positive
negative
Patients
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
57
months post transplantation


Panel C
CMV in BAL
positive (DNA ≥10³ copies/mL)
negative
Patients
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
3
6
9
12
18
24
27
30
33
36
39
42
45
48
51
54
57
months post transplantation