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The importance of intermediate-dose Valacyclovir in primary CMV prophylaxis after Allogeneic-stem cell transplantation, and the advantages of step-wise pre-emptive treatment in CMV reactivation

Osman Kara[1]*, Sinem Acar[1], Tayfun Elibol[2]

1 Bahcesehir University School of Medicine Medical Park Goztepe Training Hospital- Department of Hematology, Istanbul, TR
2 Medeniyet University School of Medicine Goztepe Training and Research Hospital- Department of Hematology, Istanbul, TR

*** Corresponding Author:** Osman Kara **E-mail:** dr_osmankara@hotmail.com

ABSTRACT

Objective: Cytomegalovirus (CMV) reactivation and disease are still one of the most important causes of morbidity and mortality after allogeneic stem cell transplantation (ASCT). Letermovir prophylaxis has been clearly shown to be effective and well-tolerated. Drug interactions and cost are limitations. Alternative regimens such as Valacyclovir 3g-6g a day are of interest. In our study, we investigated the clinical results of intermediate dose (3 gr/d) valacyclovir after ASCT in primary CMV prophylaxis.

Material and Methods: The data of 70 patients who underwent ASCT between 2019-2020 were retrospectively analyzed. Valacyclovir was given at a dose of 3 g/day to all patients for primary CMV prophylaxis after ASCT. If CMV reactivation developed during Valacyclovir prophylaxis, therapeutic oral Valganciclovir or parenteral Ganciclovir was gradually switched according to CMV DNA copy numbers.

Results: The mean age of the patients included in the study was 45.5 years. The D+/R+ seropositivity was 97.2%. CMV reactivation developed in 37/70 (52.8%) patients within the first 100 days after transplantation. While CMV negativity could be achieved with oral VValganciclovir in 17 of the reactive patients (45.9%), hospitalization was required for parenteral ganciclovir use in 20 (28.1%) of them. The median PFS of patients with and without CMV reactivation was 10 months and 18 months, with a one-year PFS were 49.9% and 80.9%, respectively. One-year overall survival rates of patients with and without CMV reactivation were 52.9% and 92.9% respectively.

Conclusion: It has become more important to prevent infections that may develop after ASCT with prophylaxis rather than treating. Post-transplant intermediate-dose Valacyclovir as primary prophylaxis has been shown to reduce CMV reactivation/disease rates at desired levels and reduce hospitalizations.

Keywords: allogeneic stem cell transplantation, ASCT, Cytomegalovirus, CMV, hospitalization, prophylaxis, valaciclovir

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INTRODUCTION

Although allogeneic stem cell transplantation (ASCT) is the only treatment method that can cure many hematological malignancies, complications that increase post-transplant morbidity and mortality continue to cause uneasiness in using this treatment modality. The most threatening post-transplant complications are acute and chronic graft versus host disease (GVHD) and opportunistic infections (bacterial, viral, and fungal).

CMV seropositivity rate in healthy adults is around 70% across the world (1), while in developing countries such as Turkey this rate rises to about 90-99% (2). In immunocompromised patients after ASCT, CMV mostly appears only as reactivation (30-37%), (3) while it is observed as CMV disease at a rate of 1.4-10 % (4, 5). It is not surprising that these rates are high in developing countries. The most common forms of CMV infection in immunosuppressed patients are pneumonia, enteritis, hepatitis, and retinitis (6).

Some non-pharmacological (using CMV negative, leuko-depleted, and filtered blood products) and pharmacological (using prophylaxis or pre-emptive therapy) policies have been adopted to minimize the reactivation and infection risk of CMV (7, 8).

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Although studies are showing the advantages of prophylaxis in terms of reducing CMV reactivation and disease, there is no consensus on the routine use of prophylaxis yet. It is the most valid, objective and constantly updated ECIL (European Conference on Infections in Leukemia) guideline to follow on the complications of CMV after allogeneic transplantation.While pre-emptive treatment was recommended instead of CMV prophylaxis in the first published ECIL guideline, Letermovir prophylaxis was agreed upon in the most recent ECIL-7 guideline ([8], [9]). However, such expensive prophylaxis like Letermovir cannot be used in first-line CMV reactivation due to reimbursement rules of governments in developing countries. So, the treatment approach has been left to the experience and selection priorities of transplant centers (10).

Only high-dose oral Acyclovir, Valacyclovir, Letermovir and parenteral Ganciclovir are proven to be first-line agents that are effective and convenient in CMV prophylaxis (10-12). The use of ganciclovir requires hospitalization due to its parenteral nature and the toxic effects of high dose acyclovir on renal functions have brought Valacyclovir one step ahead in prophylaxis. Based on this awareness, we planned to evaluate the effectiveness of intermediate dose (3 gr/d) valacyclovir in primary CMV prophylaxis after ASCT by comparing our results with recent literature.

MATERIAL and METHODS

The files of 83 patients who underwent ASCT (from related or unrelated donors) due to high-risk hematological malignancy (AML, ALL, MDS, NHL, HL, MM, AA) between January 2019 and December 2020 were retrospectively evaluated within the scope of the study. Based on the inclusion criteria, 13 patients with early mortality in the first 100 days were excluded from the study to rule out the confusion of unknown CMV or transplant-relatedness. The remaining 70 cases were analyzed retrospectively. All protocols, experimental studies, and clinical trials involving human subjects were approved by the ethics committee of the institution before the study began, and that the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Inclusion criteria were given in **Supplementary 1.**

The primary endpoints of our study were CMV reactivation and disease rate, and post-transplant CMV reactivation time. Secondary endpoints were; late CMV reactivation rate, CMV-related hospitalization, and LOS in hospital, and also impact of CMV reactivation on progress-free survival (PFS) and overall survival (OS).

All patients were monitored weekly starting from post-transplant +7 days for CMV DNAemia with an internationally standardized PCR Kit using a whole blood sample with a linear interval of 65-13.000.000 IU/ml (1 IU/ml= 1.2 copies/ml). CMV reactivation is defined as the isolation of the virus or evidence of viral replication ≥1000 copies/ml in the blood or other body fluids in two consecutive measurements in an asymptomatic patient without organ-specific abnormalities.

CMV disease is defined as isolation of the virus from blood or body fluids in patients with symptoms and/or histological evidence of tissue involvement. Late CMV reactivation is defined as the reactivation status of CMV DNA after +100 days from transplantation (7, 8).

An intermediate dose (3 gr/d) of Valacyclovir was started simultaneously with the initiation of the conditioning regimen in all enrolled patients, and the dose was adjusted according to renal function during follow-up. Valacyclovir 3 g/day was planned to be given up to +100 days post-transplant. CMV IgG was studied for CMV serology screening in all recipients and donors before transplantation. D(+)/R(+) and D(+)/R(-) status were admitted as high-risk for CMV reactivation. As a step-wise pre-emptive treatment strategy, asymptomatic patients with CMV reactivation with CMV DNA copies/ml between 1000-5000 were treated with 1800 mg/day oral valganciclovir in an outpatient setting.In patients whose CMV DNA copies/ml ≥ 5000/ml at any time or patients with CMV disease were treated with iv ganciclovir 10 mg/kg/day in an inpatient setting. Pre-emptive treatment was continued until CMV DNA negativity was achieved in two consecutive blood samples. CMV DNA measurements were evaluated at each visit after +100 days depending on the patient's clinical findings and systemic steroid use.

Creatinine (BUN) and creatinine clearance (CrCl) were checked from blood samples at each visit to monitor the most known renal-adverse effects of Valacyclovir. Scoring systems of EBMT (13) for acute GVHD and NIH-consensus 2014 criteria (14) for chronic GVHD were used. All patients received Methotrexate-Cyclosporine A or Post-transplant high-dose Cyclophosphamide combined with Tacrolimus and Mycophenolate Mofetil as GVHD prophylaxis for a minimum of 100 days after transplant. Systemic steroids (1-2 mg/kg/day) were used in acute GVHD grade 3 and above.

Statistical Analysis

IBM SPSS Statistics 25.0 was used for statistical analysis. Descriptive statistics were carried out to assess the central tendency and distribution of study variables (e.g. mean, median, standard deviation, frequencies, minimum/maximum values). Mann-Whitney U test was done to compare the two non-normally distributed variables. Chi-square and Fisher exact tests were used to evaluate the relationship between variables. A binary logistic regression test was performed to ascertain the effects of variables. While OS event was defined as death from any cause, PFS event was defined as relapse or death from any cause. Kaplan-Meier curves were generated for survival analyses, and Log-rank tests were used to assess differences in OS and PFS between study groups. The Cox-regression test was used for the analyses of treatment and prognostic effects of data and assumes a constant hazard ratio. A p-value ≤0.05 was considered statistically significant.

The X-tile model (Version 3.6.1) was used to determine the cutoff values of the CMV DNA copy. Survival curves were plotted using the Kaplan-Meier method, and differences among the individual groups were defined using the log-rank test.

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RESULTS

Patient Characteristics & Outcomes of ASCT

The demographic and Clinical Characteristics of the patients are given in table 1. Patients with high risk for CMV reactivation who underwent ASCT between January 2019 and December 2020 were conducted in our study. Patients characteristics, ASCT characteristics, outcomes of ASCT were summarized in supplementary 1. Median length of stay (LOS) in the hospital for transplantation was 28 days (10-89). When the patients with CMV reactivation were evaluated, secondary graft failure was seen more frequently in patients who were treated with iv ganciclovir (p=0.008).

CMV Reactivation Characteristics

Characteristics of CMV reactivation are given in table 2. No grade 3-4 side effect was seen with valacyclovir prophylaxis. Ganciclovir resistance was not observed in any patient who developed CMV reactivation after valacyclovir prophylaxis. While there was no positive correlation between the use of Fludarabine or ATG use in the conditioning regimen and CMV reactivation (p: 0.157 and p: 0.714, respectively), a statistically significant correlation (p: 0.003) was found between the systemic steroid use (2 mg/kg/d and above) and CMV reactivation. CMV reactivation was observed in all 9 patients using systemic steroids for acute GVHD. In addition, a significant relationship was observed between acute GVHD and CMV reactivation (p: 0.022), independent of steroid use.

Binomial logistic regression was performed to ascertain the effects of acute GVHD, secondary graft failure, donor type on the likelihood that participants have CMV reactivation. The logistic regression model was statistically significant X2(4) = 17.652, p: 0.001. The model explained %29.8 (Nagelkerke R2) of the variance in CMV reactivation and correctly classified %72.9 of cases. Of the four predictor variables, only two were statistically significant: transplantation from a mismatch-unrelated donor (HR: 4.67) and transplantation from a haploidentical donor (HR: 7.97) (table 3).

CMV Reactivation Outcomes

Overall, no statistically significant relationship was found between CMV reactivation and disease progression (p=0.592). Also, when the patients diagnosed with acute leukemia (AML and ALL) were evaluated separately, no relationship was observed between CMV reactivation and disease progression (p: 0.224 and p: 0.635, respectively). However, while CMV reactivation did not affect OS in AML, it was statistically significantly decreased in patients with ALL (p: 0.043). However, this relationship could not be confirmed by cox-regression analysis (p: 0.086). While the mean PFS duration was 10 months in patients with CMV reactivation, it was 18 months in patients who did not develop CMV reactivation. (p=0.093). The 1-year PFS rates were 49.9% and 80.9% in patients with and without CMV reactivation, respectively (figure 1). One year OS of patients with and without CMV reactivation was 52.9% and 92.9% (p: 0.012) respectively; median time for OS for both groups has not been reached (figure 2). Only acute GVHD, CMV reactivation, and disease progression were observed as factors affecting OS in univariate regression analysis. In multivariate regression analysis, both CMV reactivation and disease progression were observed as negative factors for OS with an HR 4.33 and 3.54, respectively (Table 4).

Finding Significant Maximum CMV DNA Copy

There was no association between maximum CMV DNA copy amount and OS (p=0.499). The X-tile model was used to determine the cutoff values of the CMV DNA copy. According to the CMV DNA copy, patients with CMV reactivation were divided into 2 categories: CMV DNA ≤ 7493 copies/ml (n:20), CMV-DNA >7493 copies/ml (n: 17) using the X-tile model. CMV DNA copies/ml >7493 copy were found to have a negative effect on OS with an HR 9.721 (p: 0.004, %95 CI: 2.090 – 45.207) (figure 3A and 3B).

Supplementary 1: Inclusion criteria

1. Being ≥18 years old at the time of transplant
2. Diagnosed with a high-risk hematological malignancy (ALL, AML, etc.)
3. Treated with a myeloablative or reduced-intensity conditioning regimen
4. No previous history of CMV reactivation or disease before transplant
5. Creatinine clearance must be ≥ 50 ml/min
6. Should be no previous solid organ transplantation history
7. Liver enzymes (AST, ALT) must be ≤ 3X higher than normal limits at the transplant

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Table 1. Demographic and Clinical Characteristics of the patients

	CMV Reactivation (+) (n: 37)	CMV Reactivation (-) (n: 33)	Total (n: 70)	*P value*
Age, years (median, range)	45 (18-61)	44 (23-67)	44.5 (18-67)	0.855
Male, sex (n, %)	24 (64.9)	18 (54.5)	42 (60)	0.379
Diagnosis (n, %)				0.884
ALL	11 (29.7)	10 (30.3)	21 (30)	
AML	14 (37.8)	15 (45.5)	29 (41.4)	
MDS	5 (13.5)	3 (9.1)	8 (11.4)	
HL	1 (2.7)	1 (3)	2 (2.8)	
NHL	3 (8.1)	3 (9.1)	6 (8.5)	
AA	1 (2.7)	1 (3)	2 (2.8)	
Conditioning Regimen (n, %)				0.220
MAC	17 (45.9)	20 (60.6)	37 (52.9)	
RIC	20 (54.1)	13 (39.4)	33 (47.1)	
Fludarabine (n, %)	32 (86.5)	30 (90.9)	62 (88.6)	0.714
TBI (n, %)	16 (43.2)	8 (24.2)	24 (34.3)	0.095
ATG (n, %)	7 (18.9)	2 (6.1)	9 (12.9)	0.157
Harvesting (n, %)				1.000
Peripheral	36 (97.3)	32 (97)	68 (97.1)	
Bone marrow	1 (2.7)	1 (3)	2 (2.9)	
Donor (n, %)				**0.005**
Match-related	13 (35.1)	21 (63.6)	34 (48.6)	
Match-unrelated	2 (5.4)	6 (18.2)	8 (11.4)	
Mismatch-unrelated	12 (32.4)	4 (12.1)	16 (22.9)	
Haploidentical	10 (27)	2 (6.1)	12 (17.1)	
Chimerism > %95 (n, %)	35 (94.6)	31 (93.9)	66 (94.3)	1.000
IgG CMV Status D/R (n, %)				0.219
D+/R+	37 (100)	31 (93.9)	68 (97.2)	
D+/R-	-	2 (6.1)	2 (2.8)	
Engraftments (median, range)				
Neutrophil	15 (9-26)	14 (10-26)	15 (9-26)	0.649
Lymphocyte	26 (11-50)	28 (14-62)	27 (11-62)	0.097
Thrombocyte	26 (15-102)	24 (16-66)	25.5 (15-102)	0.762
Engraftment Failure (n, %)				**0.035**
None	21 (56.8)	28 (84.8)	49 (70)	
Primary	5 (13.5)	1 (3)	6 (8.5)	
Secondary	11 (29.7)	4 (12.1)	15 (21.4)	
Acute GVHD (n, %)	13 (35.1)	4 (12.1)	17 (24.2)	**0.025**
Chronic GVHD (n, %)	13 (38.2)	6 (18.2)	19 (27.1)	0.069
CMV Disease (n, %)				
Retinitis	1 (2.7)	-		
Nephritis	2 (5.4)	-		
Colitis	1 (2.7)	-		

CMV: Cytomegalovirus, ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloblastic Leukemia, MDS: Myelodysplastic Syndrome, HL: Hodgkin Lymphoma, NHL: Non-Hodgkin Lymphoma, AA: Aplastic Anemia, MAC: Myeloablative Conditioning, RIC: Reduced-intensity Conditioning, TBI: Total Body Irradiation, ATG: Anti-thymocyte Globulin, GVHD: Graft versus Host Disease

Table 2: CMV reactivation and CMV disease

Time, day (Median, range)	30 (2-194)
CMV Disease (n, %)	
Retinitis	1 (1.4)
Nephritis	2 (2.9)
Colitis	1 (1.4)
Late CMV Reactivation (n, %)	4 (5.7)
CMV Reactivation Treatment (n, %)	
Ganciclovir	20 (28.5)
Valganciclovir	17 (24.2)
Length of Stay for Ganciclovir, day (median, range)	30.5 (8-74)
CMV DNA, maximum copies/ml (mean + std)	33.941 ± 47.483
CMV clearance, day (median, range)	21 (7-61)

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Table 3: Logistic regression analysis for CMV reactivation

	B	SE	p	HR	95.0% CI for Exp(B)	
Secondary Graft Failure	0,555	0,736	0,451	1,742	0,412	7,366
Mismatch-Unrelated Donor	1,542	0,692	**0,026**	4,674	1,204	18,155
Haploidentical Donor	2,076	0,863	**0,016**	7,972	1,469	43,268
Acute GVHD	1,242	0,692	0,072	3,464	0,893	13,439

Table 4. Univariate and multivariate analysis for overall survival.

Univariate Cox-regression Analysis

	Mean	B	SE	p	HR	95.0% CI for Exp(B)	
CMV Reactivation	0,522	1,466	0,637	**0,021**	4,330	1,242	15,098
Acute Leukemia	0,71	-0,280	0,534	0,959	0,973	0,341	2,771
Conditioning	1,464	0,457	0,487	0,348	1,580	0,608	4,104
Donor Source Match-Related (Reference)				0,233			
Donor Source Match-Unrelated	0,116	-0,270	1,081	0,803	0,763	0,092	6,355
Donor Source Mismatch-Unrelated	0,232	0,594	0,606	0,328	1,811	0,552	5,941
Donor Source Haploidentical	0,174	1,159	0,609	0,057	3,187	0,967	10,509
Graft Failure None (Reference)				0,233			
Primary Graft Failure	0,087	0,632	0,784	0,42	1,882	0,405	8,754
Secondary Graft Failure	0,217	0,879	0,529	0,096	2,410	0,855	6,795
Acute GVHD	0,246	1,016	0,496	**0,04**	2,763	1,046	7,298
Disease Progression	0,159	1,094	0,498	**0,028**	2,986	1,126	7,919

Multivariate Cox-regression Analysis

	Mean	B	SE	p	HR	95.0% CI for Exp(B)	
CMV Reactivation	0,522	1,466	0,679	**0,031**	4,330	1,144	16,386
Acute GVHD	0,246	0,475	0,528	0,368	1,609	0,571	4,530
Disease Progression	0,159	1,265	0,502	**0,012**	3,544	1,326	9,477

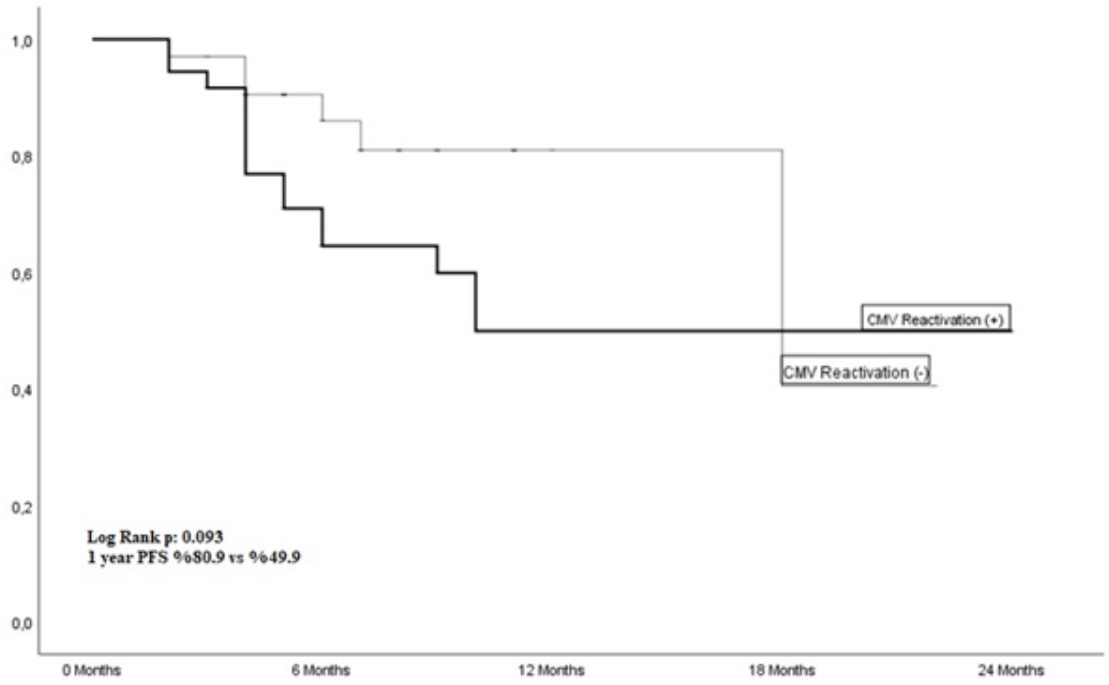

Figure 1. Progression-free survival (PFS) of the patients with and without CMV reactivation

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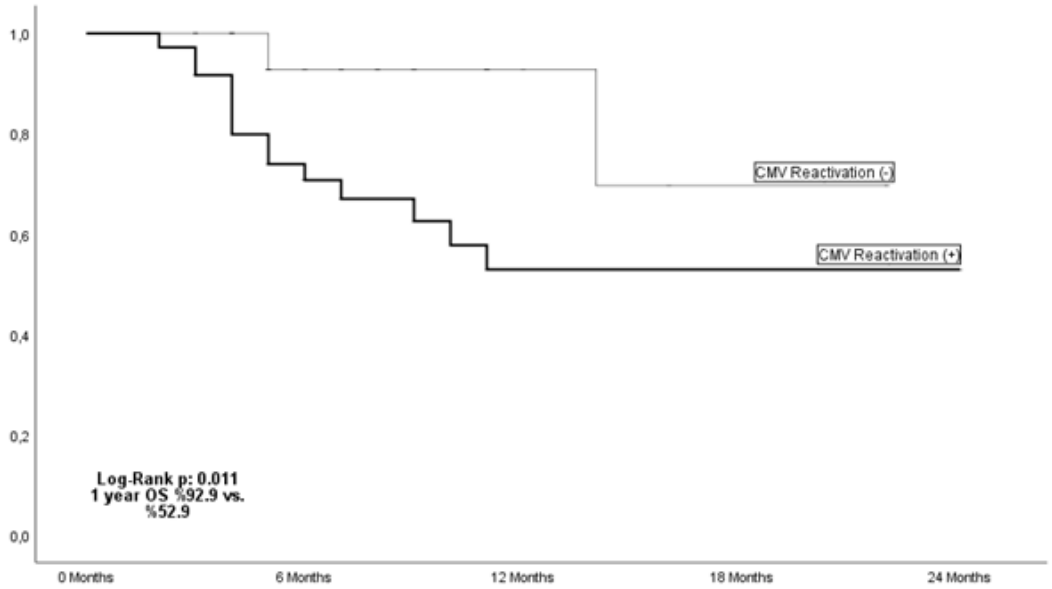

Figure 2. Overall survival (OS) of the patients with and without CMV reactivation

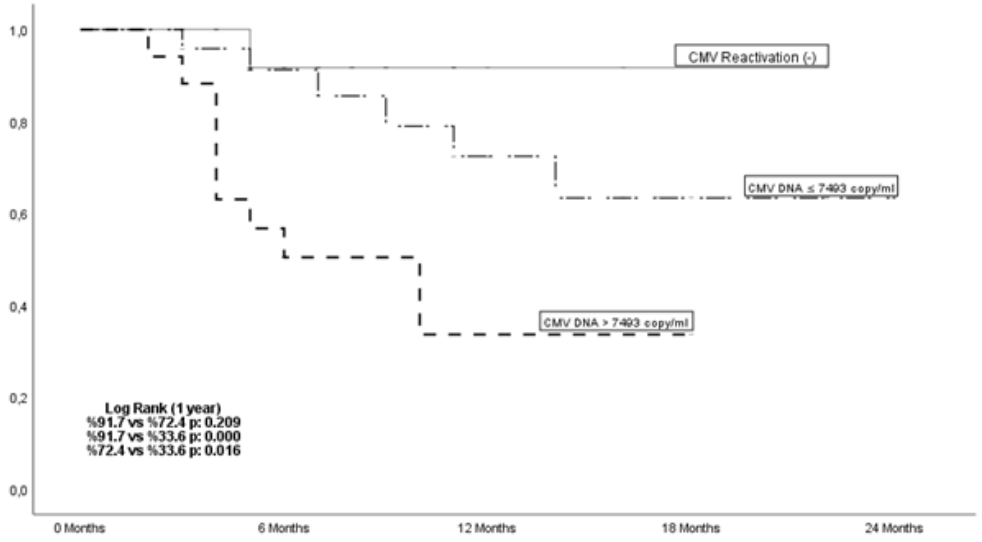

Figure 3A. Overall survival (OS) analysis according to CMV viral load

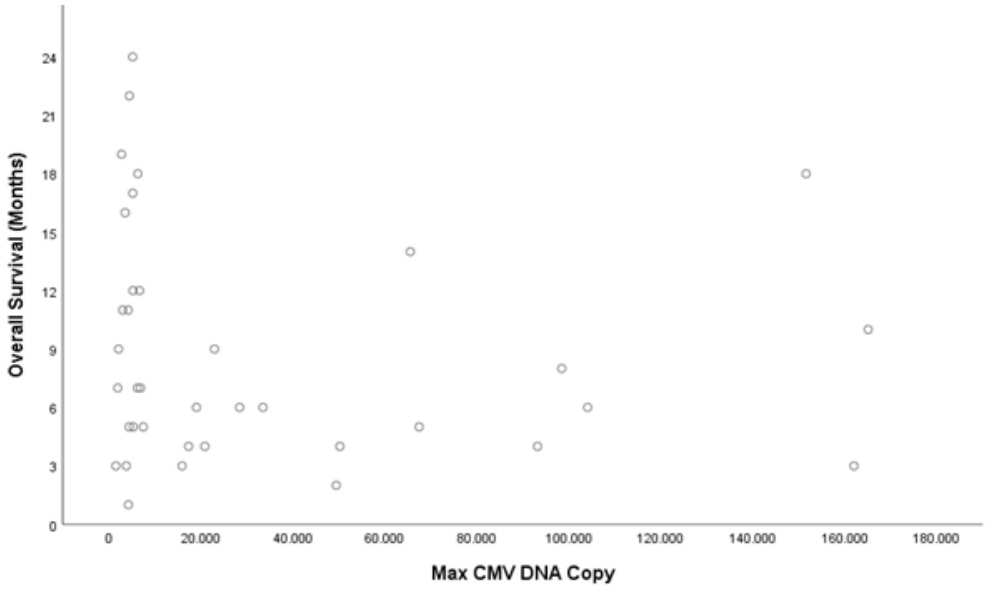

Figure 3B. CMV DNA copy number distribution and overall survival

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DISCUSSION

CMV reactivation and disease occurring after allogeneic stem cell transplantation still pose an important risk on mortality and morbidity (15). Although the approval of Letermovir (16) by the FDA and EMA in 2017 for primary CMV prophylaxis after transplant seems to have marked a breakthrough in OS regard, LTV is not reimbursed in the first-line treatment for CMV prophylaxis by health authorities in many countries. As such, transplant centers continue to work on finding the most suitable conventional antiviral agent for primary CMV prophylaxis, in line with their own experiences.

Considering the CMV seropositivity rates of the population in our country (2) and the recipient/donor CMV serostatus included in the study, our results obtained with the intermediate dose (3 gr/d) Valacyclovir for primary CMV prophylaxis after allogeneic SCT are quite gratifying when compared with both current literature and our historic data. The CMV serostatus of donor and recipient (D+/R+) was 97.2% in the study. Of the 70 patients included in the study, CMV reactivation was observed in 37 patients (52.9%), while CMV disease was detected in only 4 patients (5.7%). According to the step-wise pre-emptive treatment model based on CMV DNA copy number at the time of reactivation, 17 of 37 reactivated patients (24.2%) were successfully treated with oral Valganciclovir, while 20 patients (28.5%) required parenteral ganciclovir and were hospitalized. The average LOS for parenteral ganciclovir was 30.5 days. Ganciclovir resistance was not observed in any patient who developed CMV reactivation after valacyclovir prophylaxis. Late-term CMV reactivation was observed in 4 patients (5.7%). No statistically significant relationship was observed between CMV reactivation and primary disease progression. One-year PFS of patients with and without CMV reactivation were 49.9% and 80.9 respectively (p:0.093). One-year OS of patients with and without CMV reactivation were 52.9% and 92.9% (p: 0.012), respectively; median time for OS for both groups has not been reached.

It was seen that D+/R+ rates of our study (97.2%) are significantly higher than the studies in the literature. In addition, the fact that almost all recipients' seropositivity of CMV puts all patients at high risk for CMV reactivation. While this rate was 77% in the study conducted by Diaz et al. (17) in Latin America, it was found to be 57% in the study conducted by Ljungman et al. (18) from Europe. Post-transplant CMV reactivation rate of 52.8% in this study was not surprising with such high seropositivity of D/R compared to the literature. In a study conducted by Winston et al (19), high-dose Valacyclovir (8 g/d) and ganciclovir were compared after the use of standard Acyclovir in primary prophylaxis, and the CMV reactivation rate was found as low as 14%. The low sensitivity method (urine and blood culture screening) used to detect CMV reactivation in this study, as well as the 54% D/R seropositivity in the study population, may be the main reasons for the differences between these two studies. CMV reactivation developed in 3 (25%) of 12 patients who received primary protection with valacyclovir 3 gr/d in the study performed by Vusirikala et al.20 In this study, pp65 antigenemia searching method, which is much less sensitive than the PCR technique, was used in CMV monitoring. In addition, both low numbers of patients enrolled in the study (12 patients) and low D+/R+ seropositivity (75%) also led to a difference. In a multicenter study conducted by Ljungman et al. (18), high-dose Valacyclovir (8 g/d) and high-dose Acyclovir were compared as primary CMV prophylaxis after using standard parenteral acyclovir treatment in both groups, and the CMV reactivation rate was found to be 33% in the valacyclovir arm. The reasons for the difference between the results can be counted as not using only the PCR technique as a CMV DNA monitoring, low D/R seropositivity (57%) of the study population, dosage of the Valacyclovir used for primary prophylaxis and stem cells source of the patients (most of them from MRD).

With ganciclovir ± low-dose valacyclovir treatment, which we used for primary CMV prophylaxis after ASCT between 2015 and 2018 in our transplant center in a similar patient group (D+/R+ seropositivity 95%), CMV reactivation rate was 61% and our CMV-related disease rate was around 10%. Although it seems that we have achieved very partial success in CMV reactivation and related disease rates with the intermediate dose valacyclovir treatment, we achieved a significant improvement in CMV-related hospitalization rates (from 45.1% to 28.5%) with intermediate-dose valacyclovir prophylaxis with a step-wise preemptive therapy model. This decrease in hospitalization rates had enabled us to cope with both economically and at a time when it was difficult to find a hospital bed like the Covid-19 pandemic.

The rate of CMV-related disease (5.7%) in our study was similar to the literature. In the studies mentioned above (18-20) the rates of CMV disease vary between 2.4-8.3%. There may be two reasons for finding similar CMV disease development rates after Allo-SCT in almost all of the studies in the literature in which different prophylactic anti-viral agents were used; the fact that effected tissue sampling is the only standard method for demonstrating CMV disease and that the frequency of CMV disease development can be reduced at almost similar rates with different anti-viral agents including LTV.

The onset of CMV reactivation was found to be median 30 days after transplant, in line with the literature (19). Late-term (+100 day) reactivation, which is most related to prolonged immunosuppressive use, occurred in only 4 of 37 (5.7%) patients with CMV reactivation. Fludarabine, total body irradiation (TBI), or anti-thymocyte globulin (ATG) used as a part of the conditioning regimen, did not have a negative impact on CMV reactivation. Although a significant relationship was found between Fludarabine and CMV reactivation in a study conducted by Junghanss et al. (21), this correlation was not shown in our study. A significant relationship was observed between systemic steroid use for acute GVHD treatment and CMV reactivation (p: 0.003), consistent with the literature. In patients with CMV reactivation, the mean maximum number of CMV DNA copies/ml was 33.941 + 47.483. Although, we cannot find a correlation between viral load and OS (p: 0.499) in general terms, when we take the CMV DNA copies/ml number as a threshold value for 7493 which was found by X-tile model; it was found that OS was worse in patients with 7493 or more copies (p: 0.004, HR 9.721, %95 CI: 2.090 – 45.207). There are few articles in the literature showing the increment of CMV infections and the decrement of OS time when the CMV DNA copy number exceeds 8200/ml (22).

One of the most important points we want to emphasize in our study is the hospitalization rates and hospital stay processes associated with CMV reactivation/disease. Hospitalization for parenteral ganciclovir treatment was required in 20 of 37 CMV reactivated patients (28.5%). The average hospital stay was 30.5 days (8-74 days). Although there is not much information about CMV-related hospitalization rates and LOS in the literature, in a multicenter study designed by Schelfout et al (23) the first hospitalization period associated with CMV in the first 100 days after transplant was 31.9 days which overlaps with our data. A retrospective study on patients receiving their first ASCT found the incidence of CMV episodes during the first year related to a higher total LOS (average of 26.4 additional days) when compared to those without CMV infection (24). Our hospitalization rates were 45.1% due to CMV-related complications after Ganciclovir±Valacyclovir primary prophylaxis strategy (2015-2018). In times of difficult hospitalization processes, the importance of primary CMV prophylaxis with suitable oral anti-viral agents after ASCT is once again revealed.

The relationship between the prophylactic approach and OS has been pointed in many studies and it has been shown that most of the anti-viral agents except high dose acyclovir and letermovir do not provide an advantage over OS (12, 25-27). Since, we included only the patients transplanted in the last 24 months in our study, the follow-up period was found to be an average of 7 months (1-24 months). In this study 1-year OS rates in patients with and without CMV reactivation were 52.9% and 73.9% ($p = 0.012$), respectively and 1-year PFS was 76.9% and 90.5% (10 months vs 18 months, $p = 0.093$) respectively. In a study conducted by Dwabe et al, (28) 1-year OS and 1-year PFS were found to be 85% and 87%, respectively in the LTV prophylaxis group. Although there are reports that CMV reactivation prevents especially AML recurrence by increasing NK cell activity and triggering the graft versus leukemia effect after transplantation, (29, 30) no effect of CMV reactivation on disease progression was found in any patient group in this study. However, it is too early to say whether there will be a decrease in long-term disease recurrence.

Although there are studies in the literature mentioning grade 3-4 side effects (such as renal dysfunction, mental status changes, persistent nausea, and vomiting) that can cause drug cessation or dose adjustments during high-dose valacyclovir prophylaxis, no such side effects were observed in our study.

Another reason for relatively high CMV reactivation rates after Allo-SCT in our study was the diversity of donor sources used in the transplant setting; HLA full-match relative (MRD) 48.6%, full-match or one-mismatch unrelated (MUD) 34.3%, and HLA haploidentical 17.1%. As is very common in the literature, (31) post-transplant CMV reactivation rates from haploidentical and mismatch unrelated donors were found to be significantly higher in this study ($p=0.05$).

Main limitations of our study were that it was planned in a retrospective design and performed with a limited number of patients. In addition, the absence of another study in the literature conducted with an isolated valacyclovir intermediate dose (3 g/d) made it difficult for us to compare our data fully.

Again, the high CMV IgG rates (90% and above) in our country may negatively affect all study data. Finally, our average follow-up (7 months) period may be considered insufficient.

CONCLUSION

In an environment where CMV reactivation rate can reach up to 80% (32) in patients with ASCT for whom primary CMV prophylaxis is not administered, and where the negative effects of CMV reactivation on mortality and morbidity with/without causing disease are well-known, valacyclovir 3 g/d is effective in primary CMV prophylaxis in case of being unable to use LTV. Another point that should not be forgotten is in cases where hospital occupancy rates are high, it has become more important to prevent infections that may develop after ASCT with prophylaxis rather than treatment. Considering its success in reducing CMV disease and hospitalization periods rather than preventing CMV reactivation, we can say that primary CMV prophylaxis with intermediate-dose Valacyclovir is as successful and cost-effective as Letermovir.

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REFERENCES

1. Ljungman P, Brand R. Factors influencing CMV seropositivity in stem cell transplant patients and donors. haematologica. 2007 Aug 1;92(8):1139-42.

2. Akpınar H, Arslan H, Artuk C, et al. Consensus Report On CMV Diagnosis and Treatment in TURKEY. EKMUD. 2020;

3. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, Green JS, Saad A, Antin JH, Savani BN, Lazarus HM. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood, The Journal of the American Society of Hematology. 2016 May 19;127(20):2427-38.

4. Green ML, Leisenring W, Xie H, Mast TC, Cui Y, Sandmaier BM, Sorror ML, Goyal S, Özkök S, Yi J, Sahoo F. Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. The Lancet Haematology. 2016 Mar 1;3(3):e119-27.

5. Schuster MG, Cleveland AA, Dubberke ER, Kauffman CA, Avery RK, Husain S, Paterson DL, Silveira FP, Chiller TM, Benedict K, Murphy K. Infections in hematopoietic cell transplant recipients: results from the organ transplant infection project, a multicenter, prospective, cohort study. InOpen forum infectious diseases 2017 (Vol. 4, No. 2, p. ofx050). US: Oxford University Press.

6. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, Pikis A, Razonable RR, Miller V, Griffiths PD, Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clinical Infectious Diseases. 2016 Sep 28:ciw668.

7. Ljungman P, De La Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P, Styczynski J, Ward K. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. Bone marrow transplantation. 2008 Aug;42(4):227-40.

8. Ljungman P, de la Camara R, Robin C, Crocchiolo R, Einsele H, Hill JA, Hubacek P, Navarro D, Cordonnier C, Ward KN. Guidelines for the management of cytomegalovirus infection in patients with haematological malignancies and after stem cell transplantation from the 2017 European Conference on Infections in Leukaemia (ECIL 7). The Lancet Infectious Diseases. 2019 Aug 1;19(8):e260-72.

9. Tissot F, Agrawal S, Pagano L, Petrikkos G, Groll AH, Skiada A, Lass-Flörl C, Calandra T, Viscoli C, Herbrecht R. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. haematologica. 2017 Mar;102(3):433.

10. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study.

11. Meyers JD, Reed EC, Shepp DH, Thornquist M, Dandliker PS, Vicary CA, Flournoy N, Kirk LE, Kersey JH, Thomas ED, Balfour Jr HH. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. New England Journal of Medicine. 1988 Jan 14;318(2):70-5.

12. Prentice HG, Gluckman E, Powles R, Ljungman P, Milpied NJ, Fernandez-Ranada JM, Mandelli F, Kho P, Bell AR, Kennedy L. Impact of long-term acyclovir on cytomegalovirus infection and survival after allogeneic bone marrow transplantation. The Lancet. 1994 Mar 26;343(8900):749-53.

13. Reshef R, Saber W, Bolaños-Meade J, Chen G, Chen YB, Ho VT, Ponce DM, Nakamura R, Martens MJ, Hansen JA, Levine JE. Acute GVHD diagnosis and adjudication in a multicenter trial: a report from the BMT CTN 1202 Biorepository Study. Journal of Clinical Oncology. 2021 Jun 10;39(17):1878-87.

14. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, Palmer J, Weisdorf D, Treister NS, Cheng GS, Kerr H. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biology of Blood and Marrow Transplantation. 2015 Mar 1;21(3):389-401.

15. Schmidt-Hieber M, Labopin M, Beelen D, Volin L, Ehninger G, Finke J, Socié G, Schwerdtfeger R, Kröger N, Ganser A, Niederwieser D. CMV serostatus still has an important prognostic impact in de novo acute leukemia patients after allogeneic stem cell transplantation: a report from the Acute Leukemia Working Party of EBMT. Blood, The Journal of the American Society of Hematology. 2013 Nov 7;122(19):3359-64.

16. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, Haider S, Ullmann AJ, Katayama Y, Brown J, Mullane KM. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. New England Journal of Medicine. 2017 Dec 21;377(25):2433-44.

17. Diaz L, Rosales J, Rosso F, Rosales M, Estacio M, Manzi E, Jaramillo FJ. Cytomegalovirus disease in patients with hematopoietic stem cell transplantation, experience over 8 years. Hematology, Transfusion and Cell Therapy. 2020 Mar 30;42:18-24.

18. Ljungman P, De la Camara R, Milpied N, Volin L, Russell CA, Crisp A, Webster A, Valacyclovir International Bone Marrow Transplant Study Group. Randomized study of valacyclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. Blood, The Journal of the American Society of Hematology. 2002 Apr 15;99(8):3050-6.

19. Winston DJ, Yeager AM, Chandrasekar PH, Snydman DR, Petersen FB, Territo MC, Valacyclovir Cytomegalovirus Study Group. Randomized comparison of oral valacyclovir and intravenous ganciclovir for prevention of cytomegalovirus disease after allogeneic bone marrow transplantation. Clinical infectious diseases. 2003 Mar 15;36(6):749-58.

20. Vusirikala M, Wolff SN, Stein RS, Brandt SJ, Morgan DS, Greer JP, Schuening FG, Dummer JS, Goodman SA. Valacyclovir for the prevention of cytomegalovirus infection after allogeneic stem cell transplantation: a single institution retrospective cohort analysis. Bone marrow transplantation. 2001 Aug;28(3):265-70.

21. Junghanss C, Storb R, Maris MB, Carter RA, Sandmaier BM, Maloney DG, McSweeney PA, Corey L, Boeckh M. Impact of unrelated donor status on the incidence and outcome of cytomegalovirus infections after non-myeloablative allogeneic stem cell transplantation. British journal of haematology. 2003 Nov;123(4):662-70.

22. Yoon JH, Lee S, Kim HJ, Jeon YW, Lee SE, Cho BS, Lee DG, Eom KS, Kim YJ, Min CK, Cho SG. Impact of cytomegalovirus reactivation on relapse and survival in patients with acute leukemia who received allogeneic hematopoietic stem cell transplantation in first remission. Oncotarget. 2016 Mar 29;7(13):17230.

23. Schelfhout J, Bonafede M, Cappell K, Cole AL, Manjelievskaia J, Raval AD. Impact of cytomegalovirus complications on resource utilization and costs following hematopoietic stem cell transplant. Current Medical Research and Opinion. 2020 Jan 2;36(1):33-41.

24. Cordonnier C, Maury S, Esperou H, Pautas C, Beaune J, Rodet M, Lagrange JL, Rouard H, Beaumont JL, Bassompierre F, Glückman E. Do minitransplants have minicosts? A cost comparison between myeloablative and nonmyeloablative allogeneic stem cell transplant in patients with acute myeloid leukemia. Bone marrow transplantation. 2005 Oct;36(7):649-54.

25. Sharma P, Gakhar N, MacDonald J, Abidi MZ, Benamu E, Bajrovic V, Purev E, Haverkos BM, Tobin J, Kaiser J, Chase S. Letermovir prophylaxis through day 100 post transplant is safe and effective compared with alternative CMV prophylaxis strategies following adult cord blood and haploidentical cord blood transplantation. Bone Marrow Transplantation. 2020 Apr;55(4):780-6.

26. Lin A, Maloy M, Su Y, Bhatt V, DeRespiris L, Griffin M, Lau C, Proli A, Barker J, Shaffer B, Giralt SA. Letermovir for primary and secondary cytomegalovirus prevention in allogeneic hematopoietic cell transplant recipients: Real-world experience. Transplant Infectious Disease. 2019 Dec;21(6):e13187.

27. Karam E, LaPorte J, Sizemore C, Zhang X, Holland HK, Solh M, Morris LE, Bashey A, Solomon SR. Real world outcomes of letermovir prophylaxis in unselected high risk CMV seropositive hematopoietic stem cell transplant recipients. Blood. 2019 Nov 13;134:3269.

28. Dwabe S, Hsiao M, Yaghmour G. Real-World Experience Using Letermovir for CMV Prophylaxis in High-Risk Allogeneic Hematopoietic Stem Cell Patients in the Setting of Using T-Cell Depletion As Gvhd Prophylaxis and the Impact on CMV Reactivation, 1-Year Overall Survival, and 1-Year Gvhd-Free-Relapse-Free Survival. Blood. 2020 Nov 5;136:29.

29. Löunnqvist B, Ringdegn O, Ljungman P, Wahren B, Gahrton G. Reduced risk of recurrent leukaemia in bone marrow transplant recipients after cytomegalovirus infection. British journal of haematology. 1986 Aug;63(4):671-9.

30. Béziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Björklund AT, Retière C, Sverremark-Ekström E, Traherne J, Ljungman P, Schaffer M. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. Blood, The Journal of the American Society of Hematology. 2013 Apr 4;121(14):2678-88.

31. Ljungman P, Perez-Bercoff L, Jonsson J, Avetisyan G, Sparrelid E, Aschan J, Barkholt L, Larsson K, Winiarski J, Yun Z, Ringdén O. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. haematologica. 2006 Jan 1;91(1):78-83.

32. Boeckh M. Current antiviral strategies for controlling cytomegalovirus in hematopoietic stem cell transplant recipients: prevention and therapy. Transplant infectious disease. 1999 Sep;1(3):165-78.