Mycophenolate mofetil is associated with inferior overall survival in cytomegalovirus-seropositive patients with acute myeloid leukemia undergoing hematopoietic cell transplantation

A combination of a calcineurin-inhibitor (CNI) with mycophenolate mofetil (MMF) or methotrexate (MTX) is commonly used as graft-*versus*-host disease (GvHD) prophylaxis for HLA-matched donor hematopoietic cell transplantation (HCT). However, the use of MMF, which targets both T- and B-lymphocytes, is associated with a higher risk of cytomegalovirus (CMV) reactivation as compared to MTX.[1] Moreover, CMV reactivation is associated with an increased risk of non-relapse mortality (NRM) and worse overall survival (OS).[2] We, therefore, postulated that the use of MMF as compared to MTX may increase these risks, especially in CMV-seropositive recipients. To investigate this hypothesis, we compared the HCT outcomes of four groups: MTX/CMV$^-$ (n=916), MTX/CMV$^+$ (n=1,527), MMF/CMV$^-$ (n=267), and MMF/CMV$^+$ (n=395).

We used an existing Center for International Blood and Marrow Transplant Research (CIBMTR) publicly available dataset[3] from a previous publication.[4] Our study population included patients ≥18 years with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic neoplasm (MDS) who underwent HCT with peripheral blood grafts from 10/10 HLA-matched unrelated donors between 2008 and 2017. All patients received tacrolimus with either MMF or MTX. Patients who received *ex-vivo* T-cell-depleted/CD34$^+$ cell-selected grafts or post-transplant cyclophosphamide (PTCy) were excluded. Our primary outcome of interest was OS. Secondary outcomes included grades III-IV acute GvHD, chronic GvHD, relapse, and NRM. The Kaplan-Meier method was used to estimate OS probability. The cumulative incidence method accounting for competing risks was used to estimate the incidence of GvHD, NRM, and relapse. Competing risks considered were death or disease relapse for GvHD; disease relapse or relapse-related deaths for NRM; and death before relapse (NRM) for relapse. Predictors in univariate and multivariable analyses were evaluated using Cox proportional hazards regression for OS, and Fine-Gray regression accounting for competing risks for GvHD, NRM, and relapse. Factors significant at the 5% level (P≤0.05) were retained in the final model, except the main effect (GvHD prophylaxis/recipient CMV serostatus), which was retained in the final model irrespective of the level of statistical significance. Bonferroni-adjusted P values are reported for multivariate analyses for the main effect to account for multiple testing. Interaction effects between the main effect and other statistically significant co-variables were tested and accounted for as indicated in multivariable regression analyses. All reported outcomes are at 3 years, except acute GvHD which is at day 180. The proportionality of hazards assumption was tested graphically and statistically. All statistical analyses were performed using STATA/IC 16.1 (StataCorp LLC, College Station, TX, USA). As the data analysis was carried out at The MD Anderson Cancer Center, the local Institutional Review Board approved this study (protocol: 2022-0684), which was conducted in accordance with the Declaration of Helsinki. The dataset is publicly available[3] for data sharing, in accordance with CIBMTR guidelines.

The study population's baseline characteristics are shown in Table 1. In patients with AML (n=1,572), OS was significantly (P<0.001) inferior in CMV$^+$ recipients who received MMF prophylaxis. Overall survival at 3 years was only 31% (95% confidence interval [95% CI]: 25-37) in the MMF/CMV$^+$ group, compared with 54% (95% CI: 45-63) in the MMF/CMV$^-$ group, 51% (95% CI: 48-55) in the MTX/CMV$^+$ group, and 58% (95% CI: 53-63) in the MTX/CMV$^-$ group (Figure 1A). This effect persisted in multivariate analysis: compared to the overall mortality in the MTX/CMV$^-$ group, the risk was 1.8-fold higher in the MMF/CMV$^+$ group, while it did not differ significantly in other groups (Table 2, *Online Supplementary Table S1*). Inferior OS in the MMF/CMV$^+$ group was driven by a higher risk of NRM (Figure 1B). The risks of relapse (Figure 1C) and grade III-IV acute and chronic GvHD (Figure 1D) did not differ significantly between the groups.

In MDS patients (n=1,080), GvHD prophylaxis/recipient CMV serostatus had no significant association with OS, or the risk of relapse, but NRM varied by the Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) score (Table 2, *Online Supplementary Figure S1A*). Among those with an HCT-CI score ≥3, the MTX/CMV$^+$ group had a higher risk of NRM than had the MTX/CMV$^-$ group, while no statistically significant differences were seen in patients with an HCT-CI score 0-2. The risk of chronic GvHD was higher in the MMF/CMV$^-$ group than in the MTX groups.

In ALL patients (n=453), GvHD prophylaxis/recipient CMV serostatus had no significant associations with OS, NRM, or relapse (Table 2, *Online Supplementary Figure S1B*). The risk of chronic GvHD was higher in the MMF/CMV$^-$ group than in the MTX groups.

There were no significant predictors of grade III-IV acute GvHD in any disease type.

Next, we tested whether the use of PTCy prophylaxis altered these observations. We used a separate CIBMTR matched unrelated donor peripheral blood HCT cohort[5] of patients with AML (n=136), ALL (n=42) or MDS (n=64) who received PTCy/CNI/MMF prophylaxis (*Online Supplementary Table S2*). In this population too, in multivariate analysis, CMV$^+$ recipients had a significantly worse OS than CMV$^-$ recipients, but only if they had AML (hazard ratio [HR]=2.7, 95% CI: 1.1-6.4; *P*=0.03) and not if they had ALL (HR=0.4, 95% CI: 0.1-1.5; *P*=0.2), or MDS (HR=0.7, 95% CI: 0.3-1.5; *P*=0.3). The risk of relapse and NRM did not differ by CMV serostatus in any disease group, although these analyses were limited by the small number of events.

In summary, in the CNI study population of patients who all received tacrolimus for GvHD prophylaxis with either

Table 1. Baseline characteristics.

N of patients	MTX/CMV$^+$	MTX/CMV$^-$	MMF/CMV$^+$	MMF/CMV$^-$
	1,527	916	395	267
Recipient age in years				
Median (range)	57 (18-78)	56 (19-78)	62 (20-83)	61 (19-78)
≤40, N (%)	278 (18)	168 (18)	34 (9)	34 (13)
41-50, N (%)	231 (15)	142 (15)	38 (10)	32 (12)
51-60, N (%)	382 (25)	250 (27)	87 (22)	56 (21)
>60, N (%)	636 (42)	356 (39)	236 (60)	145 (54)
Disease, N (%)				
AML	836 (55)	391 (43)	223 (56)	122 (46)
ALL	239 (16)	135 (15)	44 (11)	35 (13)
MDS	452 (30)	390 (43)	128 (32)	110 (41)
Conditioning intensity, N (%)				
MAC	889 (58)	506 (55)	104 (26)	93 (35)
RIC/NMA	631 (41)	408 (44)	290 (73)	174 (65)
Missing	7	2	1	0
In vivo T-cell depletion, N (%)				
Yes	624 (41)	594 (65)	190 (48)	140 (52)
No	903 (59)	322 (35)	205 (52)	127 (48)
Donor/recipient gender, N (%)				
Male/male	652 (43)	443 (48)	170 (43)	134 (50)
Male/female	489 (32)	227 (25)	105 (27)	59 (22)
Female/male	155 (10)	123 (13)	55 (14)	36 (13)
Female/female	230 (15)	123 (13)	65 (16)	38 (14)
Missing	1	0	0	0
Disease status at HCT, N (%)				
Early	753 (49)	395 (43)	170 (43)	106 (40)
Intermediate	193 (13)	114 (12)	54 (14)	27 (10)
Advanced	563 (37)	397 (43)	164 (41)	132 (49)
Missing	18 (1)	10 (1)	7 (2)	2 (1)
HCT-CI, N (%)				
0-2	734 (48)	497 (54)	164 (41)	121 (45)
≥3	793 (52)	419 (46)	231 (58)	146 (55)
Donor age, N (%)				
>35 years	408 (27)	216 (24)	96 (24)	51 (19)
Donor CMV, N (%)				
Seronegative	884 (58)	681 (74)	214 (54)	204 (76)
Seropositive	643 (42)	235 (26)	181 (46)	63 (24)
Year of HCT, median (range)	2014 (2008-17)	2014 (2008-17)	2013 (2008-17)	2014 (2008-17)
Follow-up among survivors, in months, median (IQR)	48 (36-65)	48 (36-68)	50 (36-73)	49 (36-72)

MTX: methotrexate; CMV: cytomegalovirus; MMF: mycophenolate mofetil; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MDS: myelodysplastic neoplasms; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; NMA: non-myeloablative conditioning; HCT: hematopoietic cell transplantation; HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index; IQR: interquartile range.

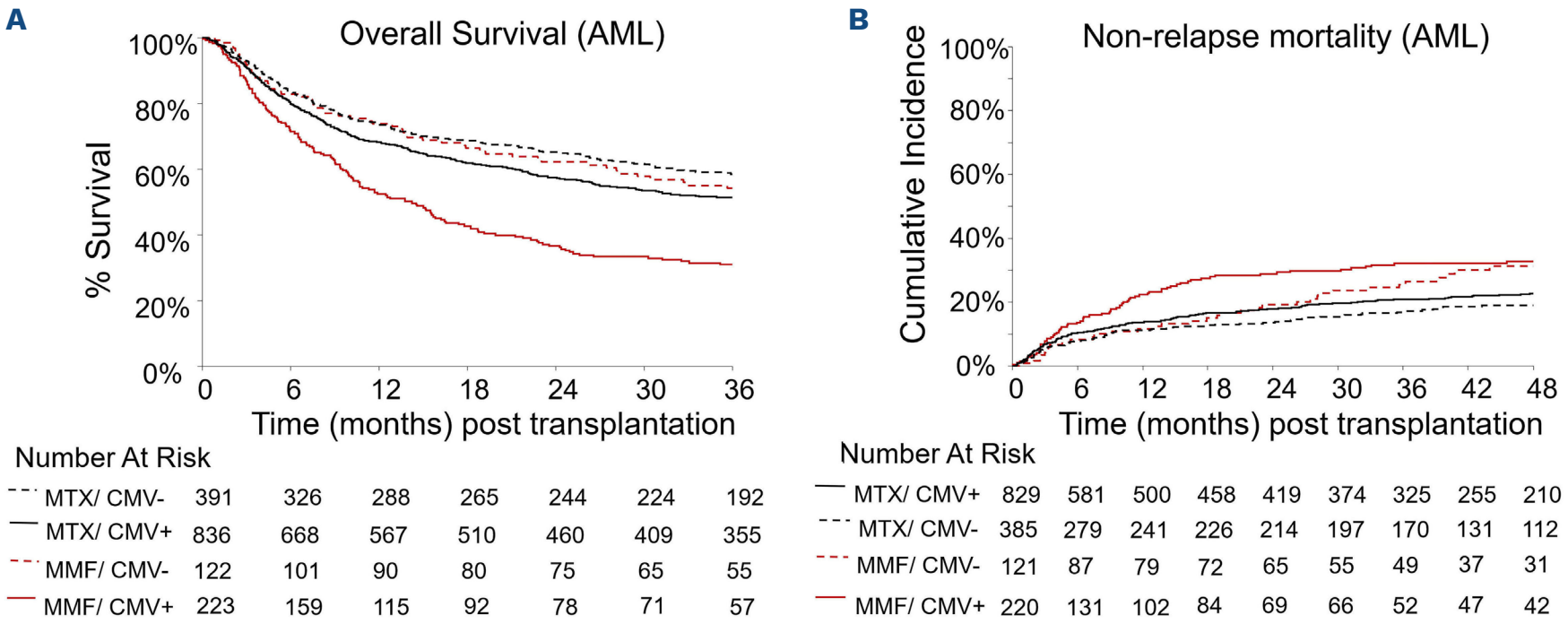

Figure 1. Outcomes of patients according to their cytomegalovirus status and whether they received methotrexate or mycophenolate mofetil for graft-*versus*-host disease prophylaxis. Among patients with acute myeloid leukemia, four groups were studied: patients given methotrexate (MTX) who were cytomegalovirus (CMV) seronegative (MTX/CMV$^-$, dotted black), patients given MTX who were CMV seropositive (MTX/CMV$^+$, solid black), patients given mycophenolate mofetil (MMF) who were CMV seronegative (MMF/CMV$^-$, dotted maroon) and those given MMF who were CMV seropositive (MMF/CMV$^+$, solid maroon). Outcomes include (A) overall survival, (B) non-relapse mortality, (C) relapse, and (D) chronic graft-*versus*-host disease. AML: acute myeloid leukemia; GvHD: graft-*versus*-host disease.

MMF or MTX and underwent matched unrelated donor HCT with peripheral blood grafts, we showed that MMF was associated with a significantly higher risk of NRM and worse OS in CMV$^+$ individuals but only in AML patients, and not in those with MDS or ALL. The underlying mechanism of this observation is unclear, but one hypothesis is as follows. It is known that CMV induces an exaggerated proliferation of natural killer cells, γ/δ T cells, and cytotoxic T cells.[6,7] As MMF inhibits both B- and T-lymphocytes,[1] it is associated with broader immunosuppressive effects than MTX, and CMV-induced lymphocytic expansion[8,9] is suppressed by MMF more than by MTX.[10] Moreover, MMF is administered for an extended period after HCT, while MTX is given for a short course. It is, therefore, conceivable that MMF may thwart CMV-driven expansion of both innate and adaptive immune cells, which may not only negate any potential protective effects of CMV on relapse but may also increase the risk of infections and NRM. Several studies have shown that CMV is associated with a reduced risk of relapse, but predominantly in AML patients[11-13] and not in those with lymphoid malignancies,[11,13,14] or even MDS.[12,13] The additional inhibition

Table 2. Multivariate analysis.*

	OS		NRM		Relapse		Chronic GvHD	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
AML								
MTX/CMV$^-$	1.0		1.0		1.0		1.0	
MTX/CMV$^+$	1.2 (0.9-1.4)	0.7	1.2 (0.9-1.6)	0.8	0.9 (0.8-1.2)	0.9	0.9 (0.7-1.1)	0.9
MMF/CMV$^-$	0.9 (0.7-1.3)	0.9	1.4 (0.9-2.1)	0.7	0.8 (0.5-1.0)	0.1	1.4 (1.05-1.8)	0.2
MMF/CMV$^+$	1.8 (1.4-2.3)	<0.001	1.9 (1.3-2.6)	0.002	1.2 (0.9-1.6)	0.1	0.9 (0.7-1.2)	0.9
ALL								
MTX/CMV$^-$	1.0		1.0		1.0		1.0	
MTX/CMV$^+$	0.9 (0.7-1.3)	0.9	1.5 (0.8-2.8)	0.9	0.9 (0.5-1.3)	0.9	1.03 (0.7-1.4)	0.9
MMF/CMV$^-$	0.8 (0.4-1.4)	0.9	1.02 (0.5-2.1)	0.9	0.7 (0.3-1.7)	0.9	2.1 (1.3-3.3)	0.01
MMF/CMV$^+$	1.2 (0.7-1.9)	0.9	0.9 (0.6-1.4)	0.9	0.8 (0.4-1.6)	0.9	1.7 (1.04-2.8)	0.2
MDS								
MTX/CMV$^-$	1.0		1.0**		1.0		1.0	
MTX/CMV$^+$	1.2 (0.99-1.5)	0.3	1.6 (1.1-2.2)	0.03	0.9 (0.8-1.3)	0.9	1.01 (0.8-1.2)	0.9
MMF/CMV$^-$	1.1 (0.8-1.5)	0.9	1.6 (0.9-2.6)	0.2	0.8 (0.5-1.2)	0.9	1.6 (1.2-2.1)	0.01
MMF/CMV$^+$	1.3 (1.0-1.7)	0.2	1.6 (1.05-2.4)	0.1	1.01 (0.7-1.5)	0.9	1.4 (1.1-1.9)	0.1

*Full models are shown in *Online Supplementary Table S1*. There were no significant predictors of grade III-IV acute GvHD in any disease type. **In patients with a Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) score ≥3. Among those with an HCT-CI 0-2, taking the MTX/CMV$^-$ group as the reference, the hazard ratios (with 95% confidence intervals) were 0.9 (0.6-1.3) for the MTX/CMV$^+$ group (*P*=0.5), 0.9 (0.4-1.7) for the MMF/CMV$^-$ group (*P*=0.7), and 1.6 (0.9-2.6) for the MMF/CMV$^+$ group (*P*=0.09). OS: overall survival; NRM: non-relapse mortality; GvHD: graft-*versus*-host disease; CI: confidence interval; HR: hazard ratio; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MTX: methotrexate; CMV: cytomegalovirus; MMF: mycophenolate mofetil; MDS: myelodysplastic neoplasms.

of humoral and T-cell immunity in the presence of MMF would be predicted to promote CMV reactivation with its associated highly suppressive immunological imprinting and infectious sequelae.[15]

The effect of MMF when used with PTCy remains unknown. In our *ad hoc* analysis of the PTCy cohort, our findings were similar to those in the CNI cohort (not given PTCy), i.e., increased mortality in CMV$^+$ recipients with AML, but not in those with ALL or MDS. However, the independent effects of MMF and CMV on this association could not be determined as all patients received PTCy/CNI/MMF. These findings are hypothesis-generating and provide foundational data for further studies to assess the interaction between MMF and CMV serostatus in patients with AML, *versus* other hematologic malignancies, receiving PTCy-based prophylaxis.

Limitations of our study are the lack of data on CMV reactivation and causes of death. Because we lacked these data, we do not know the precise reason for the worse OS in the MMF/CMV$^+$ group. Of note, almost all patients in the CNI cohort and a majority in the PTCy cohort underwent HCT in the era before letermovir was approved by the US Food and Drug Administration. Whether or not the use of letermovir alters these conclusions also remains to be investigated.

In conclusion, our data suggest that the use of MMF with CNI prophylaxis should be avoided in CMV-seropositive AML patients undergoing matched unrelated donor HCT. Further studies are needed to assess the interaction between MMF and CMV serostatus when PTCy is used for GvHD prophylaxis, and in the setting of letermovir prophylaxis.

Authors

Rima M. Saliba,[1] Stephanie J. Lee,[2] Paul A. Carpenter,[2,3] Geoffrey R. Hill,[4,5] Catherine J. Lee,[2] Amin Alousi,[1] May Daher,[1] George Chen,[1] Richard E. Champlin,[1] Katayoun Rezvani,[1] Elizabeth J. Shpall[1] and Rohtesh S. Mehta[2]

[1]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX; [2]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA; [3]Division of Pediatric Hematology, Oncology, Bone Marrow Transplant & Cellular Therapy, Seattle Children's Hospital, Seattle, WA; [4]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, WA and [5]Clinical Research Division, Fred Hutchinson Cancer Center, Houston, TX, USA

Correspondence:
R.S. MEHTA - rmehta@fredhutch.org

https://doi.org/10.3324/haematol.2023.284501

Received: October 19, 2023.
Accepted: February 16, 2024.
Early view: February 29, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

RMS performed the statistical analysis, interpreted data, and wrote the statistical section of the manuscript. SJL, PAC, JRH, CJL, AA, GC, MD, REC, KR, and EJS reviewed and interpreted the data, reviewed the manuscript, and provided critical feedback. RSM and RMS conceptualized the study design and interpreted data. RSM wrote the manuscript with the help of RMS. RMS and RSM had full access to the raw data which is publicly available. All authors approved the manuscript. The corresponding author had the final responsibility for submitting the manuscript for publication.

Acknowledgments

We thank the Center for International Blood and Marrow Transplant Research (CIBMTR) staff for providing the dataset used in this study.

Funding

This dataset was collected by the CIBMTR which is supported primarily by the Public Health Service U24CA076518 from the National Cancer Institute; the National Heart, Lung, and Blood Institute; the National Institute of Allergy and Infectious Diseases; 75R60222C00011 from the Health Resources and Services Administration; N00014-21-1-2954 and N00014-23-1-2057 from the Office of Naval Research; the National Marrow Donor Program/Be The Match; and the Medical College of Wisconsin.

Data-sharing statement

The data are publicly available and accessible at https://cibmtr.org/CIBMTR/Resources/Publicly-Available-Datasets.

References

1. Ueda Oshima M, Xie H, Zamora D, et al. Impact of GVHD prophylaxis on CMV reactivation and disease after HLA-matched peripheral blood stem cell transplantation. Blood Adv. 2023;7(8):1394-1403.
2. Teira P, Battiwalla M, Ramanathan M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood. 2016;127(20):2427-2438.
3. CIBMTR. https://cibmtr.org/CIBMTR/Resources/Publicly-Available-Datasets#. Accessed 9/6/2023.
4. Arrieta-Bolanos E, Crivello P, He M, et al. A core group of structurally similar HLA-DPB1 alleles drives permissiveness after hematopoietic cell transplantation. Blood. 2022;140(6):659-663.
5. Gooptu M, Romee R, St Martin A, et al. HLA-haploidentical vs matched unrelated donor transplants with posttransplant cyclophosphamide-based prophylaxis. Blood. 2021;138(3):273-282.
6. Nachbaur D, Bonatti H, Oberaigner W, et al. Survival after bone marrow transplantation from cytomegalovirus seropositive sibling donors. Lancet. 2001;358(9288):1157-1159.
7. Elmaagacli AH, Koldehoff M. Cytomegalovirus replication reduces the relapse incidence in patients with acute myeloid leukemia. Blood. 2016;128(3):456-459.
8. Beziat V, Liu LL, Malmberg JA, et al. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. Blood. 2013;121(14):2678-2688.
9. Foley B, Cooley S, Verneris MR, et al. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. Blood. 2012;119(11):2665-2674.
10. Ohata K, Espinoza JL, Lu X, Kondo Y, Nakao S. Mycophenolic acid inhibits natural killer cell proliferation and cytotoxic function: a possible disadvantage of including mycophenolate mofetil in the graft-versus-host disease prophylaxis regimen. Biol Blood Marrow Transplant. 2011;17(2):205-213.
11. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94(1):333-339.
12. Green ML, Leisenring WM, Xie H, et al. CMV reactivation after allogeneic HCT and relapse risk: evidence for early protection in acute myeloid leukemia. Blood. 2013;122(7):1316-1324.
13. Manjappa S, Bhamidipati PK, Stokerl-Goldstein KE, et al. Protective effect of cytomegalovirus reactivation on relapse after allogeneic hematopoietic cell transplantation in acute myeloid leukemia patients is influenced by conditioning regimen. Biol Blood Marrow Transplant. 2014;20(1):46-52.
14. Mariotti J, Maura F, Spina F, et al. Impact of cytomegalovirus replication and cytomegalovirus serostatus on the outcome of patients with B cell lymphoma after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(6):885-890.
15. Degli-Esposti MA, Hill GR. Immune control of cytomegalovirus reactivation in stem cell transplantation. Blood. 2022;139(9):1277-1288.