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Infectious Disease

Use of Leflunomide for Treatment of Cytomegalovirus Infection in Recipients of Allogeneic Stem Cell Transplant

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A B S T R A C T

Cytomegalovirus (CMV) reactivations are common after allogeneic stem cell transplants, and pre-emptive therapy has been found to be effective. However, in India, treatment options are limited because of high cost and toxicity of ganciclovir and unavailability of cidofovir and foscarnet. Leflunomide is a cheap and easily available anti-rheumatoid arthritis drug that has been shown to have anti-CMV properties both in vitro and in vivo. It also has been used effectively for CMV reactivation after renal transplants. In this retrospective analysis, we analyzed 70 allogeneic stem cell transplants that were conducted between April 2015 and February 2017. There were 49 episodes of CMV reactivations in 43 patients in this period. Leflunomide was used in 24 episodes. It was effective in CMV clearance in 9 of the 24 episodes (38%). When the CMV copy number was $<2 \times 10^3$ copies/mL, leflunomide was effective in 9 of 17 (53%) episodes, but when the copy number was $>2 \times 10^3$, leflunomide was ineffective in all of the 7 episodes. This difference was statistically significant (P= .022 by Fisher exact test), suggesting that leflunomide may be more effective in clearance of CMV when copy numbers are low.

INTRODUCTION

Cytomegalovirus (CMV) reactivation is a common cause of morbidity and mortality after allogeneic hematopoietic stem cell transplant (aHSCT). The incidence of reactivation varies with the type of donor and the source of stem cells and is highest in haploidentical transplants (HITs) (50% to 60%), followed by umbilical cord blood transplants (45% to 50%) and matched unrelated donor (MUD) transplants (25% to 35%), and is least in matched sibling donor (MSD) transplants (15% to 25%) [1–3]. The presence of antibody against CMV (immunoglobulin G) also determines risk of reactivation [4]. In India, almost all stem cell transplant recipients and donors are seropositive for CMV antibody and are at high risk of CMV reactivation [5,6].

Ganciclovir is the drug of choice for pre-emptive therapy but is associated with significant cytopenias and nephrotoxicity, making it difficult for use in the periengraftment period and in immunocompromised patients [7]. Second-line drugs such as foscarnet and cidofovir are not available in India and have to be imported at a high cost, leaving few therapeutic options for patients with CMV infections. Leflunomide is an anti-rheumatoid arthritis drug that has been used in CMV infections after renal transplant [8]. However, there are only a few case reports about its utility post-aHSCT [9,10]. We report here our experience with the use of leflunomide in CMV reactivations post-aHSCT.

METHODS

Study Population

This is a retrospective study conducted at the stem cell transplant unit of a tertiary referral cancer center in India. All patients who underwent an allogeneic stem cell transplant at our center between April 2015 and February 2017 were included in this study.

Transplant Procedure

Serologic evaluation for CMV infection was done for all patients and donors at baseline. All patients underwent Hickman catheter insertion before start of the conditioning regimen and were isolated in high-efficiency particulate arrestance filtered rooms and nursed using strict barrier nursing. Conditioning regimens used were either myeloablative with >12 Gy total-body irradiation (TBI) along with cyclophosphamide or reduced-intensity conditioning regimens that included fludarabine with melphalan or cyclophosphamide or treosulfan or 7.2 Gy TBI. Two Gy TBI was used in reduced-intensity conditioning regimens for some patients. Graft-versus-host disease

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prophylaxis used was either cyclosporine or tacrolimus along with either methotrexate or mycophenolate mofetil for MSD and MUD transplants. In addition, post-transplant cyclophosphamide (50 mg/kg for 2 days) was used for HITs. Antithymocyte globulin was used in MUD transplants and in patients who had received multiple transfusions. All patients received antifungal prophylaxis with either posaconazole or voriconazole. Aciclovir at a dose of 400 mg thrice daily was used for herpes simplex prophylaxis. In case of fever, all patients received broad-spectrum antibiotics. Complete blood count and renal and liver functions were monitored daily.

CMV Monitoring

Quantitative PCR (QPCR) was used to monitor for CMV reactivations and response to therapy. Peripheral blood was collected twice per week from day 0 to day +120 (longer if patient was taking steroids or had active graft-versus-host disease) for QPCR analysis.

We used the COBAS AmpliPrep/COBAS TaqMan CMV Test (Roche diagnostics, USA) for CMV detection in plasma. This is a nucleic acid amplification test for the quantitation of CMV DNA in EDTA human plasma using the COBAS AmpliPrep instrument for automated specimen processing and COBAS TaqMan 48 Analyzer (Roche diagnostics, Germany) for automated amplification and detection. The test can quantitate CMV DNA over the range of 150 to 10,000,000 copies/mL.

CMV Therapy

Patients who were detected to have a positive QPCR of >500 copies/mL at 2 consecutive time points were started on pre-emptive therapy. Intravenous ganciclovir (5 mg/kg twice daily) was the preferred agent for pre-emptive therapy. However, in case of ongoing cytopenias, leflunomide was used. In case of clinical resistance (see next paragraph for definition) to ganciclovir, leflunomide was added as a second-line treatment. Second-line drug was added for CMV infection if there was a log increase in CMV copy numbers while on therapy with the first antiviral agent or failure to clear (no log decrease) in CMV copy number despite adequate treatment with the first antiviral agent or development of end-organ involvement while on therapy with the first agent or toxicities caused by the first agent requiring stoppage of the drug. For adult patients, leflunomide was started at a loading dose of 100 mg once a day for 3 days followed by a daily maintenance dose of 20 mg until clearance of CMV or intolerance. First-line agent was continued unless significant toxicities required stoppage of the drug. The dose of leflunomide used in pediatric patients weighing <20 kg was a 100-mg loading dose on the first day followed by 10 mg on alternate days. Those weighing 20 to 40 kg received a 100-mg loading dose for 2 days followed by 10 mg once per day. Patients weighing >40 kg received a full dose of 100 mg for 3 days followed by 20 mg daily. If the patient was taking steroids, leflunomide was continued until steroids were tapered to low doses or stopped. Invasive procedures such as bronchoalveolar lavage and colonoscopic biopsies were used to detect CMV disease when clinically suspected.

Definitions

CMV reactivation was defined as a QPCR result of >500 copies/mL at 2 successive time points without clinical evidence of CMV disease. CMV clearance was defined as undetectable or below the quantifiable limit of CMV DNA on QPCR for at least 2 consecutive time points after starting a particular therapy. The lower limit of quantitation of our assay was 150 copies/mL. Time to clearance was the time between start of treatment and CMV clearance. Clinical resistance or failure of therapy was defined as >1 log increase in CMV copy number at any time while on therapy or if another anti-CMV agent was added because of CMV disease or nonclearance.

Statistics

The primary objective was to find out the clearance rate of leflunomide in patients with CMV reactivations post-aHSCT. The data were collected by assessing patient charts and records and tabulating them in an Excel sheet format. Chi-squared test and Fisher exact test were used to compare the difference in response between different groups.

RESULTS

Seventy patients underwent aHSCT during this period at our center. The demographics are summarized in Table 1. Twenty-one were female and 49 were male. The age range for the entire cohort was 3 to 54 years. The cohort included 47 MSD transplants, 18 HITs, and 5 MUD transplants. The median age was 28.5 years. The most common indication for transplant was chronic myeloid leukemia (28.5%), followed by acute myeloid leukemia (25.7%), acute lymphoblastic leukemia (22.9%), Hodgkin and non-Hodgkin lymphoma (10.0%), and others (12.9%, including myelofibrosis, myelodysplastic syndrome, juvenile myelomonocytic leukemia, and aplastic anemia). Almost all transplant recipients (95.7%) and donors (98.5%) had a positive serology for CMV at baseline.

There were 49 episodes of CMV reactivation in 43 patients (61%). Table 2 shows characteristics of all 43 patients. The incidence was highest in HITs, with 17 of 18 (95%) patients developing reactivation, followed by MUD (60%) and MSD (53%). The median time to CMV reactivation from day of transplant was 43 days (range, 0 to 496). CMV disease was seen in 9 patients. One patient had CMV hepatitis, whereas the rest had CMV colitis. Ganciclovir was the drug of choice for CMV reactivation and was used as the first-line drug in 23 episodes (Figure 1).

Leflunomide was used in 24 episodes: first line in 15, second line in 8, and third line in 1. The median CMV copy number/mL was 0.82×10^3 (range, 0 to 13×10^3) on the day of starting leflunomide. The median duration of use of leflunomide for all patients was 34 days (range, 6 to 85 days), whereas for those who responded to leflunomide, it was 30 days. It was used pre-emptively in 20 episodes and as a therapeutic (ie, patient had end-organ involvement) in 4. When used as a first-line treatment, leflunomide was able to clear CMV in 7 of the 15 episodes (46%), whereas ganciclovir was able to clear it in 12 of the 23 (52%). As a second-line agent, leflunomide could clear CMV in only 2 of 9 episodes. Overall CMV was cleared in 9 of the 24 episodes (38%). Figure 2 shows a dot plot curve showing trend of response to leflunomide in 20 of these 24 episodes. Seven of the 24 episodes had an initial CMV burden of $>2 \times 10^3$ copies/mL. Leflunomide failed in all of these, while there was clearance in 9 of the 17 (53%) episodes that had CMV $<2 \times 10^3$ copies/mL. This difference was statistically significant by Fisher exact test (P= .022). None of the patients who received leflunomide in a therapeutic setting cleared CMV (0 of 4), compared with 9 of 20 with pre-emptive leflunomide, although this difference was not statistically significant. The median time taken to clear CMV with leflunomide was 21 days (range, 7 to 50 days). Of all patients who responded to leflunomide, only 1 patient relapsed within 15 days of clearing CMV with leflunomide. He was rechallenged with leflunomide and responded again. The other patients had sustained remissions. Besides CMV levels, none of the other factors (including the underlying disease, type of transplant, donor/recipient serostatus, timing of reactivation, type of conditioning, and immune suppression used) showed statistically significant correlation with response to leflunomide. Of 12 episodes of CMV

Table 1
Median Age, Type of Transplant, Conditioning Regimens, and CMV Reactivation Rates

Type of Transplant	No. of Patients	Conditioning Regimen		Median Age, yr	No. (%) of Patients with CMV Reactivation
		Myeloablative	Reduced Intensity		
MSD	47	11	36	33	25 (53.0)
MUD	5	1	4	23	3 (60.0)
HIT	18	0	18	21.5	17 (94.4)

Table 2
Details of CMV Reactivations: Type of Transplant, Baseline Patient and Donor CMV Serostatus, and Response to Leflunomide

Patient No.	Episode No.	Type of Transplant	Age, yr	Baseline Patient Status	Baseline Donor Status	Day of BMT When CMV Reactivated	Leflunamide, Y/N	Leflunomide, First/Second/ Third Line	Type of Therapy	CMV Clearance with Leflunomide
1	1	MSD	38	Positive	Positive	35	N			
1	2	MSD	38	Positive	Positive	77	Y	First	Pre-emptive	Y
2	1	MSD	52	Positive	Positive	261	N			
2	2	MSD	52	Positive	Positive	306	N			
3	1	MSD	49	Positive	Positive	197	N			
4	1	MSD	20	Positive	Positive	28	N			
5	1	HIT	11	Positive	Positive	25	N			
6	1	MSD	51	Positive	Positive	207	N			
7	1	MSD	28	Positive	Positive	396	N			
8	1	HIT	13	Positive	Positive	34	N			
9	1	MSD	21	Positive	Positive	13	N			
9	2	MSD	21	Positive	Positive	496	N			
10	1	MSD	33	Positive	Positive	117	N			
10	2	MSD	33	Positive	Positive	160	N			
11	1	HIT	15	Positive	Positive	212	Y	First	Pre-emptive	N
12	1	MSD	30	Positive	Positive	455	Y	First	Pre-emptive	Y
13	1	MSD	27	Positive	Positive	42	Y	Second	Therapeutic	N
14	1	MSD	22	Positive	Positive	68	Y	First	Pre-emptive	N
15	1	HIT	24	Positive	Positive	43	Y	First	Pre-emptive	Y
16	1	MSD	54	Positive	Negative	24	Y	Second	Therapeutic	N
17	1	MSD	41	Positive	Positive	29	Y	Second	PREMPTIVE	Y
17	2	MSD	41	Positive	Positive	82	Y	First	Pre-emptive	Y
18	1	HIT	7	Positive	Positive	54	Y	First	Pre-emptive	Y
19	1	MSD	46	Positive	Positive	70	Y	Second	Therapeutic	N
20	1	MUD	29	Positive	Positive	0	Y	Second	Pre-emptive	Y
21	1	MSD	36	Positive	Positive	62	Y	First	Pre-emptive	N
22	1	HIT	23	Positive	Positive	48	Y	First	Pre-emptive	N
23	1	HIT	46	Positive	Positive	28	Y	Second	Pre-emptive	N
24	1	HIT	16	Positive	Positive	18	Y	First	Pre-emptive	N
25	1	HIT	27	Positive	Positive	39	Y	First	Pre-emptive	N
26	1	HIT	35	Negative	Positive	34	Y	Third	Pre-emptive	N
27	1	HIT	30	Positive	Positive	30	Y	Second	Pre-emptive	N
28	1	HIT	31	Positive	Positive	29	Y	First	Pre-emptive	N
28	2	HIT	31	Positive	Positive	155	Y	First	Therapeutic	N
29	1	MUD	23	Positive	Positive	169	Y	First	Pre-emptive	Y
30	1	HIT	10	Positive	Positive	22	Y	Second	Pre-emptive	N
31	1	MSD	19	Positive	Positive	169	Y	First	Pre-emptive	Y
32	1	HIT	46	Positive	Positive	40	N			
33	1	MSD	29	Positive	Positive	35	N			
34	1	MSD	27	Positive	Positive	380	N			
35	1	MSD	38	Positive	Positive	8	N			
36	1	MSD	42	Positive	Positive	60	N			
37	1	HIT	31	Positive	Positive	26	N			
38	1	MSD	39	Positive	Positive	33	N			
39	1	MUD	19	Positive	Positive	22	N			
40	1	HIT	17	Positive	Positive	21	N			
41	1	MSD	45	Positive	Positive	55	N			
42	1	MSD	14	Positive	Positive	12	N			
43	1	HIT	16	Positive	Positive	47	N			

BMT indicates bone marrow transplant; Y, yes; N, no.

reactivations in HIT patients, 2 cleared CMV, whereas of the 12 episodes in MSD/MUD transplants, 7 cleared CMV with leflunomide. Although on face value, HIT patients seemed to be responding less favorably, the difference was not statistically significant by Fisher exact test.

None of the patients had toxicity requiring stoppage of the drug. In totality, leflunomide was able to clear only 9 of 24 CMV infections (38%). Of the 9 patients who had cleared CMV on leflunomide, 1 died of bacterial sepsis and septic shock. Of the remaining 15 patients, 11 died of uncontrolled CMV

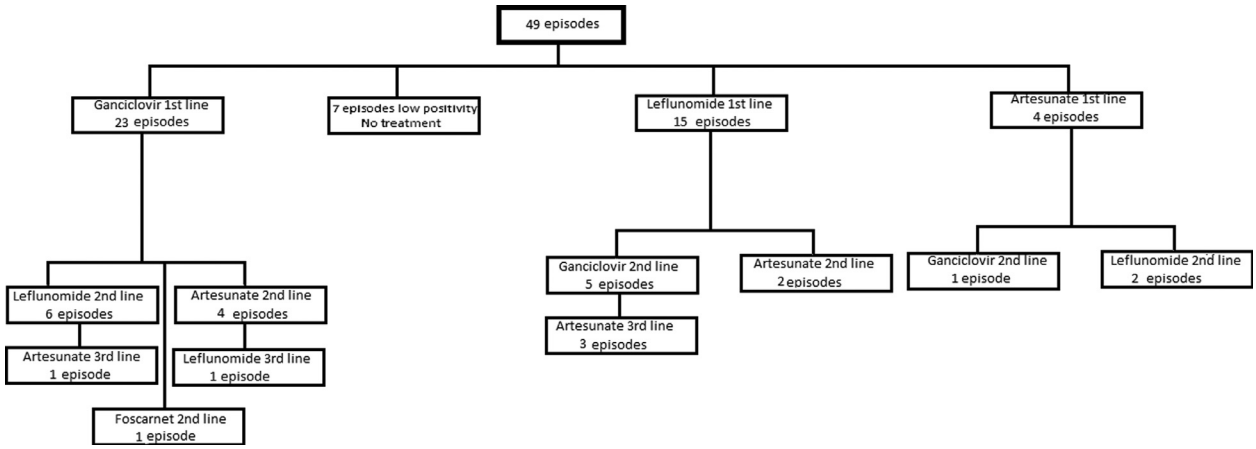

Figure 1. Flowchart representing CMV treatment drugs used in patients.

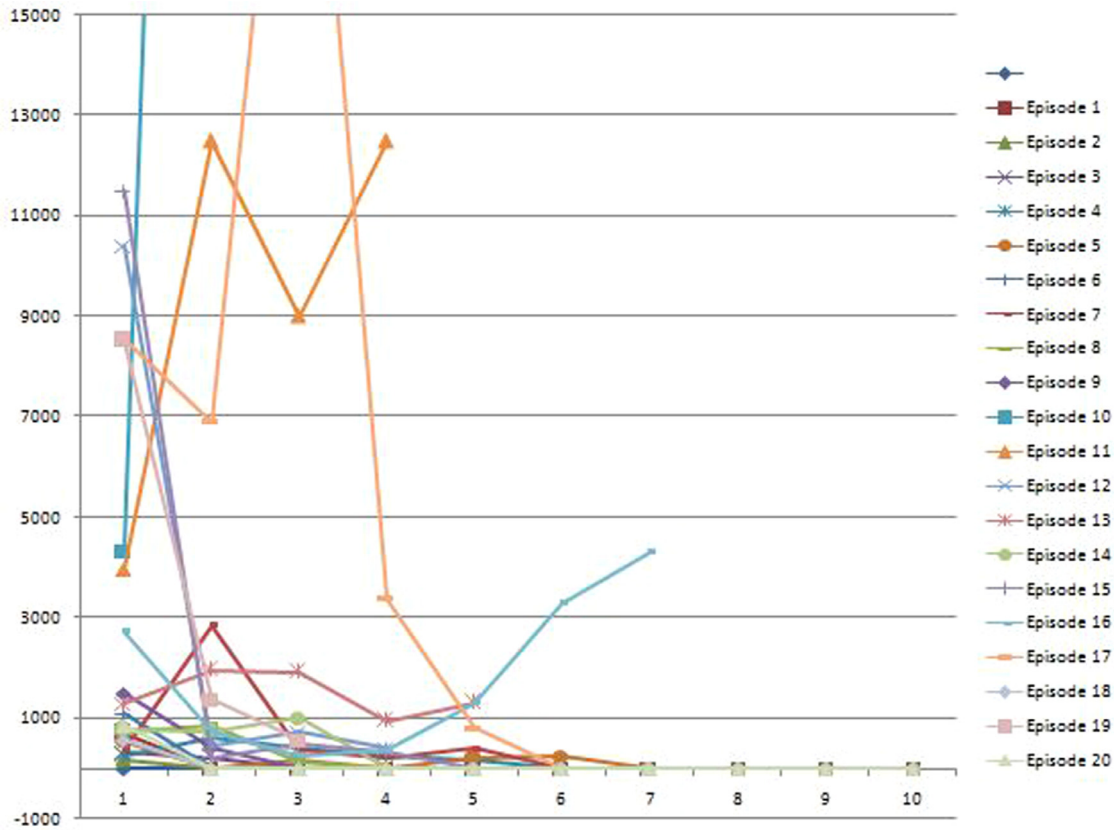

Figure 2. Dot plot showing trend of CMV viremia after leflunomide. Twenty episodes are included of 24 (3 patients had end-organ involvement without viremia and 1 patient discontinued all treatment).

infection. Three of the 4 patients with CMV colitis who had been taking leflunomide died.

DISCUSSION

Hematopoietic stem cell transplants offer curative potential for many hematologic disorders. CMV reactivation is a common and potentially fatal complication post-aHSCT. CMV infection is highly prevalent in India, and approximately 95% of the Indian population is seropositive for CMV antibodies [5]. Allogeneic transplants are becoming more common in India, and with the nonavailability of foscarnet and cidofovir, treating ganciclovir-resistant CMV infections becomes a herculean task. There is sparse literature about post-aHSCT CMV reactivation rates in India, and it is estimated at between 10% and 39% [6,11,12]. The reactivation rates post-HIT seem to be more than umbilical cord transplants [2,3]. Unavailability of matched unrelated donors for the Indian population and cost constraints are making HITs more popular. However, the CMV reactivation rates post-HIT in India are not known. Few case reports with a limited number of patients estimate it at around 35% to 45%, which is less than the reported incidence of 50% to 65% in developed and other developing countries [2,6,13,14]. In this study, CMV reactivation rates were high, especially in HIT, in which almost all patients reactivated.

The mortality among patients with uncontrolled CMV infection in this study was high. CMV reactivation has been associated with lower overall survival in other studies as well [4,11]. Ganciclovir is the only easily available treatment option in India. Not only is it expensive, but it also causes significant cytopenias and renal dysfunction, making it difficult to use in the periengraftment period. Given this situation, it is important to find alternative, cheap drugs for the treatment of CMV.

Leflunomide is an anti-rheumatoid arthritis drug that is cheap and easily available. Waldman and colleagues [15] showed the antiviral efficacy of leflunomide in human umbilical vein epithelial cell lines and human foreskin fibroblast monolayers. They demonstrated a reduction in CMV-related cytopathic effects as well as reduction in CMV production using plaque reduction and viral assays when these CMV-infected cell lines were exposed to A77 1726 (*N*-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotoamide), which is the active metabolite of leflunomide. The mechanism of action of this drug is likely through its effect on virion assembly of the CMV particle during replication, as demonstrated using electron microscopy and not by inhibition of DNA polymerase, which is the mechanism of other anti-CMV drugs [15]. Hence, leflunomide also may be effective against ganciclovir-resistant CMV strains because of its novel mechanism of action. Indeed, Waldman and colleagues [15] were also able to demonstrate this effect in ganciclovir-resistant CMV D16 strains using human foreskin fibroblast cultures. The same team also demonstrated that leflunomide induced CMV clearance in vivo in rat models.

Leflunomide also has been used and demonstrated to be safe and effective in CMV clearance in post-renal transplant patients in India. In a prospective trial, CMV clearance was demonstrated in 15 of 17 patients [8]. However, there are only occasional case reports of successful use of leflunomide after allogeneic transplants [9,16,17]. This case series, to our knowledge, provides the largest reported data about leflunomide in post-aHSCT CMV infection.

This study showed that leflunomide was effective in CMV clearance in only 38% of cases. However, its success rate was significantly higher (53%) when it was used in patients with CMV copy numbers $<2 \times 10^3$/mL. Leflunomide was also ineffective when used in patients with end-organ disease, although this was not statistically significant. This indicates that although leflunomide has anti-CMV action, it is probably not effective in patients with a high CMV burden. Given the limitation of this study being a retrospective analysis, more prospective studies are indicated to confirm this finding. Leflunomide does offer a therapeutic option for post-transplant CMV infections, especially in resource-limited settings where access to newer drugs and adoptive cellular therapies are limited. Being more effective at a low CMV burden, leflunomide may have a role as a prophylactic agent in HITs, especially in India, where the CMV reactivation rates seem to be much higher than previously reported.

CONCLUSIONS

Leflunomide has considerable activity against CMV, but it is best used when CMV copies are <2000/mL in a pre-emptive setting. In a therapeutic setting, its role seems limited. A larger prospective study is warranted.

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REFERENCES

1. Goldsmith SR, Slade M, Lawrence SJ, et al. Cytomegalovirus viremia and relapse after haploidentical hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:S320.
2. Hammerstrom AE, Lombardi LR, Pingali SR, et al. Prevention of cytomegalovirus reactivation in haploidentical stem cell transplantation. *Biol Blood Marrow Transplant*. 2018;24:353–358.
3. Zaia JA. Cytomegalovirus infection. In: Forman SJ, Negrin RS, Antin JH, Appelbaum FR, eds. *Thomas' Hematopoietic Cell Transplantation*. West Sussex, UK: Wiley-Blackwell; 2016:1069–1079.
4. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *J Infect Dis*. 2002;185:273–282.
5. Kothari A, Ramachandran VG, Gupta P, Singh B, Talwar V. Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. *J Health Popul Nutr*. 2002;20:348–351.
6. Uppuluri R, Subburaj D, Jayaraman D, et al. Cytomegalovirus reactivation posthematopoietic stem cell transplantation (HSCT) and type of graft: A step toward rationalizing CMV testing and positively impacting the economics of HSCT in developing countries. *Pediatr Blood Cancer*. 2017;64:e26639.
7. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. *Hematol Oncol Clin North Am*. 2011;25:151–169.
8. John GT, Manivannan J, Chandy S, et al. A prospective evaluation of leflunomide therapy for cytomegalovirus disease in renal transplant recipients. *Transplant Proc*. 2005;37:4303–4305.
9. Avery RK, Bolwell BJ, Yen-Lieberman B, et al. Use of leflunomide in an allogeneic bone marrow transplant recipient with refractory cytomegalovirus infection. *Bone Marrow Transplant*. 2004;34:1071–1075.
10. Battiwalla M, Paplham P, Almyroudis NG, et al. Leflunomide failure to control recurrent cytomegalovirus infection in the setting of renal failure after allogeneic stem cell transplantation. *Transpl Infect Dis*. 2007;9:28–32.
11. George B, Devasia A, Mathews V, et al. Lower incidence of CMV reactivation following allogeneic stem cell transplantation despite a high seroprevalence: a single centre experience. *Biol Blood Marrow Transplant*. 2014;20:S222–S223.
12. Sharma SK, Kumar S, Agrawal N, et al. Cytomegalovirus reactivation following hematopoietic stem cell transplantation. *J Infect Dev Ctries*. 2013;7:1003–1007.
13. Jaiswal SR, Chakrabarti A, Chatterjee S, et al. Haploidentical peripheral blood stem cell transplantation with post-transplantation cyclophosphamide in children with advanced acute leukemia with fludarabine-, busulfan-, and melphalan-based conditioning. *Biol Blood Marrow Transplant*. 2016;22:499–504.
14. Lin X, Ou Y, Long H, et al. Cytomegalovirus infection after haploidentical stem cell transplantation may reduce relapse risk in leukemia. *Zhonghua Nei Ke Za Zhi*. 2016;55:107–110. [in Chinese].
15. Waldman WJ, Knight DA, Blinder L, et al. Inhibition of cytomegalovirus in vitro and in vivo by the experimental immunosuppressive agent leflunomide. *Intervirology*. 1999;42:412–418.
16. Mori N, Vanichanan J, Tverdek F, et al. Use of leflunomide in the treatment of recalcitrant CMV infection in immunocompromised patients. *Biol Blood Marrow Transplant*. 2014;20:S242.
17. Ehlert K, Groll AH, Vormoor J. Rapid clearance of CMV-antigenemia with combination treatment of foscarnet and leflunomide in a 2-year old boy with refractory CMV disease after allogeneic HSCT for JMML. *Blood*. 2005;106:5379.