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Incidence of CMV Replication and the Role of Letermovir Primary/Secondary Prophylaxis in the Early Phase After Allogeneic Hematopoietic Stem Cell Transplantation – A Single Centre Study

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Abstract. *Background/Aim: Cytomegalovirus (CMV) replication may cause life-threatening complications after allogeneic haematopoietic stem cell transplantation (allo-HSCT). The aim of the study was to characterize CMV events, and the outcome of letermovir (LTV) CMV prophylaxis. Patients and Methods: In this retrospective analysis of patients treated with an allo-HSCT between 2010 and 2020, we determined plasma CMV events, as well as associated risk factors. Results: We identified 423 patients who had undergone allo-HSCT between 2010 and 2020. CMV DNAemia was found in 130/423 (30.7%) of patients. CMV reactivation rate was significantly higher in patients with acute graft-versus-host disease, HLA mismatch, and CMV IgG seropositivity of donors and recipients. Among 42 patients receiving LTV prophylaxis those, 5 (11.9%) showed CMV DNAemia under LTV versus 87/353 (24.6%) in a control group. Conclusion: Despite the development of better approaches with weekly monitoring and early treatment initiation, CMV reactivations play an important role after allo-HSCT.*

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Cytomegalovirus (CMV) belongs to the most frequent viral infection events after allogenic hematopoietic stem cell transplantation (allo-HSCT) (1). Progress in CMV diagnosis and management, such as the introduction of sensitive polymerase chain reaction–based CMV viral load assays and the commonly used strategy of pre-emptive antiviral therapy (PET), have reduced the risk of development of CMV infection and disease, particularly in the first months after HSCT (2). Key factors increasing the risk for CMV replication are seropositive recipient, particularly when the donor is seronegative, unrelated donor with HLA mismatches, and acute graft-*versus*-host disease (GvHD) (3-6).

So far, HSCT recipients with the abovementioned risk combinations are monitored closely for CMV infection. Patients with detectable viral load are treated with antiviral drugs such as ganciclovir, foscarnet or valganciclovir. This PET strategy is prioritised since existing antiviral agents don't seem to be suitable for prophylactic treatment, especially regarding their toxicity (7). Despite PET, non-relapse mortality after HSCT is significantly higher among patients who experience reactivation of CMV, in particular when patients additionally suffer from GvHD or have HLA-mismatched donors (8). CMV disease can affect different organ systems. The manifestations include pneumonia, gastroenteritis, gastrointestinal ulcers, hepatitis, retinitis and seldom encephalitis (9, 10). Indirect CMV mediated effects have been associated with GvHD, opportunistic bacterial or fungal infections and decreased graft and patient survival (2, 6). A pre-emptive CMV strategy is designed to minimize the toxic side effects of the antiviral therapy through a targeted approach (6).

In 2018, the non-nucleoside viral agent letermovir (LTV) was approved for prophylaxis from CMV infections in HSCT recipients. LTV is the first CMV DNA terminase complex inhibitor, yet inactive against all other human herpesviruses (7). The terminase is a novel antiviral target as it facilitates processing and packaging of the CMV genome (11). Several studies have shown a significant reduction in clinically significant CMV infection and a tendency to lower all-cause mortality in allo-HSCT recipients who received LTV prophylaxis (12-14). A double-blind randomised phase III study has examined LTV prophylaxis in seropositive allo-HSCT recipients. The participants who received a dosage of 480 mg/day (or 240 mg/day when patients were on cyclosporine A) from the first day after allo-HSCT through day +100 post-transplant showed significantly less clinically significant CMV infections both at week 14 and 24 after transplantation compared to the placebo group. In addition, all-cause mortality was significantly lower in the LTV group at week 24 post-transplant (12).

LTV has been approved for primary CMV prevention in CMV seropositive recipients after the results of a phase 3 trial by Marty *et al*. showed a significant reduction in clinically relevant CMV infections and a lower risk for all-cause mortality at 24 weeks after HSCT (12, 15). Furthermore, LTV was associated with only mild side effects and did not elicit an increase in the rate of nephrotoxic or myelotoxic events (12).

A study by Lin *et al*. has provided support for the use of LTV for primary CMV prevention for the first 14 weeks and implies that LTV maintains its efficacy after this initial time period. It additionally encouraged the use of LTV for secondary prophylaxis (15).

The aim of this study was to examine the incidence and risk factors of CMV replication, end-organ disease, therapy, and the use of LTV in CMV prophylaxis.

Patients and Methods

Patient population and study design. This retrospective, single-center cohort study was performed at the Department of Hematology of the University Hospital Basel according to regulations of the local ethics committee. We included patients 1) with hematological disease, 2) who received an allo-HSCT at our institution between 2010 and 2018 (without LTV; n=381) and 2019 to 2020 (LTV group; n=42), 3) with available data of CMV IgG serostatus of donor/recipient, conditioning regimen, stem cell source, T cell depletion, graft-*versus*-host disease, and CMV events (viral load)/organ disease and therapy; 4) who received LTV for primary and secondary prophylaxis; 5) a historical control-group without LTV who underwent PET strategy with twice weekly monitoring of blood CMV DNA levels was identified for comparison; resulting in a cohort of altogether 423 patients (Table I).

Our electronic database was used to extract demographic data such as age and sex as well as other relevant parameters including hematologic diagnosis, donor type (HLA match/mismatch, genetically related/unrelated), stem cell source (bone marrow, peripheral blood, cord blood), data concerning acute and chronic GvHD, and treatments amongst other parameters. Concerning CMV, we assessed the CMV serology (IgG) status of donors and recipients before allo-HSCT and the maximum CMV load, the date of maximum CMV load, the time interval from HSCT; and/or CMV end-organ disease, as well as anti-viral therapy. In patients receiving LTV, we also examined CMV load regularly.

Conditioning regimens and graft-versus-host disease prophylaxis. Myeloablative conditioning (MAC) regimens included cyclophosphamide in combination with busulfan, cyclophosphamide and total body irradiation (TBI) ≥8Gy, cytarabine, carmustine, etoposide and melphalan (BEAM), and other protocols. Reduced-intensity conditioning (RIC) regimens consisted of fludarabine with low-dose TBI <6 Gy, fludarabine combined with busulfan or melphalan, and other protocols. Reasons for RIC were advanced age or relevant comorbidities. GvHD prophylaxis administered along with the MAC was cyclosporine A and methotrexate as well as anti-T-cell globulins (ATGs) in cases of unrelated donors and in matched related donors ≥40 years. In patients with RIC, GvHD prophylaxis consisted of cyclosporine A, methotrexate, and ATGs in cases of unrelated donors and in matched related donors ≥40 years, according to institutional standards (if RIC was fludarabine/busulfan), or cyclosporine A and mycophenolate mofetil (MMF) (if RIC was fludarabine/low-dose TBI) (16). The diagnosis of acute and chronic GvHD was based on clinical symptoms and/or biopsies (skin, oral, mucosal, liver or gut) and graded according to consensus criteria for acute and chronic GvHD (17, 18). Corticosteroid treatment (methylprednisolone, *i.v.*, 2 mg/kg/d) was initiated at diagnosis of aGvHD grade ≥2. Cyclosporine A was continued in patients with aGvHD.

Supportive care and treatment of post-HSCT complications. The routine antimicrobial prophylaxis used in our cohort to cover Pneumocystis jirovecii and Toxoplasmosis consisted of trimethoprim/sulfamethoxazole (160/800 mg, three times weekly) and fluconazole (400 mg once weekly) against yeast infections. Valacyclovir (500 mg bid orally) was given in patients showing positive serology for herpes simplex virus. Mold-active prophylaxis was not administered regularly but patients were treated empirically or pre-emptively following a diagnostic-driven approach based on galactomannan in serum and regularly performed imaging by chest CT scans. Invasive fungal infections were treated by anti-fungal therapy and broad-spectrum antibiotics were used to treat active infections.

Definitions of CMV replication and CMV end-organ disease. CMV reactivation after allo-HSCT was defined as detectable plasma virus load (*i.e*. CMV DNAemia) using quantitative real-time polymerase chain reaction assay (PCR) (19). It was considered positive when >300 copies (before July 2012) or >137 IU/ml (after July 2012) were detectable in EDTA plasma. The cut-offs were comparable after a multicenter study had demonstrated equivalence between both methods (20). Plasma CMV load was monitored by quantitative CMV PCR, starting from the time point of conditioning and continued weekly until day + 100.

Clinically relevant CMV event for this study was defined as a CMV DNAemia of >1,000 copies/ml (determined twice in an interval from 3-4 days) and/or evidence of CMV syndrome/disease requiring initiation of PET or targeted treatment with other-than-LTV CMV-acting antiviral agent according to guidelines (21). CMV end-organ disease was defined as (1) pneumonia, (2) gastrointestinal disease, (3)

Table I. *Patient baseline characteristics*.

	Total (n=423)	No CMV DNAemia (n=293; 69.3%) >1,000 copies: 119 <1,000 copies: 11	With CMV DNAemia (n=130; 30.7%)	*p*-Value
Gender				
Female	183 (43.2%)	131 (44.6%)	52 (40%)	0.533
Male	240 (56.6%)	162 (55.1%)	78 (60%)	
Age median (range)	49 (19-71)	47 (22-68)	48 (20-790)	
Diagnosis				
AML	152 (35.8%)	109 (37.1%)	43 (33.1%)	0.562
ALL	47 (11.1%)	31 (10.5%)	16 (12.3%)	
MDS/MPN	81 (19.1%)	58 (19.7%)	23 (17.7%)	
Lymphoma/myeloma	85 (20.0%)	59 (20.1%)	26 (20%)	
CLL	27 (6.4%)	17 (5.8%)	10 (7.7%)	
CML	15 (3.8%)	8 (3.1%)	7 (5.4%)	
Others	16 (3.8%)	11 (3.7%))	5 (3.8%)	
Stem cell source				
PBSC	376 (88.9%)	262 (89.1%)	114 (87.7%)	0.451
BM	45 (10.6%)	31 (10.5%)	14 (10.8%)	
CB	2 (0.5%)	1 (0.3%)	1 (0.77%)	
Donor				
Identical sibling	160 (36.6%)	126 (44.9%)	34 (26.2%)	0.008
Unrelated	238 (56.3%)	152 (52%)	86 (66.2%)	
Haplo-identical	25 (5.9%)	15 (5.1%)	10 (76.7%)	
Conditioning				
Myeloablative	321 (75.8%)	220 (75.1%)	101 (63.8%)	0.287
Reduced intensity	102 (24.1%)	73 (24.8%)	29 (22.3%)	
TBI used	157 (37.0%)	106 (36.1%)	51 (39.2%)	0.727
GvHD				
Acute GvHD ≥ grade 2	234 (55.2%)	144 (49.0%)	90 (69.2%)	<0.0001
Median time to aGvHD		29 days (5-417)	25 days (4-435)	0.089
Chronic GvHD	163 (38.4%)	106 (36.1%)	57 (43.9%)	0.184
Donor CMV IgG				
Positive	177 (43.1%)	107 (36.7%)	70 (53.8%)	0.001
Negative	235 (56.9%)	176 (59.9%)	59 (45.4%)	
Recipient CMV IgG				
Positive	262 (63.5%)	139 (47.6%)	123 (94.6%)	0.0001
Negative	151 (36.5%)	145 (49.3%)	6 (4.6%)	
Median CMV replication (range)		0	7,004 (263-361,000)	0.0001
Median time to max. CMV replication (range)		0	49 days (1-2,071)	
1st line therapy for CMV reactivation/organ disease#				
Ganciclovir		2/2 (100%)*	40/114 (35.1%)	
Foscarnet		0	11/114 (9.6%)	
Valganciclovir		0	63/114 (55.3%)	
2nd line				
Ganciclovir		0	22/60 (36.7%)	
Foscarnet		0	19/60 (31.7%)	
Valganciclovir		1/1 (100%)*	19/60 (31.7%)	
3rd line				
Ganciclovir		0	10/34 (29.4%)	
Foscarnet		0	7/34 (20.6%)	
Valganciclovir		0	17/34 (50.0%)	
4rd line				
Ganciclovir		0	5/11 (45.5%)	
Foscarnet		0	5/11 (45.5%)	
Valganciclovir		0	1/11 (9.0%)	
CMV organ disease	36 (8.5%)	2/293 (0.7%)	34/130 (26.2%)	0.0001

*Patients with CMV organ disease. #from patients with available therapy data. CMV: Cytomegalovirus; IgG: immunoglobulin G; GvHD: acute graft-versus-host disease; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; TBI: total body irradiation; HSCT: hematopoietic stem cell transplantation; PBSC: peripheral blood stem cells; BM: bone marrow; CB: cord blood.

hepatitis, (4) retinitis and (5) combined (multiple organs). End-organ disease were defined using current definitions (21).

CMV treatment and prophylaxis. In general, if immunosuppressed patients showed plasma CMV DNAemia or if they were diagnosed with CMV disease, immunosuppression (when possible) was reduced. CMV treatment consisted of different strategies such as PET, targeted therapy in case of organ manifestation, primary or secondary CMV prophylaxis. Antiviral therapy included the administration of valganciclovir (Valcyte®), ganciclovir (Cymevene®), foscarnet (Foscavir®) and/or letermovir (Prevymis®). Early (preemptive) therapy in HSCT patients was administered if plasma CMV DNAemia was detected (>1,000 copies/ml determined twice) in order to prevent CMV disease. If CMV plasma DNAemia was <1,000 copies/ml, weekly monitoring was performed. The antiviral treatment of choice was ganciclovir with a therapeutic dosage (in normal kidney function) of 5 mg/kg given *i.v.* as a 1-h-infusion every 12 h at least for 14 days, respectively until CMV was undetectable in plasma with PCR (determined twice). In case of hematotoxic side effects with neutropenia <1,000/μl, the administration of G-CSF was considered. If response to G-CSF administration was inadequate, we considered switching therapy to foscarnet or interrupting therapy until neutrophils recovered. In case of thrombocytopenia <25,000/μl, therapy was switched or interrupted.

When plasma CMV load continue to increase under therapy, respectively did not decrease after 10-14 days, development of resistance to therapy was considered. In many cases foscarnet has proven effective in cases of clinically relevant ganciclovir-resistant CMV.

Outpatients in good general condition showing asymptomatic CMV DNAemia received valganciclovir as an early therapy for two weeks (2×900 mg p.o.) and were monitored once a week with CMV PCR.

In case of detection of CMV organ disease, antiviral treatment was administered until clinical symptoms disappeared and plasma CMV was undetectable with PCR. The most common treatment regime included an induction therapy followed by a secondary prophylaxis. Induction therapy consisted of ganciclovir 5 mg/kg *i.v.* every 12 h for 21 days in combination with polyspecific immunoglobulins (IVIG, Privigen®) 0.5 g/kg *i.v.* once a week for 2 to 4 weeks. Secondary prophylaxis generally consisted of valganciclovir 900 mg once daily for 3 months or LTV since 2019. Foscarnet could be used as alternative antiviral treatment.

Until 2019 no primary prophylaxis was administered after HSCT. Since 2019, LTV was administered in the LTV group as antiviral prophylaxis after allo-HSCT in recipients with risks constellation for CMV reactivation (D–/R+) or after haplo-identical HSCT. Generally, the administration was scheduled for a period of 100 days starting from the day of the engraftment when no CMV DNA was detectable in peripheral blood. LTV was switched to systemic antiviral treatment when CMV DNA was detectable.

Secondary prophylaxis, generally using valganciclovir, was indicated for 3 months after antiviral treatment of CMV organ disease (see above). Secondary prophylaxis using LTV was performed in some patients with CMV organ disease. To compare plasma CMV DNAemia incidence under LTV we used patients from our cohort (n=423) transplanted from 2010 to 2018 and survived at least until day +180 (n=353) without LTV prophylaxis (historical control group).

Patients who achieved virologic suppression (undetectable viral load or <137 IU/ml in peripheral blood) on systemic antiviral therapy for CMV could be switched to LTV for secondary prophylaxis. The primary outcome was the proportion of patients who experienced reactivation of CMV under LTV. Patients were followed through March 2020 or death, whichever occurred first.

Statistical analysis. Statistical analyses, including distribution analysis and descriptive statistics, were performed with the IBM SPSS 25.0 (IBM, Armonk, NY, USA). Comparison between groups was performed using χ^2-test for categorical variables and Mann-Whitney *U*-test or Student's *t*-test for continuous variables. Kaplan–Meier curves were calculated for survival estimates and the log rank statistics were used to determine differences between groups for categorical variables and the Cox proportional hazards regression analysis for continuous variables. *p*-Values <0.05 were considered as significant. Two-sided tests were used throughout.

Results

Patient characteristics. The 423 allo-HSCT patients had a median age of 49 years (range=19-71 years). Of these, 240 were male (56.6%) and 183 were female (43.2%), including patients receiving transplants from identical siblings (160; 36.6%), haplo-identical (25; 5.9%), and unrelated donors (238; 56.3%). Stem cell source was mostly peripheral blood (376; 88.9%), followed by bone marrow (45; 10.6%), and cord blood (2; 0.5%). The main hematologic diagnoses were acute myeloid leukemia (AML, n=152; 35.8%), acute lymphoblastic leukemia (ALL, n=47; 11.1%), chronic myeloid leukemia (CML, n=15; 3.8%), chronic lymphocytic leukemia (CLL, n=27; 6.4%), lymphoma/myeloma (n=85; 20.0%), myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN, n=81; 19.1%), and others (n=16; 3.8%) (Table I). Conditioning regimens were largely myeloablative (321; 75.8%), compared to reduced intensity conditioning (102; 24.1%). TBI was used in 157 patients (37.1%). Clinically relevant aGvHD (grade ≥2) occurred in 234 patients (55.2%). Chronic GvHD occurred in 163 patients (38.4%).

CMV reactivation. Out of the cohort of 423 patients, 130 (30.7%) showed CMV reactivation and plasma CMV DNAemia. Thereof, 119 patients had >1,000 copies/ml and 11 <1,000 copies/ml. The median peak CMV load in peripheral blood was 7004 IU/ml (range=263-361,000 IU/ml) with a median time to peak CMV load of 49 days after HSCT (range=1-2,071 days). From the 130 patients showing plasma CMV DNAemia, 83 (63.85%) were alive and 47 (36.15%) died during the follow-up period. The incidence of plasma CMV DNaemia was higher in patients with aGvHD ($p<0.0001$), a haplo-identical donor ($p=0.008$), donor CMV IgG positivity ($p=0.001$), and recipient CMV IgG positivity ($p=0.0001$) (Table I).

CMV disease and therapy. Overall, 96 of the 130 patients with CMV DNAemia (73.8%) were classified as asymptomatic, *i.e.* without associated organ disease. In total, 36 out of 423 patients (8.5%) were diagnosed with CMV

end-organ disease of which 9 (2.1%) presented as CMV pneumonia, 23 (5.4%) gastrointestinal disease, 1 (0.2%) retinitis, and 3 (0.7%) with multiple organs affected by CMV. Two patients diagnosed with CMV end-organ disease showed no plasma CMV DNAemia.

From the 130 patients showing plasma CMV DNAemia (119 with cr-CMV replication), 114 received first line therapy consisting of valganciclovir (63; 55.3%), ganciclovir (40; 35.1%) or foscarnet (11; 9.6%). In 60 patients second line therapy was administered including ganciclovir (22; 36.7%), foscarnet (19; 31.7%) and valganciclovir (19; 31.7%) (Table I).

First line therapy for the treatment of CMV organ disease was ganciclovir (22; 61.1%), valganciclovir (10; 27.7%), or foscarnet (4; 11.1%). Second line therapy was administered in 26 patients with CMV organ-disease including foscarnet (11; 42.3%), ganciclovir (8; 30.8%) and valganciclovir (7; 26.9%) (Table II).

From a total of 36 patients diagnosed with CMV organ-disease, 16 patients (44.44%) died from different causes. In 7 patients, CMV was described as possible cause of death.

Letermovoir as primary or secondary prophylaxis. A total of, 42 patients received LTV primary (28/42) or secondary (14/42) prophylaxis. Five out of 42 patients (11.9%) showed CMV replication under LTV compared to 87/353 (24.6%) in the historical control group without CMV prophylaxis ($p<0.003$) (Table III). Under LTV primary prophylaxis, 2/28 (7.1%) patients showed low level CMV DNAemia without CMV disease development. LTV prophylaxis was stopped in both patients and they were treated with valganciclovir. Subsequently both patients showed cGvHD without preceding aGvHD.

Under LTV secondary prophylaxis, 3/14 (21.4%) patients showed low level CMV DNAemia (219, 170 and 138 copies/ml), whereby CMV DNAemia persisted in one patient and LTV secondary prophylaxis was replaced with valganciclovir therapy. All of these 3 patients showed previous CMV organ disease.

Twenty of the 42 patients (47.6%) with LTV prophylaxis experienced aGvHD and 11/42 (26.2%) cGvHD. Median time from HSCT to aGvHD was 39 days. In total, 6/42 (14.3%) patients died during the follow-up period; 2 of them with CMV end-organ-disease. CMV was not described as possible cause of death in any of these 6 patients.

Table II. *CMV organ disease*.

	Total (n=423; 100%)
CMV organ disease	36 (8.5%)
Pneumonia	9 (2.1%)
Gastrointestinal disease	23 (5.4%)
Hepatitis	0
Combined (multiple organs)	3 (0.7%)
Retinitis	1 (0.2%)
CMV disease without CMV replication	2 (0.5%)
1st line therapy for CMV organ disease#	
Ganciclovir	22/36 (61.1%)
Foscarnet	4/36 (11.1%)
Valganciclovir	10/36 (27.7%)
2nd line	
Ganciclovir	8/26 (30.8%)
Foscarnet	11/26 (42.3%)
Valganciclovir	7/26 (26.9%)
3rd line	
Ganciclovir	7/18 (38.9%)
Foscarnet	4/18 (22.2%)
Valganciclovir	7/18 (38.9%)
4rd line	
Ganciclovir	3/7 (42.9%)
Foscarnet	3/7 (42.9%)
Valganciclovir	1/7 (14.3%)
Secondary prophylaxis	9/36 (25%)

#from patients with available therapy data. CMV: Cytomegalovirus.

Discussion

CMV diseases are major causes of death among allo-HSCT recipients (22). In the present study, we identified the incidence and risk factors for post-transplant CMV reactivation and CMV DNAemia and analysed the impact of the first CMV DNA terminase inhibitor LTV, administered as post-HSCT CMV prophylaxis, on CMV DNAemia and CMV disease.

A total of 130 (30.7%) of the 423 patients showed CMV DNAemia after allo-HSCT. These results regarding CMV DNAemia are in line with previous studies and show once again the importance of evaluating possible drugs for CMV prophylaxis such as LTV (23).

CMV IgG seropositivity of recipients and donors, HLA-mismatch, donor type and the presence of aGvHD were found to significantly correlate with the presence of CMV DNAemia in post-HSCT patients. First of all, recipient and/or donor CMV IgG seropositivity (D-/R+, D+/R+) pose a higher risk for plasma CMV DNAemia. Among the 130 patients with CMV DNAemia and/or disease, 123 (94.6%) recipients and 70 (53.8%) donors were CMV IgG positive. This most likely results from the recipient's latent CMV infection being reactivated due to the post-transplant immunosuppression (2). Several studies support this finding, whereby some studies additionally show a relatively higher ratio of CMV reactivation in D-/R+ patients (8, 24-27). This phenomenon is possibly based on the fact that when CMV seropositive recipients receive grafts from seronegative donors there is no transfer of multifunctional CMV-specific T cells and antiviral cytokines, which play a significant role in controlling the virus (24).

Table III. *Patients with letermovir prophylaxis after allo-HSCT.*

	With LTV prophylaxis (n=42; 100%)
Primary prophylaxis	28 (66.6%)
Duration median (range) days	98 (5-153)
Secondary prophylaxis	14 (33.3%)
Duration median (range) days	75 (9-162)
Previous CMV organ disease	9 (1 pneumonia, 8 gastrointestinal)
(between HSCT and LTV)	(21.4%)
Previous CMV DNAemia	13 (31%)
Median age (range)	43 (22-65)
Female	15 (35.7%)
Male	27 (64.3%)
Diagnosis	
AML	14 (32.6%)
ALL	4 (9.3%)
MDS/MPN	7 (16.3%)
Lymphoma/myeloma	11 (25.6%)
Others	3 (7%)
CML	3 (9.3%)
Conditioning	
Reduced	0
Myeloablative	42 (100%)
TBI used	11 (25.6%)
Stem cell source	
PBSC	31 (7.4%)
BM	11 (25.6%)
CB	0
Donor	
Identical sibling	10 (23.8%)
Unrelated	21 (50.0%)
Haplo-identical	11 (26.2%)
GvHD	
Acute GvHD	20 (47.6%)
Median time to acute GvHD	39 days (16- 435)
Chronic GVHD	11 (26.2%)
Donor CMV IgG	
Positive	15 (35.7%)
Recipient CMV IgG	
Positive	42 (100%)
CMV DNAemia until day	2/42 (4.7%)
+100 under LTV	day +31 (1197 copies)
1st prophylaxis	day +51 (461 copies)
Cumulative incidence CMV	7.1% (3/42)
viremia under LTV day +180	days +108 (219 copies)
2nd prophylaxis	+118 (170 copies)
	+ 295 (138 copies)
Cumulative incidence	24.6% (87/353)
CMV viremia day	
+180 historical control group n=353	
Median CMV replication (range)	6766 (381-361,000)
Median time to max. CMV	46 (1-163)
replication (range); days	

LTV: Letermovir; CMV: cytomegalovirus; IgG: immunoglobulin G; GvHD: acute graft-versus-host disease; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; TBI: total body irradiation; HSCT: hematopoietic stem cell transplantation; PBSC: peripheral blood stem cells; BM: bone marrow; CB: cord blood.

Furthermore, patients with HLA mismatched (related or unrelated) and haplo-identical donors were more likely to show CMV DNAemia (p=0.008). Previous research has shown an increased risk for CMV reactivation, CMV disease, CMV-associated death and transplant-related mortality in patients who received unrelated or mismatched donor transplants (2, 8, 28, 29). An earlier study has observed that a matched CMV serostatus may abrogate the effect of an HLA-mismatch (30).

Moreover, patients with unrelated (52% of the patients with undetectable plasma CMV load *versus* 66.2% of the patients with CMV DNAemia) or haplo-identical (5.1% *vs*. 76.7%) donors showed a significantly higher incidence of CMV reactivation than patients with identical sibling donors (44.9% *vs*. 26.2%), whereat the effect was particularly apparent in haplo-identical donors. Current literature also shows that patients with haplo-identical or unrelated donors have a greater risk of virus infection due to the higher degree of immunosuppression (31, 32).

Clinically-relevant aGvHD (grade ≥2) occurred in 234/423 patients (55.2%). Chronic GvHD occurred in 163 (38.4%) of patients. Patients with CMV DNAemia in blood had a higher rate of aGvHD than patients without CMV DNA detection (69.2% *vs*. 49%). These findings are in line with results from similar studies (26). Also, aGvHD occurred earlier after HSCT in patients with CMV DNAemia (25 days *vs*. 29 days) however, it did not meet statistical significance. The question that arises now is how aGvHD and CMV DNAemia interact. The median time interval from allo-HSCT to maximum plasma CMV loads was 49 days, whereas the median time interval from allo-HSCT to the onset of aGvHD in patients with CMV DNAemia was 25 days. These numbers suggest that, on average, aGvHD occurred before CMV was detectable in plasma. Existing data show a bidirectional interaction between plasma CMV DNAemia and aGvHD (33). Our results support these findings, with a tendency towards aGvHD occurring before CMV replication. This could be explained by the fact that aGvHD is treated with immunosuppressants, which render the immune system even more vulnerable to CMV reactivation. Reversely, in patients with CMV infection, immunosuppressive therapy has to be reduced leading to a higher risk for aGvHD. Therefore, aGvHD is a predictor of future CMV DNAemia and vice versa. The data does not show a significant correlation between chronic GvHD and CMV reactivation. This is interesting since patients with aGvHD are more likely to develop subsequent cGvHD (34, 35). Some studies show that patients with both cGvHD and early CMV replication have lower 5-year cumulative incidence of relapse and higher leukaemia-free and overall survival-rates (36). This anti-leukemic effect would be an exciting future field for research.

The incidence of CMV DNAemia in patients under LTV primary or secondary prophylaxis was significantly reduced (5/42 patients, 11.9%) compared to the historical control group (24.6%). This difference can be weighted even stronger since patients receiving LTV primary prophylaxis have CMV sero-constellations with a high risk for virus reactivation and patients in the control group include different sero-constellations. Also, it is important to keep in mind that the 5 patients under LTV only showed low level CMV DNAemia. The two patients with low plasma CMV DNA levels under LTV primary prophylaxis did not show CMV organ disease and CMV DNAemia could immediately be stopped with valganciclovir. However, both patients developed cGvHD without preceding aGvHD. This could be traced back to the abovementioned bidirectional interaction between CMV and GvHD. Two (11.8%) of the patients showing CMV DNAemia with LTV prophylaxis died during follow-up compared to 47 (19.6%) from the patients showing CMV DNAemia without LTV prophylaxis. In 7 (1.7%) patients without LTV prophylaxis CMV was described as possible cause of death, whereas in none of the patients with LTV prophylaxis CMV was considered as possible cause of death. Hence, mortality and CMV-associated mortality amongst patients with CMV DNAemia and LTV prophylaxis were lower compared to patients with CMV DNAemia without LTV prophylaxis.

Our results as well as existing literature show that LTV prophylaxis goes hand in hand with a reduced risk for plasma CMV DNAemia and lower mortality in allo-HSCT recipients (13, 15, 23).

Our results confirm the main findings of the phase 3 trial, as LTV was found to be effective at reducing occurrence of CMV events and was well tolerated (23).

A major limitation of this study is its small sample size and retrospective nature, as well as its single-centre design.

In summary, LTV is effective in CMV primary and secondary prophylaxis, reducing plasma CMV DNAemia in patients with CMV risk-constellation after HSCT as well as mortality.

Conclusion

Despite the development of better approaches with weekly monitoring and early treatment initiation, CMV reactivation plays an important role after allo-HSCT. New anti-viral strategies such as prophylaxis with LTV for CMV may avoid CMV disease in high-risk patients and thus, improve outcome.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

US, MG, and MM designed the study. US, MG, DH, MM, CL, DT, JH, SG, and JP collected or provided data that were analyzed by US, MG, MM, HH, and NK. MM and JP performed statistical analysis. JH, SG, DH, and JP treated and observed the patients. The draft of the article written by US, MG, and MM, was reviewed and approved by all Authors.

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