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Impact of letermovir on Cytomegalovirus-specific T-cells reconstitution after allogeneic hematopoietic stem cell transplantation in the post-transplant cyclophosphamide era

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RUNNING TITLE: CMV-specific T cells in alloHSCT with PTCy and LTV

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DATA SHARING STATEMENT
The source data that support the findings of this study are openly available in the San Raffaele Open Research Data Repository, DOI: 10.17632/nx2t36ng7s.1

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AUTHORS' CONTRIBUTIONS
E.T. designed the study, conducted laboratory experiments, analysed and interpreted data and wrote the paper; G.O. designed the study, provided clinical data and samples, analysed and interpreted data and wrote the paper; V.B. and V.V. conducted laboratory experiments and analysed data; M.N. designed the study and participated in the data interpretation; G.M.B., E.X., M.D., A.Ac., L.L., F.G., S.P., E.D., A.B., F.F., R.D., L.V., A.As., A.R., D.C., C.C., M.T.L.S. provided clinical data and samples and participated in the data interpretation; F.C. designed and supervised the study; C.B and R.G. designed and supervised the study and wrote the paper.

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Main text

The approval of letermovir (LTV) in prophylaxis after allogeneic-hematopoietic stem cell transplantation (allo-HSCT) has reduced the incidence of Cytomegalovirus (CMV) clinically relevant reactivations (CRE) in the early post-HSCT period, postponing these events after LTV cessation in high risk patients[1-3]. T-cell reconstitution is impaired in LTV recipients, and levels of both polyclonal and CMV-specific T cells are restored only some months after LTV treatment[4-8]. Since the presence of CMV-specific T cells correlates with protection against CRE[9-13], this delay potentially exposes patients to viral reactivations after LTV discontinuation.

To identify the factors stimulating the emergence of protective CMV-specific immunity during or immediately after LTV treatment, here we analyse the dynamics of CMV-specific $CD8^+$ T-cell reconstitution in patients receiving allo-HSCT with post-transplant cyclophosphamide (PT-Cy) and calcineurin inhibitor (CNI)-free Graft versus Host Disease (GvHD) prophylaxis. Additionally, we explored the correlation between reconstitution of CMV-specific T cells and key clinical outcomes, such as the incidence and severity of CMV reactivation and of acute and chronic GvHD (aGvHD and cGvHD). Understanding whether CMV-specific T cells can serve as a predictive biomarker may have significant implications for risk stratification and personalized management of post-HSCT patients.

In a single-center retrospective observational study we analyzed 42 CMV-seropositive adult patients with hematological malignancies undergoing allo-HSCT with sirolimus. All patients were treated according to institutional guidelines[14]. GvHD prophylaxis was based on PT-Cy, except in patients receiving graft from cord blood. Anti-thymocite globulin was not used. The conditioning regimen was Treosulfan-based; an intensified conditioning with the addition of a second alkylating agent was classified as myeloablative[14].

Fifteen patients (36%) were treated with allo-HSCT from 2019 to 2022 and received 480 mg of LTV orally once daily from day 0 until day 100 after transplant as CMV prophylaxis. The control group (no-LTV) was composed of 27 (64%) patients transplanted from 2016 to 2018, before LTV approval. Only patients with PBMC available at 90 (D90) and 180 (D180) days after HSCT and whose donors expressed at least one HLA evaluable with Dextramer® CMV-Kit were enrolled. All patients signed written informed consent in agreement within the Declaration of Helsinki and the noninterventional ALMON study approved by the San Raffaele Hospital Institutional Ethical Committee in 2007. Samples were processed and stored by the institutional Biological Resource Center (Num_ID in BBMRI-ERIC: bbmri-eric:ID:IT_1383758011993577). CMV DNAemia was quantified weekly by real-time PCR on whole blood samples until day 100, twice weekly in case of positivity. Subsequently, patients were routinely monitored for CMV viremia and/or presence of CMV-related symptoms: in detail, patients with no CMV reactivation or CMV-related symptoms were monitored for CMV viremia every 2-3 weeks until D180 as per institutional guidelines[5]. CRE was defined as onset of CMV disease or initiation of pre-emptive therapy, based on CMV DNAemia above 10'000 copies/ml and the CMV-infection risk of the patient, or repeated positive samples showing increased viral load during LTV prophylaxis[13]. CMV blips were defined as the detection of a single positivity for CMV DNA with subsequent spontaneous clearance[15]. Patients', transplants' and immune characteristics are detailed in **Table 1**. CMV-specific $CD8^+$ T cells were quantified by flow cytometry using the In Vitro Diagnostic Regulation (IVDR) Dextramer® CMV-Kit (Immudex) as previously described[10] in PBMC frozen at D90 and D180. Dextramer reagents are composed by multiple MHC-peptide complexes and fluorophores covalently bound to a dextran backbone. This tool allows the evaluation of CMV-specific immunity with a good correlation with functional assays and higher sensitivity [10]. Data were considered evaluable if at least 15'000 events in the $CD3^+CD8^+$ region were acquired. Absolute counts of CMV-specific $CD8^+$ T cells were calculated based on the percentage of

$CD3^+CD8^+Dextramer^+$ lymphocytes in frozen samples and on the absolute counts of $CD3^+$ T cells in corresponding whole fresh blood. Protective anti-viral immunity was defined based on the threshold of 0.5 CMV-specific cells/µl, which we had previously identified as an independent protective factor from subsequent CRE[10].

Absolute $CD3^+$ counts and percentages of $CD8^+$ T cells were similar in the 2 groups at both timepoints, with a trend for a slower $CD3^+$ reconstitution in LTV patients, in agreement with previous observations[4,5,8]. Only a minority of patients (n=2 at D90 and n=4 at D180) were treated with a dose of steroids with a potential impact on T-cells amount and functionality. As expected, the number and incidence of both CRE and overall CMV reactivations within D180 was lower in LTV compared with no-LTV patients (CRE, p=0.007; CMV reactivations, p=0.008) **(Table 1** and **Supplementary Figure 1A, B)**. In agreement with our previous data in a wider cohort[5], in patients receiving LTV prophylaxis we also observed a reduction in moderate-to-severe cGvHD (p=0.009) and a trend towards a lower incidence of any grade aGvHD (p=0.06) and cGvHD (p=0.07) **(Supplementary Figure 1C-E)**.

In our cohort, absolute numbers of CMV-specific $CD8^+$ T cells were enriched from D90 to D180 (p=0.02, **Supplementary Figure 2A**). Analysing separately the 2 different CMV prophylaxis groups, this increase was evident only in patients receiving LTV (p=0.03, **Figure 1A**). Accordingly, the absolute numbers of CMV-specific T lymphocytes and the percentages of patients harboring protective levels of CMV-specific $CD8^+$ T cells were lower in LTV compared to no-LTV patients at D90 (p=0.009 and p=0.04 respectively), but these values are comparable in the 2 groups at D180 as a consequence of CMV-specific immunity restoration in LTV patients **(Figure 1B, C)**. Interestingly, the incidence of cGvHD (both overall and moderate-to-severe) was not affected by the presence of CMV-specific T cells **(Figure 1D** and **Supplementary Figure 2B**) but was increased in patients experiencing CMV reactivations (including not-CRE) before either D90 or D180, especially when considering only moderate-to-severe cGvHD (**Figure 1E** and **Supplementary Figure 2C**). These data suggest that, while CMV-specific lymphocytes should not be involved in driving cGvHD onset and progression, its occurrence might be influenced by the increased inflammation associated with viral reactivation. Larger-scale investigations are needed to substantiate this hypothesis.

In bivariate analyses, the variables associated with the presence of protective CMV-specific immunity at D90 were absence of LTV prophylaxis, the percentage of $CD8^+$ T cells and the number and occourrence of CMV reactivations (including not-clinically relevant ones) before D90 (see **Table 2** for further details). For D180, statistically significant correlation was found only for viral reactivations before D180**,** with a trend towards positive correlation with absolute $CD3^+$ T-cell counts and donor CMV serostatus (**Table 2**). Of note, CMV-specific T cells were not affected by either the type of transplant or the intensity of conditioning regimen, which are the other 2 variables significantly different between LTV and no-LTV groups **(Tables 1 and 2)**. Multivariate binomial logistic regression confirmed for both timepoints a positive correlation between the presence of CMV-specific T cells above the protective threshold and previous CMV reactivation, regardless their number **(Table 2** and data not shown**)**. Interestingly, at D180 this correlation was significant only including the occurrence of CMV blips in the analysis (D90, no blips p=0.010 and with blips p=0.007; D180, no blips p=0.159 and with blips p=0.028). Furthermore, at D90 also the percentage of $CD8^+$ T cells was significantly correlated with CMV-specific immunity (p=0.025), which is possibly dependent on the type of assay, while in this cohort with good immune-reconstitution absolute T-cell counts had no impact **(Table 2)**.

In this study we quantified CMV-specific $CD8^+$ T cells by a flow cytometry IVDR kit. Compared to the detection of Interferon(IFN)-γ producing CMV-specific T cells by flow cytometry, ELISpot or QuantiFERON, previously used to evaluate CMV-specific immunity

in this setting[6-8,16], the use of this kit strongly reduces the analysis time and is characterized by standardization, ease of use and high reproducibility, in agreement with the recent European Regulation IVD-R 2017/746. Furthermore, we have recently demonstrated that the quantification of CMV-specific T cells by Dextramer staining correlates with functional assays such as IFN-γ ELISpot and QuantiFERON[10].

The GvHD prophylaxis regimen in this cohort was CNI–free. Instead of the more traditional tacrolimus or cyclosporine backbones, it relied predominantly on PT-Cy (omitted only in patients receiving cord blood units as graft source), sirolimus and mycophenolate mofetil (MMF). By omitting CNIs, this strategy aims to reduce nephrotoxicity, neurotoxicity and metabolic complications, while still harnessing the potent in vivo T-cell modulation activity of PT-Cy and the synergistic immunoregulation of sirolimus plus MMF to prevent both acute and chronic GvHD.

Our findings reinforce the protective role of LTV against clinically relevant CMV-CRE and confirm that LTV prophylaxis is associated with a delayed reconstitution of CMV-specific $CD8^+$ T cells compared to no-LTV patients. Importantly, our data underscore the pivotal role of antigen exposure—even transient and at low levels, such as during CMV blips—in promoting the expansion of protective levels of CMV-specific T lymphocytes. This suggests that minimal antigenic stimulation is sufficient to boost protective CMV-specific immune responses in the context of ongoing reconstitution. Moreover, in this cohort, four patients (LTV n=3; no-LTV n=1) achieved protective levels of CMV-specific T cells by D180 despite the absence of previous CMV positivity (data not shown). Although we cannot exclude transient CMV reactivation escaping our monitoring schedule, this observation suggests that, while antigen exposure facilitates expansion, the recovery of CMV-specific immunity is also dependent on the broader trajectory of immune reconstitution, which improves at late post-transplant timepoints. A limitation of the method used in this study is the possibility to evaluate only patients with good immune-reconstitution, due to the requirement of at least 15'000 $CD3^+CD8^+$ T cells.

These findings have several implications: first, a clearer definition of the stimuli that promote CMV-specific T-cell emergence—whether through subclinical antigen exposure or endogenous immune recovery—could help in refining patients stratification strategies. Our work supports the potential utility of combining longitudinal immune monitoring with CMV-specific Dextramer-based assay to identify patients with suboptimal CMV-specific immune reconstitution. Moreover, in the setting of extended prophylaxis that is currently explored in clinical trials, Dextramer-based assays could allow to discriminate the patients that could safely shorten the therapy duration or, on the other hand, would need even longer prophylaxis, allowing for better protection during the window of immune vulnerability.

Additionally, consistent with prior observations[5], our data suggest that the incidence of GvHD is lower in patients receiving LTV, but increases after CMV reactivation. This supports the hypothesis that reduced CMV reactivation and associated inflammation may contribute to a more favorable GvHD profile. Furthermore, our data provide indirect evidence that CMV-specific T cells are not major contributors to the pathogenesis of cGvHD, since we observed no increased risk of cGvHD in patients with higher levels of CMV-specific T cells.

Taken together, our study provides new insights into the dynamics of CMV-specific immunity under LTV prophylaxis and highlights actionable items for personalized antiviral strategies after allo-HSCT.

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Tables

Table 1

	Patients (n=42)		
	no-LTV (n=27)	**LTV (n=15)**	**p value**
Age - mean (range)	53 (20-70)	53 (22-75)	0.633
Gender - n (%)			0.927
F	13 (48.1)	7 (46.7)	
M	14 (51.9)	8 (53.3)	
Diagnosis - n (%)			0.599
AML	15 (55.6)	10 (66.7)	
MDS/MPN	7 (25.9)	4 (26.6)	
ALL	3 (11.1)	0	
Other	2 (7.4)	1 (6.7)	
Graft source - n (%)			0.909
PBSC	24 (88.9)	13 (86.7)	
BM	1 (3.7)	1 (6.7)	
CBU	2 (7.4)	1 (6.7)	
Donor type - n (%)			**0.035**
MUD	14 (51.9)	14 (93.3)	
MRD	4 (14.8)	0	
MMRD	7 (25.9)	0	
CBU	2 (7.4)	1 (6.7)	
GvHD prophylaxis - n (%)			0.400
PTCy-sirolimus-MMF	22 (81.5)	14 (93.3)	
PTCy-sirolimus	3 (11.1)	0	
sirolimus-MMF	2 (7.4)	1 (6.7)	
CMV serostatus host/donor - n (%)			0.307
pos/pos	17 (63.0)	7 (46.7)	
pos/neg	10 (37.0)	8 (53.3)	
Conditioning Intensity - n (%)			**<0.001**
MAC	22 (81.5)	4 (26.7)	
RIC	5 (18.5)	11 (73.3)	
Number of HSCT - n (%)			0.435
1	23 (85.2)	14 (93.3)	
2	4 (14.8)	1 (6.7)	
% donor chimerism ad D30 - mean (range)	98.4 (90.0-99.9)	98.7 (96.2-99.9)	0.447
% donor chimerism ad D90 - mean (range)	98.6 (87.0-100)	99.4 (96.7-100)	0.246
Absolute CD3+ counts D90 - mean (range)	731 (108-2226)	474 (82-890)	0.202
Absolute CD3+ counts D180 - mean (range)	1157 (89-3068)	948 (231-2571)	0.561
%CD8+/CD3+ D90 - mean (range)	39.1 (11.5-67.5)	32.9 (3.1-56.0)	0.306
%CD8+/CD3+ D180 - mean (range)	42.1 (13.3-77.8)	47.2 (16.1-73.2)	0.415

Number of CRE D90 - n (%)			**0.016**
0	16 (59.3)	15 (100.0)	
1	10 (37.0)	0	
2	1 (3.7)	0	
Number of CRE D180 - n (%)			**0.024**
0	14 (51.9)	14 (93.3)	
1	12 (44.4)	1 (6.7)	
2	1 (3.7)	0	
Number of CMV reactivations D90 - n (%)			**<0.001**
0	10 (37.0)	15 (100.0)	
1	15 (55.6)	0	
2	2 (7.4)	0	
Number of CMV reactivations D180 - n (%)			0.053
0	8 (29.6)	10 (66.7)	
1	11 (40.7)	5 (33.3)	
2	7 (25.9)	0	
3	1 (3.7)	0	
Number of CMV reactivations including blips D90 - n (%)			**0.007**
0	9 (33.3)	14 (93.3)	
1	12 (44.4)	1 (6.7)	
2	4 (14.8)	0	
3	1 (3.7)	0	
4	1 (3.7)	0	
Number of CMV reactivations including blips D180 - n (%)			0.128
0	6 (22.2)	7 (46.7)	
1	9 (33.3)	7 (46.7)	
2	5 (18.5)	1 (6.7)	
3	4 (14.8)	0	
4	3 (11.1)	0	
Steroids ≥ 0.5 mg/kg/day at D90 - n (%)			0.280
no	25 (92.6)	15 (100)	
yes	2 (7.4)	0	
Steroids ≥ 0.5 mg/kg/day at D180 - n (%)			0.117
no	23 (85.2)	15 (100)	
yes	4 (14.8)	0	

Table 1. Patients' and transplants' characteristics.
Statistical analyses: chi-square test for categorical variables, Spearman's correlation for continuous variables.
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; BM, bone marrow; CBU, cord blood unit; CMV, cytomegalovirus; F, female; GvHD, Graft-versus-Host Disease; HSCT, hematopoietic stem cell transplantation; LTV, letermovir; M, male; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MPN, myeloproliferative neoplasm; MRD, matched related donor; MMRD, mismatched related donor; MUD, matched unrelated donor; PBSC, peripheral blood stem cells; PTCy, post-transplant cyclophosphamide; RIC, reduced-intensity conditioning.
Values may not add up to 100% due to rounding.

Table 2

BIVARIATE ANALYSES											
	D90					D180					
	DEX+ <0.5/ul	DEX+ ≥0.5/ul	p	χ^2	Odds ratio (95% CI)		DEX+ <0.5/ul	DEX+ ≥0.5/ul	p	χ^2	Odds ratio (95% CI)
treatment			**0.042**	4.12	0.183 (0.032-1.04)	**treatment**			0.588	0.294	1.47 (0.363-5.95)
No-LTV	11	15				No-LTV	10	17			
LTV	8	2				LTV	4	10			
disease			0.537	0.380	0.549 (0.080-3.76)	**disease**			0.477	0.507	0.442 (0.0446-4.39)
myeloid	17	14				myeloid	13	23			
lymphoid	2	3				lymphoid	1	4			
type of HSCT			0.095	6.36	n.a.	**type of HSCT**			0.240	4.21	n.a.
MUD	13	11				MUD	9	18			
MRD	4	0				MRD	3	1			
MMRD	2	5				MMRD	1	6			
CBU	0	1				CBU	1	2			
donor CMV serostatus			0.270	1.22	2.16 (0.544-8.57)	**donor CMV serostatus**			0.058	3.59	3.60 (0.929-14.0)
negative	9	5				negative	9	9			
positive	10	12				positive	5	18			
conditioning intensity			0.836	0.043	1.17 (0.271-5.02)	**conditioning intensity**			0.443	0.588	1.72 (0.428-6.90)
MAC	14	12				MAC	10	16			
RIC	5	5				RIC	4	11			
aGvHD			0.765	0.089	1.22 (0.327-4.56)	**aGvHD**			0.424	0.638	0.588 (0.159-2.17)
no	11	9				no	7	17			
yes	8	8				yes	7	10			
absolute CD3+ counts D90			0.476	n.a.	n.a.	**absolute CD3+ counts D180**			0.050	n.a.	n.a.
mean (range)	592 (237-1510)	802 (110-2226)				mean (range)	844 (406-2571)	1198 (89-3068)			
%CD8+/CD3+ D90			**0.022**	n.a.	n.a.	**%CD8+/CD3+ D180**			0.061	n.a.	n.a.
mean (range)	33.0 (11.5-61.6)	46.7 (19.8-67.5)				mean (range)	35.4 (15.2-71.6)	48.1 (13.3-77.8)			
CMV reactivation before D90			**<0.001**	11.1	12.2 (2.53-58.7)	**CMV reactivation before D180**			**0.017**	5.70	5.14 (1.28-20.7)
no	15	4				no	9	7			
yes	4	13				yes	5	20			
number of CMV reactivations before D90			**<0.001**	14.3	n.a.	**number of CMV reactivations before D180**			0.174	4.97	n.a.
0	16	4				0	9	8			
1	2	12				1	3	13			
2	1	1				2	2	5			

3	0	0				3	0	1			
CMV reactivation before D90 including blips			**<0.001**	13.5	17.5 (3.31-92.5)	**CMV reactivation before D180 including blips**			**0.005**	7.98	7.67 (1.71-34.3)
no	15	3				no	8	4			
yes	4	14				yes	6	23			
number of CMV reactivations before D90 including blips			**0.008**	13.9	n.a.	**number of CMV reactivations before D180 including blips**			**0.023**	11.4	n.a.
0	15	3				0	8	4			
1	3	9				1	4	12			
2	1	3				2	0	6			
3	0	1				3	2	2			
4	0	1				4	0	3			

MULTIVARIATE ANALYSES

DEX+ ≥0.5/ul D90						**DEX+ ≥0.5/ul D180**					
	p	**Odds-Ratio (95% CI)**		**p**	**Odds-Ratio (95% CI)**		**p**	**Odds-Ratio (95% CI)**		**p**	**Odds-Ratio (95% CI)**
CMVreact before D90 (no blips)			**CMVreact before D90 (including blips)**			**CMV react before D180 (no blips)**			**CMV react before D180 (including blips)**		
1 – 0	**0.010**	53.575 (2.545-1127.726)	1 – 0	**0.007**	54.979 (2.922-1034.328)	1 – 0	0.159	3.004 (0.651-13.86)	1 – 0	**0.028**	6.702 (1.233-36.416)
treatment			**treatment**			**donor CVM status**			**donor CVM status**		
1 – 0	0.862	0.817 (0.084-7.950)	1 – 0	0.975	0.963 (0.092-10.113)	pos-neg	0.137	3.169 (0.694-14.47)	pos-neg	0.060	4.569 (0.936-22.299)
%CD8+/CD3+ D90			**%CD8+/CD3+ D90**			**absolute CD3+ counts D180**			**absolute CD3+ counts D180**		
	0.017	1.115 (1.020-1.219)		**0.025**	1.106 (1.013-1.208)		0.246	1.001 (0.999-1.00)		0.356	1.001 (0.999-1.002)

Table 2. Correlation between the presence of protective CMV-specific T cells and clinical/biological variables.

Statistical analyses: bivariate, chi-square test for categorical variables, Spearman's correlation for continuous variables; multivatiate, binomial logistic regression.

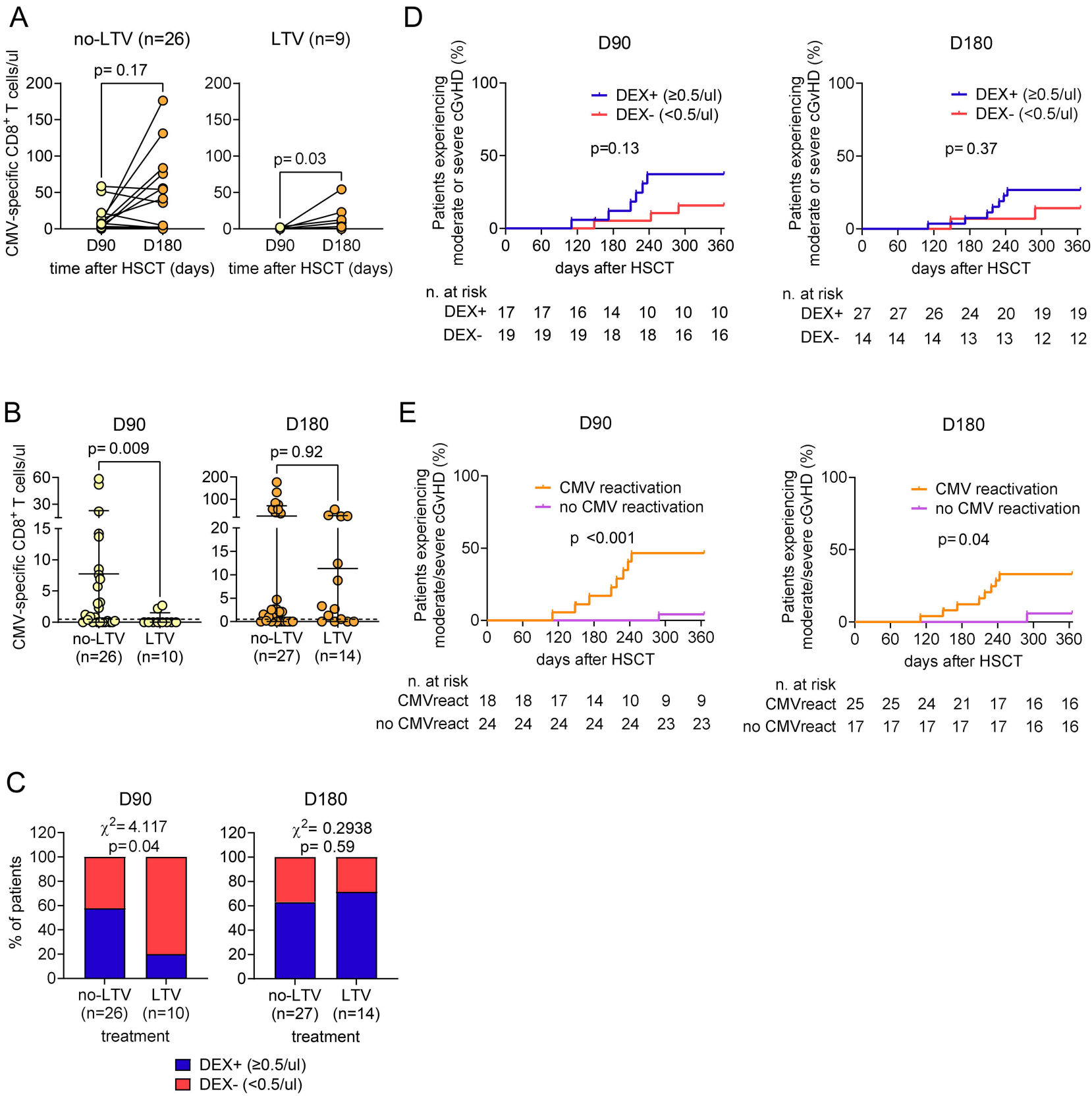
Only patients with evaluable samples (≥15'000 $CD8^+$ T cells acquired) at either D90 or D180 were included. The columns "DEX- <0.5/ul" and "DEX+ ≥0.5/ul" contain the number of patients in each group.

aGvHD, acute Graft-versus-Host Disease; CBU, cord blood unit; CMV, Cytomegalovirus; DEX, $Dextramer^+CD8^+$ T cells; HSCT, hematopoietic stem cell transplantation; LTV, Letermovir; MAC, myeloablative conditioning; MRD, matched related donor; MMRD, mismatched related donor; MUD, matched unrelated donor; RIC, reduced-intensity conditioning.

Figure legends

Figure 1. Dynamics of CMV-specific T cells differ according to letermovir (LTV) treatment without impact on moderate-to-severe cGvHD. A, longitudinal variation in the absolute values of CMV-specific $CD8^+$ T cells between D90 and D180 in patients receiving (right panel) or not (left panel) LTV as CMV prophylaxis. Only patients with evaluable samples (≥15'000 $CD8^+$ T cells acquired) at both D90 and D180 were included. Means of CMV-specific T cells: no-LTV, 7.8 cells/µl at D90 and 26.0 cells/µl at D180; LTV, 0.5 cells/µl at D90 and 9.6 cells/µl at D180. Statistical analyses by Wilcoxon test. **B** and **C**, comparison of the absolute counts of CMV-specific lymphocytes (B) and of the percentages of patients with levels of CMV-specific T cells above the protective threshold of 0.5/µl (C) at D90 and D180 after allo-HSCT between patients treated or not with LTV. Only patients with evaluable samples (≥15'000 $CD8^+$ T cells acquired) at either D90 or D180 were included. Means of CMV-specific T cells in panel B: D90, 7.8 cells/µl in no-LTV and 0.5 cells/µl in LTV; D180, 25.0 cells/µl in no-LTV and 11.3 cells/µl in LTV. Statistical analyses by Mann-Whitney test in B and chi-square test in C. **D,** incidence of moderate-to-severe cGvHD according to the presence of protective levels of CMV-specific T cells (DEX+≥0.5/µl) at D90 (left graph) or D180 (right graph). **E,** incidence of moderate-to-severe cGvHD according to the occurrence of CMV reactivations before D90 (left graph) or D180 (right graph). D and E, statistical analyses by Log-rank test. Lines indicate censored data.

Figure 1


A
no-LTV (n=26)
LTV (n=9)
p= 0.17
p= 0.03
CMV-specific CD8+ T cells/ul
D90
D180
time after HSCT (days)
B
D90
D180
p= 0.009
p= 0.92
no-LTV (n=26)
LTV (n=10)
no-LTV (n=27)
LTV (n=14)
C
D90
D180
χ2= 4.117
p=0.04
χ2= 0.2938
p= 0.59
% of patients
treatment
DEX+ (≥0.5/ul)
DEX- (<0.5/ul)
D
D90
D180
Patients experiencing moderate or severe cGvHD (%)
DEX+ (≥0.5/ul)
DEX- (<0.5/ul)
p=0.13
p= 0.37
days after HSCT
n. at risk
DEX+ 17 17 16 14 10 10 10
DEX- 19 19 19 18 18 16 16
DEX+ 27 27 26 24 20 19 19
DEX- 14 14 14 13 13 12 12
E
D90
D180
Patients experiencing moderate/severe cGvHD (%)
CMV reactivation
no CMV reactivation
p <0.001
p= 0.04
days after HSCT
n. at risk
CMVreact 18 18 17 14 10 9 9
no CMVreact 24 24 24 24 24 23 23
CMVreact 25 25 24 21 17 16 16
no CMVreact 17 17 17 17 17 16 16

Supplementary Material for

Impact of letermovir on cytomegalovirus-specific T-cells reconstitution after allogeneic hematopoietic stem cell transplantation in the post-transplant cyclophosphamide era

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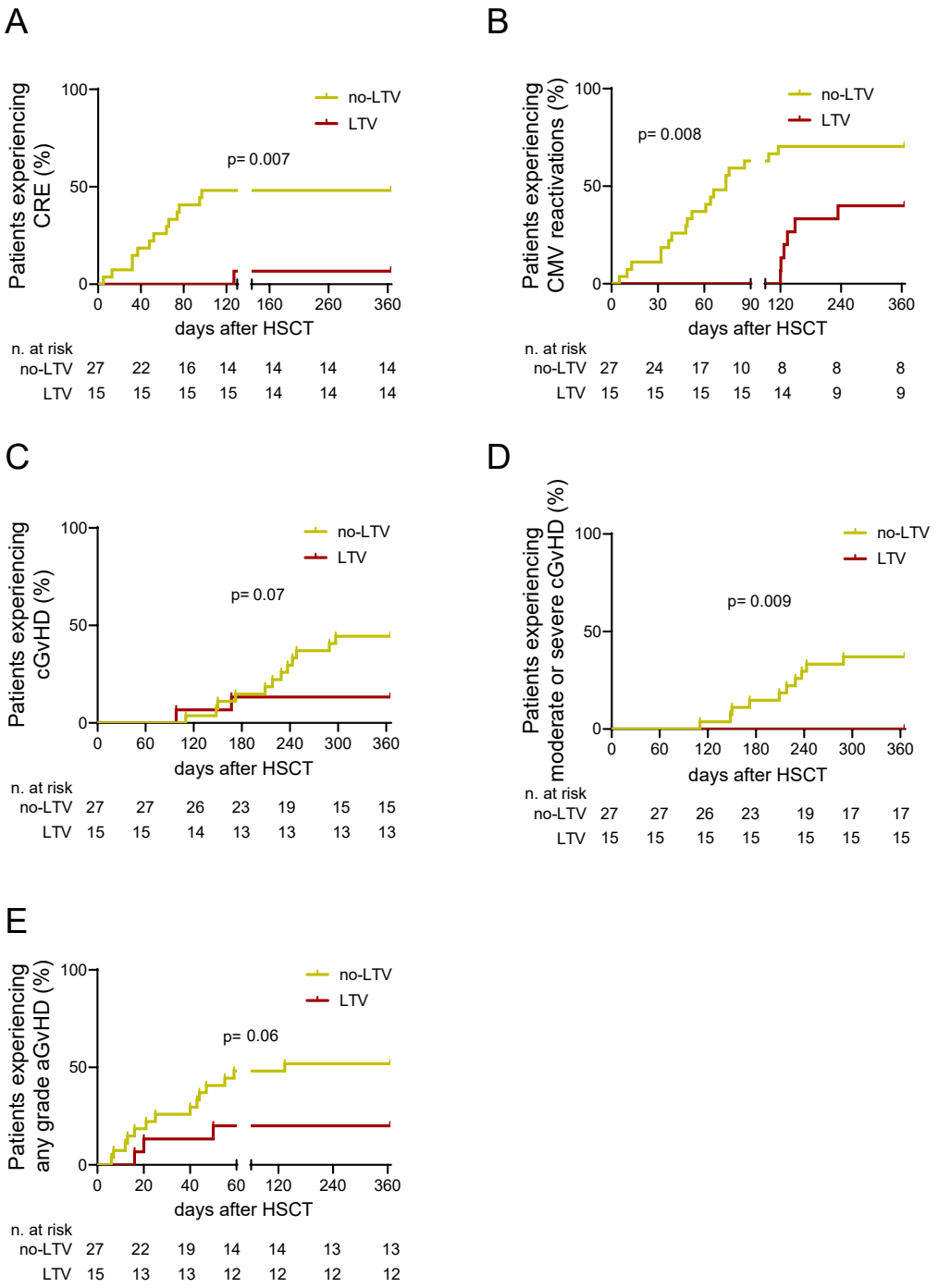
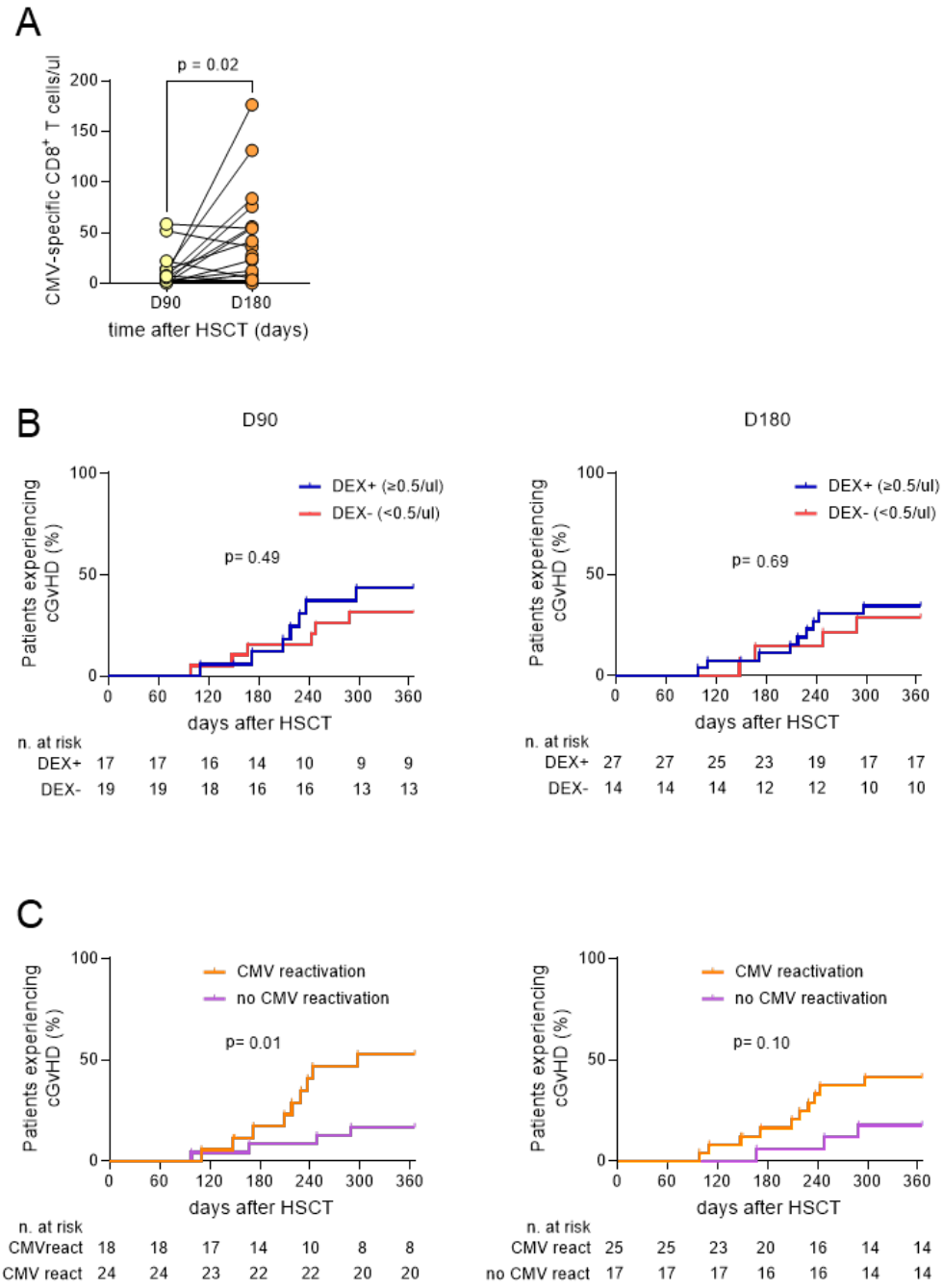
Supplementary Figures

Supplementary Figure 1



Supplementary Figure 2



Legends to Supplementary Figures

Supplementary Figure 1. LTV prophylaxis is associated with reduced incidence of CRE, CMV reactivations and moderate-to-severe cGvHD. Incidence of CRE (**A**), overall CMV reactivations (**B**), cGvHD of any grade (**C**) or moderate-to-severe (**D**) and of aGvHD of any grade (**E**) in patients receiving or not LTV as CMV prophylaxis. Statistical analyses by Log-rank test, lines indicate censored data.

Supplementary Figure 2. Dynamics of CMV-specific T cells in the overall population without impact on cGvHD. A, longitudinal variation in the absolute values of CMV-specific $CD8^+$ T cells between D90 and D180 in the overall patients' cohort (n=35). Only patients with evaluable samples (≥15'000 $CD8^+$ T cells acquired) at both D90 and D180 were included. Means of CMV-specific T cells: 5.7 cells/μl at D90 and 20.3 cells/μl at D180. Statistical analysis by Wilcoxon test. **B,** incidence of overall cGvHD according to the presence of protective levels of CMV-specific T cells (DEX+≥0.5/μl) at D90 (left graph) or D180 (right graph). **C,** incidence of overall cGvHD according to the occurrence of CMV reactivations before D90 (left graph) or D180 (right graph). B and C, statistical analyses by Log-rank test. Lines indicate censored data.