Incidence, features and outcomes of Cytomegalovirus DNAemia in unmanipulated haploidentical allogeneic hematopoietic stem cell transplantation with post-transplantation cyclophosphamide

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***Running Title*:** CMV DNAemia in haploidentical allo-HSCT.

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Abstract

Background: Conflicting data have been published as to the risk of cytomegalovirus (CMV) DNAemia and CMV disease in patients undergoing haploidentical hematopoietic stem cell transplantation (Haplo-HSCT) with post-transplantation cyclophosphamide. **Methods**: We conducted a multicenter retrospective study including 118 patients subjected to unmanipulated Haplo-HSCT to further clarify this issue. An historic cohort comprising 165 patients undergoing other transplant modalities (HLA-matched related, matched unrelated or mismatched) was built for comparison purposes. Plasma CMV DNA monitoring was performed using two highly sensitive real-time PCR assays. **Results**: Overall, the cumulative incidence of CMV DNAemia, recurrent CMV DNAemia and CMV DNAemia requiring preemptive antiviral therapy in patients undergoing Haplo-HSCT was 63.9%, 34.9% and 50.1%, respectively. These figures were rather comparable for other transplant modalities (P=0.22, P=0.13 and P=0.72, respectively). A trend towards longer duration of episodes and shorter CMV DNA doubling times was observed in Haplo-HSCT patients in comparison with other transplant modalities. Furthermore, median CMV DNA peak load was significantly higher in Haplo-HSCTs (P=0.008), yet overall mortality by day 180 and 365 was the same across comparison groups. There were five cases of CMV disease and all occurred in Haplo-HSCT patients. This latter observation is worrying and merits further investigation.

Conclusions: The incidence of initial and recurrent episodes of CMV DNAemia either requiring or not antiviral therapy in unmanipulated Haplo-HSCT was comparable to other transplant modalities in our cohort.

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Key words: Cytomegalovirus (CMV), CMV DNAemia, haploidenticalhematopoietic stem cell transplantation, overall mortality.

Introduction

Haploidentical hematopoietic stem cell transplantation (Haplo-HSCT) with posttransplantation cyclophosphamide (PT-Cy) is being increasingly used as the first-choice platform for treatment of high-risk hematological malignancies, in the absence of a suitable HLA-matched donor. The potential advantages of Haplo-HSCT lie in the wide and rapid availability of donors and comparable clinical outcomes to other alternative allogeneic stem cell transplant (allo-HSCT) modalities[1-5].

Cytomegalovirus (CMV) remains a threat for allo-HSCT recipients despite the availability of effective strategies for the prevention of end-organ disease (i.e. preemptive therapy)[6], since viral DNAemia itself may be detrimental in terms of survival[7-11]. Conflicting data have been published as to the incidence of CMV DNAemia and CMV disease in haplo-HSCT recipients, with some studies suggesting that this transplant modality harbors a significantly higher risk for both events when compared to HLA-matched related or unrelated allo-HSCTs[12-22]; yet this appeared not to have an impact on overall (OM) and non-relapse (NRM) mortality[18,23-25]. In the current multicenter study we estimated the incidence of CMV DNAemia, CMV disease OM and NRM in Haplo-allo-HSCT and compared to that in other transplant modalities. Additionally, we provide

comparative data regarding the dynamics of CMV DNAemia across donor types, which to our knowledge have not been thoroughly investigated.

Patients and methods

Study population

In this multicenter, observational study, we retrospectively reviewed CMV DNA PCR results and clinical charts of 283 patients who underwent allo-HSCT in four Spanish institutions.

A total of 118 consecutive patients who underwent Haplo-HSCT with cyclophosphamide (50mg/kg/day on days 3 and 5 after transplantation) at the Hospital Clínico Universitario of Valencia-HCUV- (n=63), Hospital Regional Universitario of Málaga, Spain –HRUM- (n=16), Hospital Clínico Universitario of Salamanca, Spain-HCUS- (n=30), or Hospital General Universitario Gregorio Marañón of Madrid, Spain –HGUGM- (n=9), between January 2016 and December 2018 were included in the current study. In turn, a historical cohort was built that included a total of 165 consecutive patients undergoing other allo-HSCT modalities at two of these centers (HCUV, n=137 from May 2012 to June 2018, and HRUM, n=28 from January 2016 to February 2018). Of these, 76 were HLA-matched-related (MRD), 62 HLA-matched unrelated (MUD) and 27 HLA-mismatched either related-MMRD (n=24) or unrelated –MMUD- (n=3) (n=27). All but 8 patients were followed up through day 365. These 8 patients were followed up for a median of median 294 days (227-328). This study was approved by the Hospital Clínico, Fundación INCLIVA Ethics Committee. Signed informed consent was obtained from participants.

Management of CMV infection

CMV infection was managed in all patients by a preemptive antiviral therapy approach. Plasma CMV DNA load was monitored using the CMV Real*Time* CMV PCR (Abbott Molecular, Des Plaines, IL, USA), whose limit of detection (LOD) is approximately 31 IU/ml (95% C.I.)[26], at all centers except at HCUS in which the Affigene CMV Trender kit (Cepheid AB, Bromma, Sweden) was used[27]; the LOD of this assay is 137 IU/ml, according to the manufacturer. CMV DNA load monitoring was conducted on a weekly basis through day +100. From day +100 until day +365 patients at risk for recurrent episodes of CMV DNAemia were also monitored on a weekly basis, while the remaining patients were monitored at each planned visit. Preemptive antiviral therapy with oral valganciclovir (900 mg/12 h), iv. ganciclovir (5 mg/kg/12 h), or foscarnet (60mg/kg/12 h) was initiated at HCUV when the plasma CMV DNA load reached levels of ≥1,500 IU/ml, or when the CMV doubling time (dt) was ≤2.0 days, whichever occurred first (this latter strategy since May 2014)[28]. CMV DNA threshold for inception of preemptive therapy was ≥600 IU/ml at HCUS, ≥156 IU/ml at HRUM and any level at HGUGM. CMV disease was diagnosed and treated as previously detailed[6].

CMV DNA kinetics parameters

The CMV DNA doubling time was given by $dt = (t2 - t1) \times [\ln 2/\ln(q2/q1)]$, with q1 and t1 representing the CMV DNA (copies/ml) at the time of the first positive qRT-PCR (in days), respectively, and q2 and t2 representing CMV DNA at the time of the second positive qRT-PCR (in the absence of antiviral treatment)[29]. Only episodes showing an increase between the first and second CMV DNA load values of >3-fold were considered for analysis. The kinetics of CMV DNA load clearance is expressed by the equation $y_t = y_0 e^{-kt}$, where y0 is the peak CMVDNA load, t is time elapsed between y_0 and $y_t$and k is the decay constant[29].

Definitions

CMV DNAemia was defined as detection of CMV DNA at any level in one or more plasma specimens. The overall duration of a given episode of viral DNAemia was the time elapsed between the day of first detection of viral DNA in plasma and that of the first negative (undetectable) PCR result. Recurrent episodes were those that developed at least 15 days after clearance of the previous one. Acute graft versus host disease (aGvHD) was diagnosed and graded as previously reported[30].

Statistical analysis

The cumulative incidence of events of interest was estimated by the cumulative incidence method using the statistical software R (http://www.r-project.org/) with the survival and cmprsk packages. The Fine-Gray test was used for comparisons. Overall mortality (OM) was defined as the total number of deaths from any cause. Non-relapse mortality (NRM) was defined as the total number of deaths without relapse or recurrence; the occurrence of relapse or progression was considered a competitive event and censored. The cumulative incidence of CMV DNAemia was calculated considering death and relapse as competitive events. Cox proportional hazards regression models were used to assess the potential risk factors for OM and NRM, as previously reported[10]. Post-transplant events such as CMV DNAemia and acute Graft versus Host Disease (aGvHD) were treated as time-dependent variables. For multivariate analyses, only variables with parameter estimates showing a P value ≤ 0.10 in the univariate analyses were included; two-sided P-values= <0.05 were deemed to be significant. Differences between medians were compared using the Mann-Whitney U-test and the Kruskal-Wallis test, when appropriate. The latter statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics of patients

Demographics, baseline and post-transplant characteristics of patients undergoing different transplant modalities are shown in Table 1. Sex, age, underlying diseases, conditioning regimen used and incidence of aGvHD were comparable across groups, which in turn differed in parameters linked to the allo-HSCT platform selected (i.e. use of ATG), or aGvHD prophylaxis regimens (i.e. use of PT-Cy). Of note, the donor/recipient CMV serostatus pair D-/R+ was underrepresented in patients undergoing haploidentical and MRD transplantation

Incidence of CMV DNAemia in unmanipulated Haplo-HSCT and in other transplant modalities

Overall, the cumulative incidence of CMV DNAemia in patients undergoing Haplo-HSCT was 63.9% (95% CI, 55%-72.6%). This figure was lower at HCUS (48.4%- 95% CI, 30%-67%) than at the other participating centers: HCUV (71%; 95% CI, 60%-83%, HRUM 56% (95% CI, 29%-84%), and HGUGM78% (95% CI, 44%-100%). In this sense it is noteworthy that the LOD of the Affigene PCR assay, used at HCUS, is higher (less sensitive) than that of the Abbott PCR assay (used at the remaining centers). For patients subjected to MRD, MUD and MMUR or MMRD allo-HSCTs (all monitored with the Abbott PCR assay) the cumulative incidence of CMV DNAemia was 73.7% (95% CI, 63.6%-83.8%), 80.7% (95% CI, 70.5%-90.8%) and 70.4% (95% CI, 52%-88.8%), respectively. Figure 1 shows the data for patients monitored with the Abbott PCR assay (n=252): the cumulative incidence of CMV DNAemia appeared to be comparable across the different transplant modalities *(P*=0.22). In our series, no risk factors for the

occurrence of CMV DNAemia in Haplo-HSCT were identified other than recipient CMV seropositivity. This was so both when the entire cohort was considered for the analyses (Table 2) and when only patients monitored with the Abbott PCR assay were included (data not shown)

Overall, the cumulative incidence of recurrent CMV DNAemia in Haplo-HSCT was 34% (95% CI, 23%-45%); this figure was not significantly different (*P*=0.13) from that seen in patients who underwent MRD (44.6%; 95% CI, 31%-58%), MUD (44%; 95% CI, 30%-58%) and MMRD or MMUD (63%; 95% CI, 39%-87%). Nevertheless, the cumulative incidence of recurrent episodes receiving PET was higher in MMRD and MMUD than in haploidentical, MUD and MRD modalities (Figure 2).

We next assessed the incidence of episodes of CMV DNAemia that required administration of preemptive antiviral therapy (CMV PET). Overall cumulative incidence of CMV PET (including initial and recurrent episodes) was not differerent across the donor types (*P*=0.72): 50.9% (95% CI, 31.7%-72%) in Haplo-HSCT, 44.7% (95% CI, 33%-56%) in MRD, 43.6% (95% CI, 30.8%-56%) in MUD and 51.9% (95% CI, 31.7%-72%) in MMRD or MMUD. This was also the case (*P*=0.22) when only first episodes of CMV DNAemia were analyzed: 50% (95% CI, 41%-59%) in Haplo-HSCT, 36.8% (95% CI, 26%-48%) in MRD, 37% (95% CI, 25%-49% in MUD and 40.7% (95% CI, 21%-61%) in MMRD/MMUD.

To control for the potential impact of the CMV DNA threshold triggering PET in the results, we performed a subanalysis including only patients attended at the HCUV (consistent criterion for PET inception throughout the study period), which comprised the majority of patients in our series. As shown in Figure 3, no significant differences were

found, regardless of whether all episodes documented throughout the study follow-up (3A) or only initial episodes (3B) were considered for the analyses.

Incidence of CMV disease

Five of the 118 patients undergoing Haplo-HSCT developed CMV disease (cumulative incidence, 4.5%; 95% CI, 0.5-7.9%), in all cases involving the gastro-intestinal tract. Two cases were documented at HCUV, two at HCUS and one at HRUM. Relevant virological characteristics of these clinical episodes are displayed in Table 4. Of note: in one patient CMV DNAemia was not documented, preemptive antiviral therapy was started in 3 out of the 5 patients and the CMV DNA dt could only be calculated (according to established criteria) in one patient. No cases of CMV disease were diagnosed in control patients undergoing other allo-HSCT modalities (at HCUV and HRUM) (P=0.74).

Kinetics of CMV DNAemia in Haplo-HSCT and other transplant modalities

We next investigated the kinetics of initial episodes of CMV DNAemia in patients undergoing different allo-HSCT donor types. For these analyses, as detailed in the footnotes of Table 3, we selected specific subgroups of patients (episodes) to ensure homogeneity. No differences were seen across transplant modalities regarding the timeframe of CMV DNAemia documentation, the magnitude of initial CMV DNA load or the CMV DNA half-life, in either treated or untreated episodes (Table 3). A trend towards longer duration of episodes and shorter CMV DNA dt_s was observed in Haplo-HSCT patients in comparison with other transplant platforms. Furthermore, median CMV DNA peak load was significantly higher in Haplo-HSCTs (P=0.007).

CMV DNAemia, all-cause and non-relapse mortality

A total of 58 and 74 patients had died by day 180 (Haplo-HSCT, n=24; MRD, n=13; MUD, n=13 and MMUD, n=8) and 365 (Haplo-HSCT, n=33; MRD, n=18; MUD, n=15, and MMUD, n=8) after allo-HSCT. The causes of death are shown in Supplementary Table 1. As shown in Figure 4, neither OM nor NRM was different across comparison groups.

Discussion

In this retrospective multicenter study including a relatively large cohort of patients undergoing T-cell replete Haplo-HSCT we found an overall cumulative incidence of CMV DNAemia of 63.9% by day 365 after transplantation, slightly higher than reported in other fairly comparable series (ranging from 30% to 55%)[12-14,18-20]. Several non-mutually exclusive factors may account for this apparent discrepancy, including different follow-up lengths, the choice of immunosuppressive regimens for aGvHD prevention (i.e. either including or not sirolimus[31]), the blood specimen for CMV DNA surveillance (whole blood vs. plasma) or the use of real-time PCRs for CMV DNA detection displaying distinct limits of detection. Regarding the latter issue, the cumulative incidence of CMV DNAemia was lower at HCUS (48.4%), where the Affigene Trender CMV PCR kit was used, than at the other participating centers (ranging from 56% to 78%), in which a more sensitive PCR assay (Abbott) was employed. Notwithstanding, these figures are substantially lower than those reported for Haplo-HSCT with combined used of cyclophosphamide and ATG, which could be as high as 87% [15,20].

In our Haplo-HSCT cohort only recipient CMV seropositivity was associated with an increased risk of CMV DNAemia. Use of high-dose corticosteroids for severe aGvHD[14], or conditioning regimens containing myeloablative doses of busulfan[19], or preceding

HHV-6 DNAemia[32] have been reported to be associated with higher rates of CMV DNAemia in patients undergoing unmanipulated Haplo-HSCT.

In our experience, the cumulative incidence of CMV DNAemia in Haplo-HSCT patients was not different from that in patients undergoing other all-HSCT modalities, including MRD, MUD or MMRD/MMUD, in line with previous observations[14,18]. A novel finding of the current study was that the rate of recurrent CMV DNAemia as well as the percentage of episodes that required PET administration, irrespective of whether they were initial or recurrent, were rather comparable across the different transplant platforms.

Occurrence of CMV disease in patients undergoing Haplo-HSCT with or without T-cell depletion has been consistently reported as high, ranging from 11% to 17%[12-15], compared to those in other allo-HSCT modalities (less than 10%)[6]. In a recent survey across Spanish centers performing all types of allo-HSCTs in adult patients the cumulative incidence of CMV disease was 2.8%[33]. In our study, although CMV disease was documented in 4.5% of patients, all cases (n=5) developed in the context of Haplo-HSCT.

The current study makes a novel contribution to the literature in providing insight into the kinetics of CMV DNAemia in unmanipulated Haplo-HSCT. We observed a trend towards longer duration of initial episodes of CMV DNAemia and shorter CMV DNA dt_s and significantly higher median CMV DNA peak loads in Haplo-HSCT patients than in those undergoing other transplant modalities. This occurred despite the fact that the donor/recipient CMV serostatus pair D-/R+, convincingly associated with a protracted reconstitution of CMV-specific T-cell immunity[6], was underrepresented in patients undergoing Haplo-HSCT compared to other allo-HSCT platforms (MUD and MMRD/MMUD).

The above observation could be explained, at least in part, by the delay in $CD4^+$ T-cell reconstitution apparently experienced by patients undergoing Haplo-HSCT, in comparison with HLA-matched allo-HSCTs[34]. This might also account for the increased incidence of CMV disease seemingly occurring in our cohort and reported by other groups[12-15] and the high rate of CMV drug resistance among patients receiving PET after Haplo-HSCT, presumably linked to persistent viral replication[14]. In the absence of data on CMV-specific T-cell immunity, this is nevertheless merely speculative, yet our data suggest that OM and NRM in patients undergoing Haplo-HSCT by days 180 and 365 are not different from that in patients subjected to other allo-HSCT types, as has been previously reported[18,23-25].

There are several limitations in our study. The first and main one is the relatively high heterogeneity across the study and historical cohorts. In particular, no patients undergoing non-Haplo-HSCT from two centers HGUS and HGUGM were included. Nevertheless, this is in our view unlikely to have had any impact on the conclusions reached, as allo-HSCT management at these centers within the study period was largely comparable to that in the other two centers (HCUV and HRUM) (not shown). Second, different CMV PET strategies were used across centers; despite this, subanalyses performed with patients attended at HCUV led to similar conclusions to those reached when all patients in this series were taken into consideration. Third, no data on CMV-specific T-cell immunity were provided, and finally, the robustness of mortality analyses was hampered by the sample size.

In summary, our study suggests that unmanipulated Haplo-HSCT increases neither the risk of CMV DNAemia and recurrent CMV DNAemia, as documented by highly-sensitive real-time PCR assays, nor the need for preemptive antiviral therapy and OM and

NRM. Nevertheless, the data presented herein relative to certain kinetics parameters of CMV DNAemia (i.e. duration of the episodes and CMV DNA dt and peak levels) and the seemingly high incidence of CMV disease in these patients should be taken seriously into consideration and further studies to clarify this are warranted.

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Author Contribution

David Navarro and Dixie Huntley developed the study idea. Maria Jesús Pascual, Juan Carlos Hernández-Boluda, Beatriz Gago, Lourdes Vázquez, José Luis Piñana, Magdalena García, Ariadna Pérez, David Serrano, Marta Hernández and Carlos Solano revised and collected de data. Dixie Huntley and Estela Giménez performed the statistical analysis. José Luis Piñana, Carlos Solano and Eliseo Albert supported the data interpretation. All the coauthors revised the draft of the manuscript and David Navarro wrote the final version of the manuscript.

Figure Legends

Figure 1. Cumulative incidence of CMV DNAemia through day 365 after transplantation in patients undergoing different transplant modalities in whom plasma CMV DNA monitoring was performed by means of the Abbott real time PCR assay (n=252). MRD refers to matched related allo-HSCT and MUD to matched unrelated allo-HSCT. HLA-mismatched includes allo-HSCTs with unrelated and related donors.

Figure 2. Overall cumulative incidence of recurrent CMV DNAemia receiving preemptive antiviral therapy (PET) through day 365 after transplantation in patients undergoing different transplant modalities. MRD refers to matched related allo-HSCT and MUD to matched unrelated allo-HSCT. HLA-mismatched includes allo-HSCTs with unrelated and related donors.

Figure 3. Cumulative incidence of CMV DNAemia requiring preemptive antiviral therapy (PET) through day 365 after transplantation in patients attended at Hospital ClínicoUniversitario of Valencia undergoing different transplant modalities. At this center PET was incepted when the plasma CMV DNA load reached levels of ≥1,500 IU/ml, or when the CMV doubling time (dt) was ≤2.0 days, whichever occurred first. MRD refers to matched related allo-HSCT and MUD to matched unrelated allo-HSCT. HLA-mismatched includes allo-HSCTs with unrelated and related donors.

Figure 4. Cumulative incidence of all-cause (overall) mortality by day 180 and by day 365 in patients undergoing different transplant modalities. MRD refers to matched related allo-HSCT and MUD to matched unrelated allo-HSCT. HLA-mismatched includes allo-HSCTs with unrelated and related donors.

Table 1. Patient demographics and clinical characteristics according to transplant modality.						
Parameter	**no. (%)**	**Haploidentical (n=118)**	**HLA mismatched (MMRD/MMUD) (n=27)**	**MUD (n=62)**	**MRD (n=76)**	***P*value[a]**
Sex						0.34
Male	165 (58.3)	69 (59)	14 (52)	32 (52)	50 (66)	
Female	118 (41.7)	49 (41)	13 (48)	30 (48)	26 (34)	
Age (median, range)	55 (18-73)	58 (18-73)	50 (19-67)	55 (18-70)	54 (18-70)	0.59
Underlying disease						0.07
Acute leukemia	100 (35.3)	43 (36.4)	6 (22.2)	20 (32.3)	31 (40.8)	
Chronic leukemia	26 (9.2)	9 (7.6)	3 (11.1)	7 (11.3)	7 (9.2)	
Lymphoma	82 (29)	26 (22)	16 (59.3)	15 (24.2)	25 (32.9)	
MDS/ Multiple myeloma/Myelofibrosis	52 (18.4)	25 (21.2)	0	19 (30.6)	8 (10.5)	
Other	23 (8.1)	15 (12.7)	2 (7.4)	1 (1.6)	5 (6.6)	
Stem cell source						<0.001
PB	258 (91.2)	110 (93.2)	19 (70.4)	57 (91.9)	72 (94.7)	
BM	18 (6.4)	8 (6.8)	3 (11.1)	3 (4.8)	4 (5.3)	
UCB	7 (2.5)	0	5 (18.5)	2 (3.2)	0	
D/R CMV serostatus						0.001

D+/R+	162 (57.2)	73 (61.9)	12 (44.4)	24 (38.7)	53 (69.7)	
D−/R+	84 (29.7)	30 (25.4)	10 (37)	31 (50)	13 (17.1)	
D+/R−	21 (7.4)	12 (10.2)	2 (7.4)	2 (3.2)	5 (6.6)	
D-R-	16 (5.7)	3 (2.5)	3 (11.1)	5 (8.1)	5 (6.6)	
Conditioning regimen						0.55
Myeloablative	81 (28.6)	35 (29.7)	10 (37)	14 (22.6)	22 (28.9)	
Reduced intensity	202 (71.4)	83 (70.3)	17 (63)	48 (77.4)	54 (71.1)	
Containing ATG	25 (8.8)	1 (0.8)	19 (70.4)	1 (1.6)	4 (5.3)	<0.001
aGvHD prophylaxis						<0.001
Based on Cyclosporin A	134 (47.3)	44 (37.3)	11 (40.7)	18 (29)	61 (80.3)	
Based on Tacrolimus	59 (20.8)	49 (41.5)	2 (7.4)	4 (6.5)	4 (5.3)	
Tacrolimus plus sirolimus	52 (18.4)	0	13 (48.1)	34 (54.8)	5 (6.6)	
Sirolimus/Mycophenolate Mophetil/ Cyclophosphamide	30 (10.6)	20 (16.9)	1 (3.7)	5 (8.1)	4 (5.3)	
Other combinations	8 (2.8)	5 (4.2)	0	1 (1.6)	2 (2.6)	
Acute GvHD						0.08
grades 0-I	195 (68.9)	73 (61.9)	23 (85.2)	45 (72.6)	54 (71.1)	0.08
grades II-IV	88 (31.1)	45 (38.1)	4 (14.8)	17 (27.4)	22 (28.9)	
Chronic GvHD	54 (19.1)	12 (10.2)	6 (22.2)	16 (25.8)	20 (26.3)	0.01

Treatment with corticosteroids>1mg-/kg/day	77 (27.2)	31 (26.3)	5 (18.5)	17 (27.4)	24 (31.6)	0.61

[a]Statistically significant ($P<0.05$).

Abbreviations, ATG, Anti-thymocyte globulin; aGvHD, Acute Graft versus Host Disease; BM, Bone Marrow; CMV, Cytomegalovirus; D, Donor; HL, Hodgkin's Lymphoma; HLA, Human Leukocyte Antigen; MDS, Myelodysplastic Syndrome; MUD, Matched Unrelated Donor; MRD, Matched Related Donor; MMRD/MMUD, HLA mismatched related or unrelated; NHL, Non-Hodgkin's lymphoma; PB, Peripheral Blood; R, Receptor; UCB, Umbilical Cord Blood.

Table 2. Analysis of potential risk factors for Cytomegalovirus DNAemia in patients undergoing Haploidentical transplantation.

Factor	Univariate	
	HR (95% CI)	***P*value[b]**
Sex (male/ female)	1.13 (0.71-1.79)	0.60
Age (< 65 years /> 65 years)	1.02 (0.58-1.79)	0.96
Underlying disease		
Acute leukemia	0.85 (0.40-1.81)	0.67
Chronic leukemia	1.73 (0.66-4.48)	0.26
lymphoma	1.08 (0.49-2.40)	0.84
MDS/MM/MF	0.74 (0.32-1.70)	0.48
Other	1 (ref)	
Stem cell source		
PB / UCB plus BM	0.87 (0.38-2.00)	0.74
D/R CMV serostatus		
R+ vs. R-	3.42 (1.25-9.38)	0.02
D+ vs. D-	1.40 (0.82-2.35)	0.23
D+ vs. D- in R+	1.52 (0.89-2.60)	0.13
Conditioning regimen		
Reduced intensity (Yes vs. No)	0.83 (0.51-1.34)	0.44
aGvHD prophylaxis		

Based on Tacrolimus	0.78 (0.24-2.57)	0.68
Based on Cyclosporin A	1.30 (0.40-4.29)	0.66
Sirolimus/Mycophenolate Mophetil/ Cyclophosphamide	1.71 (0.50-5.87)	0.40
Other	1 (ref)	
aGvHD[a] 0-I/ vs II-IV	0.65 (0.37-1.14)	0.13

[a]Time dependent covariate

[b]Statistically significant (P<0.05).

Abbreviations,aGvHD, Acute Graft versus Host Disease; BM, Bone Marrow;CMV, Cytomegalovirus; D, Donor; HL, Hodgkin's Lymphoma; MDS, Myelodispastic Syndrome; NHL, Non-hodgkin's lymphoma; PB, Peripheral Blood; R, Receptor; UCB, Umilical Cord Blood.

Table 3. Virological features of initial episodes of Cytomegalovirus DNAemia according to the modality of allogeneic hematopoietic stem cell transplantation

Factor	Haploidentical	HLA mismatched (MMRD/MMUD)	MUD	MRD	*P* value
Patients monitored with the Abbott real-time PCR assay					
Initial CMV DNA load (IU/ml) median (range)[a]	114 (30-8,705)	80 (31-402)	104 (31-66,717)	107 (33-9,711)	0.79
All patients					
Days to CMV DNAemia, median (range)[b]	32 (-7-123)	30 (-2-126)	30 (-8-204)	26 (-7-166)	0.35
CMV doubling time, median (range)[c]	1.97 (0.76-3.66)	2.12 (0.82-3.86)	2.48 (0.57-6.76)	2.32 (0.44-5.60)	0.08
CMV DNA half life following antiviral preemptive therapy[b]	3.23 (0.95-34.8)	2.64 (0.33-6.25)	3.57 (0.66-20.5)	2.22(0.36-15.5)	0.49
Patients undergoing allo-HSCT at HCUV					
CMV peak load (IU/ml),	4,324 (133-4,268,946)	1,883 (56-14,467)	847 (47-62466)	1,182 (66-122,547)	0.007

median (range)[d]					
Duration in days, median (range)[d]	49 (7-192)	28 (7-134)	42 (7-162)	42 (3-158)	0.30
CMV DNA half life for non-treated episodes[e]	2.87 (0.95-34.8)	2.49 (0.33-6.25)	3.57 (0.81-20.5)	2.24 (0.36-15.5)	0.98

[a]From 252 patients monitored with the Abbott RealTime CMV PCR to avoid a potential bias related to the different performance of this and the Affigene PCR assays[27].

[b]All patients experiencing CMV DNAemia (n=200). As for the CMV DNA half life, the CMV DNA peak is taken as CMV DNA load at t_0 irrespective of whether or not antiviral treatment has begun[29].

[c]Calculated from a total 109 episodes meeting the criteria stated in the methods section and developing in patients attended at all participating centers (both PCRs are collinear and dt_s are calculated in the absence of antiviral therapy[28])

[d]Only patients (n=199) undergoing allo-HSCT at HCUV were included, as the duration and the CMV DNA peak load of a given episode are dependent upon the CMV DNA load at which preemptive antiviral therapy is initiated[29] (different across institutions).

[e]Only patients (n=72) undergoing allo-HSCT at HCUV were considered, since there was not sufficient number (or simply there was not) of untreated episodes across transplant types in the remaining participating centers.

Abbreviations, CMV, Cytomegalovirus; dt, doubling time; HLA, Human Leukocyte Antigen; MMRD/MMUD, HLA mismatched related or unrelated; MUD, Matched Unrelated Donor; MRD, Matched Related Donor.

Table 4. Virological characteristics of patients developing CMV disease within the study period

Patient number	CMV D/R serostatus	Days to CMV DNAemia following transplantation	Initial CMV DNA load (IU/ml)	Preemptive antiviral therapy	CMV peak load (IU/ml)	Duration of CMV DNAemia (days)	CMV DNA dt (days)
13	D+/R+	34	240	Yes	671	15	Not calculated[a]
23	D+/R+	No CMV DNAemia	-	No	-	-	-
53	D-/R+	-1	<150	No	<150	7	Not calculated[a]
132	D+/R+	7	34	Yes	26,941	152	2.06
180	D-/R+	53	47	Yes	4,268,946	86	Not calculated[a]

CMV, Cytomegalovirus; D, Donor; dt, doubling time; IU, International Units; R, Recipient.

[a]Increase between the first and second CMV DNA load values of ≤3-fold.

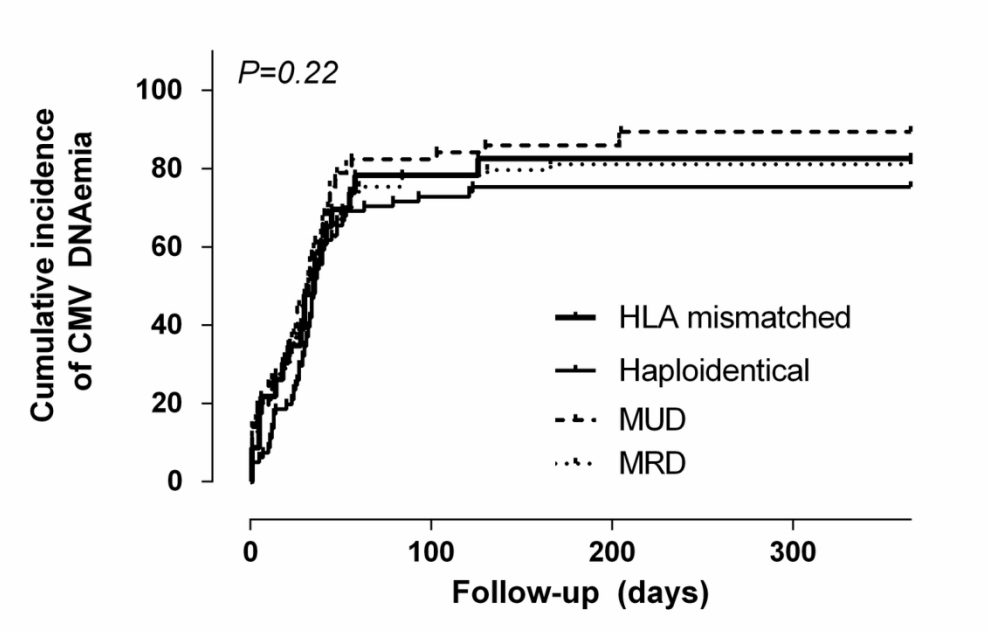

Figure 1

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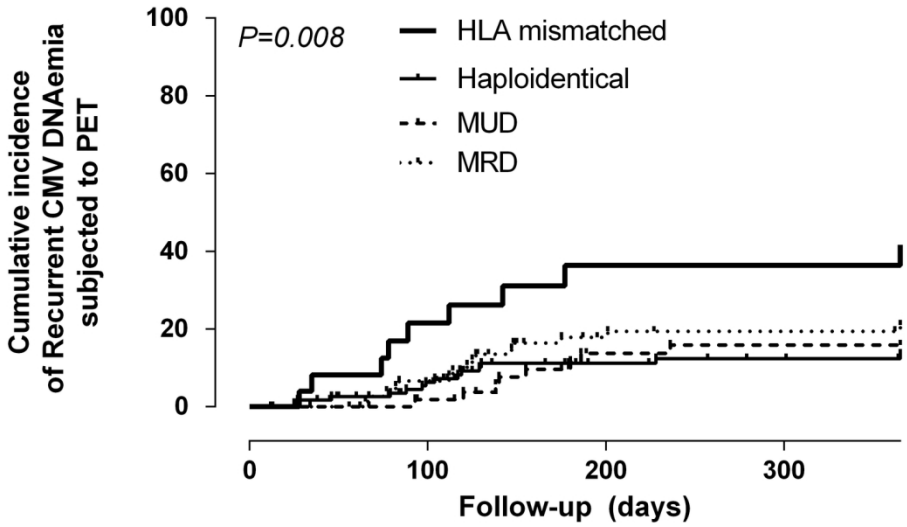

Figure 2 revised

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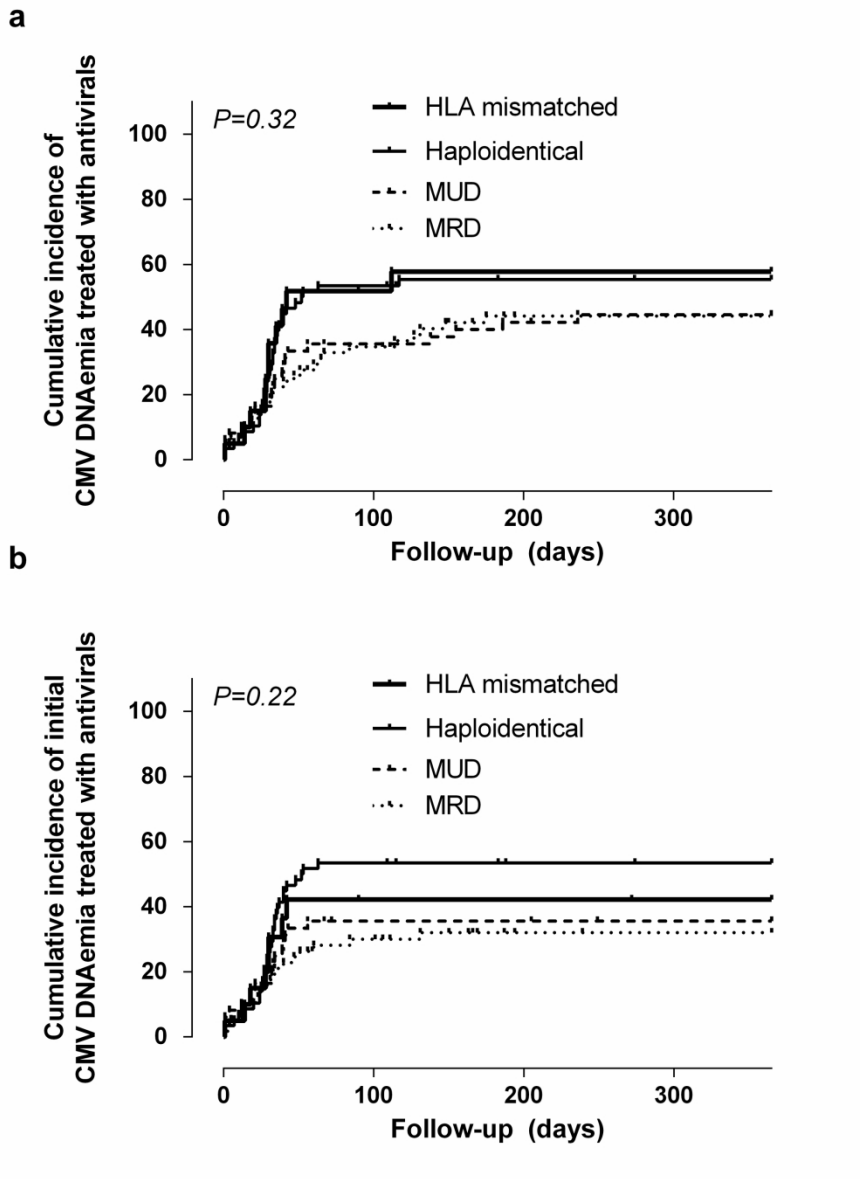

Figure 3

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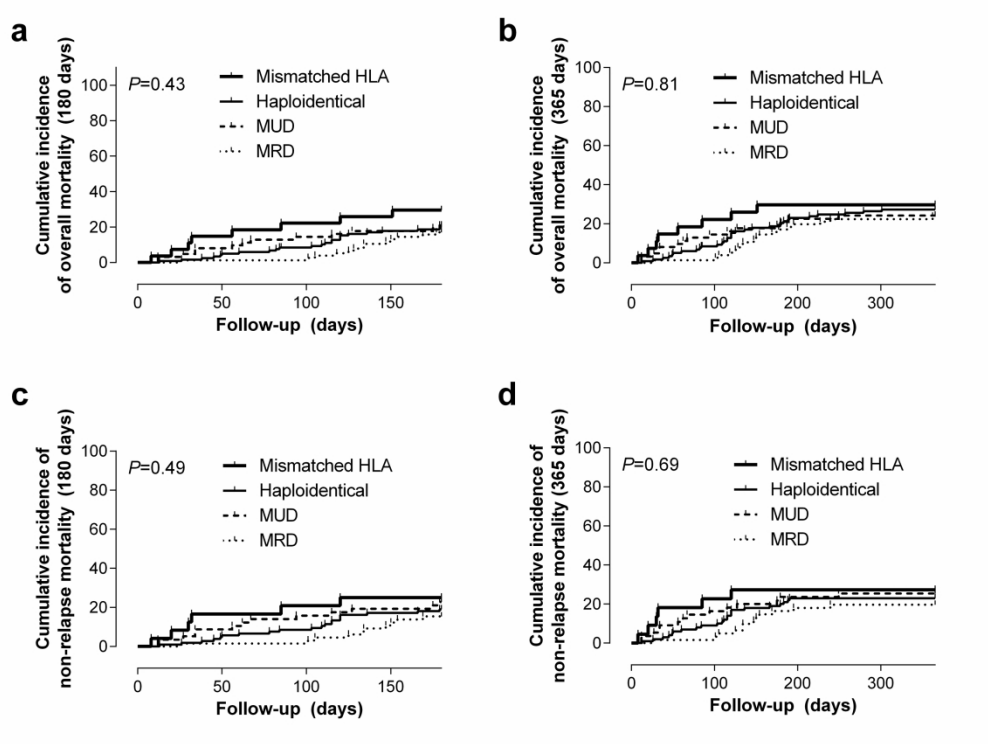

Figure 4

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Supplementary Table 1. Causes of one-year overall mortality in patients undergoing different transplant modalities.

Cause	no. (%)	HLA mismatched	Haploidentical	MUD	MRD	*P value*
Infectious complications	16 (21.6)	1 (20)	7 (20)	4 (30.8)	4 (22.2)	0.65
Relapse	14 (18.9)	1 (20)	8 (22.9)	0	5 (27.8)	
Pneumonia	9 (12.2)	1 (20)	3 (8.6)	2 (15.4)	3 (16.7)	
GvHD	6 (8.1)	0	2 (5.7)	0	4 (22.2)	
Graft failure	1 (1.4)	0	1 (2.9)	0	0	
GvHD plus infectious complications	2 (2.7)	0	1 (2.9)	1 (7.7)	0	
CMV disease	1 (1.4)	0	1 (2.9)	0	0	
Other causes[a]	25 (33.8)	5 (62.5)	12 (34.3)	6 (46.2)	2 (11.1)	
Total	74 (26.1)	8	35	13	18	

[a]Including cardiac tamponade, diffuse leukoencephalopathy, sinusoidal obstruction syndrome, severe acute liver failure, intraventricular haemorrhage, among other causes

Abbreviations. GvHD, Graft versus Host Disease**;** HLA, Human Leukocyte Antigen; MUD, Matched Unrelated Donor; MRD, Matched Related Donor.