Impact of graft composition on outcomes of haploidentical bone marrow stem cell transplantation

Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is a therapeutic option for patients without an HLA-matched donor and several studies recently demonstrated comparable outcomes following these or HLA-matched transplants.[1-3] Multiple haploidentical donors are often available, including parents, children, and half-matched siblings. While influences of several donor characteristics on post-transplant outcomes have been extensively evaluated, the impact of bone marrow cellular graft composition on patients receiving post-transplantation cyclophosphamide-based graft-*versus*-host disease (GvHD) prophylaxis has not been studied.

We hypothesized that graft composition independently affects the outcomes of haplo-HSCT and evaluated graft, donor, and recipient characteristics as predictors of outcomes in a homogeneous population of 147 patients ≥18 years old who received a fresh bone marrow graft between February 2009 and August 2015 at the MD Anderson Cancer Center (MDACC). Validation was done in a subsequent cohort of 111 patients treated between August 2015 and May 2019. All patients received the same melphalan-based conditioning regimen, post-transplantation cyclophosphamide-based GvHD prophylaxis and standard antimicrobial prophylaxis, as previously described.[4] The MDACC Institutional Review Board approved this retrospective study.

Univariable analysis using Cox proportional hazards regression analysis and Fine and Gray competing-risks regression was performed to evaluate donor, recipient, disease, and transplant characteristics and graft cellular characteristics including $CD34^+$, total nucleated cells (TNC), $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD4^+/CD3^+CD8^+$, $CD19^+$, and $CD3^-CD56^+$ cell populations for associations with outcomes. Predictors that were significant at the 0.1 level on univariable analysis were considered for multivariable analysis using classification and regression tree (CART) analysis to classify donor, recipient, and graft characteristics in order of their statistical impact and identify interaction effects among these three categories of predictors.

For the internal validation, we performed bootstrapping analysis to estimate bias-corrected confidence intervals around the relative risk assessing the association between $CD3^+CD4^+/CD3^+CD8^+$ ratio and outcomes based on 1,000 resampled datasets.

Details of graft cellular assessment and statistical methods are summarized in *Online Supplementary Material 1*. Most patients (54%) underwent haplo-HSCT for acute myeloid leukemia and myelodysplastic syndrome. Forty-three percent of patients had a high or very high disease risk index (DRI). Most patients (73%) had a hematopoietic cell transplantation comorbidity index score ≤3. The patients' characteristics are summarized in *Online Supplementary Material 2*.

The median follow-up of survivors was 37 (range, 7-80) months. Transplantation outcomes are summarized in *Online Supplementary Material 3*. Associations between outcomes and donor and recipient characteristics are presented in Online Supplementary Material 4.

The median $CD4^+/CD8^+$ cell ratio (CD4/CD8) was 1.1

Table 1. Correlation between graft cellular subsets and donor characteristics evaluated in univariate analysis.

Characteristics	N. of patients (n=147)	Median $CD34^+$ cell dose, $x10^6$/kg (IQR)	Median TNC dose, $x10^6$/kg (IQR)	% $CD4^+$ cells		% $CD8^+$ cells		CD4/CD8 ≤0.8		CD4/CD8 >1.5		% $CD19^+$ cells		% $CD56^+$ cells	
				Median (IQR)	*P* value	Median (IQR)	*P* value	%	*P* value	%	*P* value	Median (IQR)	*P* value	Median (IQR)	*P* value
Age, years															
>30	88	2.5 (1.7, 3.4)	319 (264, 407)	41 (37, 47)	0.04	35 (30, 42)	0.9	23	0.4	24	0.04	8 (6, 13)	0.4	8 (6, 13)	0.8
≤30	59	2.5 (2, 3.4)	329 (236, 400)	38 (35, 44)		35 (30, 42)		29		10		9 (6, 15)		9 (6, 12)	
>50	27	2.1 (1.5, 2.7)	315 (229, 404)	43 (38, 51)	0.01	36 (30, 45)	0.8	22	0.7	30	0.1	6 (3, 8)	<0.01	9 (7, 13)	0.3
≤50	120	*2.6 (1.9, 3.5)	321 (256, 404)	39 (35, 44)		35 (30, 42)		26		16		10 (7, 14)		9 (6, 12)	
Gender															
Female	65	2.3 (1.9, 3.1)	315 (264, 378)	42 (37, 47)	0.06	35 (31, 41)	0.9	17	0.04	17	0.7	9 (6, 13)	0.7	8 (6, 12)	0.1
Male	82	2.6 (1.7, 3.7)	332 (249, 410)	39 (33, 44)		35 (30, 44)		32		19		8 (7, 13)		10 (6, 13)	
CMV serostatus															
Reactive	94	2.5 (1.8, 3.5)	326 (268, 406)	40 (36, 46)	0.9	37 (31, 44)	0.003	31	0.04	18	0.9	8 (6, 13)	0.7	8 (6, 11)	0.06
Nonreactive	53	2.4 (1.6, 3)	**295 (209, 375)	41 (35, 46)		33 (28, 37)		15		19		9 (7, 14)		10 (7, 13)	

IQR: interquartile range; TNC: total nucleated cells; CD4/CD8: $CD4^+/CD8^+$ cell ratio; CMV: cytomegalovirus. *P=0.04. **P=0.02 for comparison of 25th percentile

A

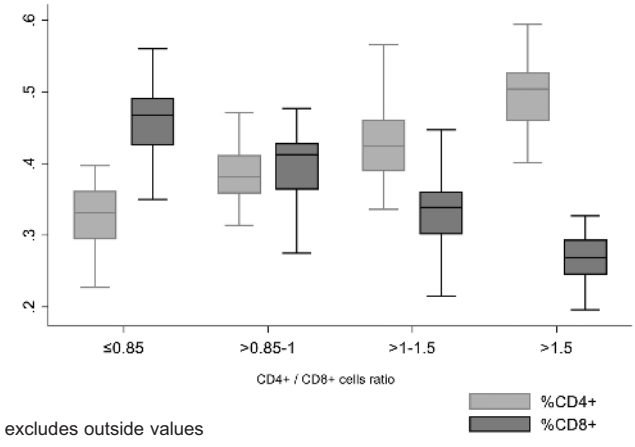
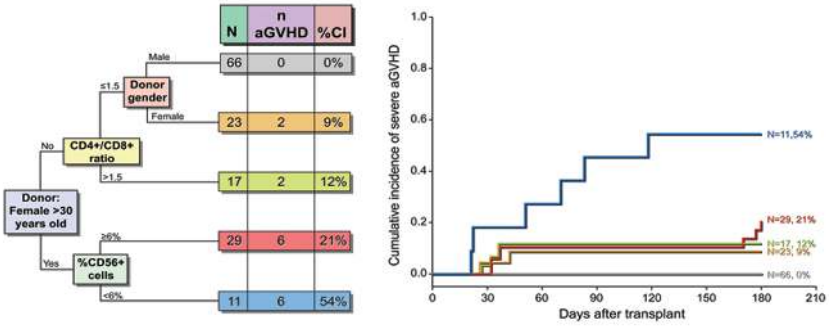


Figure 1. Legend on following page.

B



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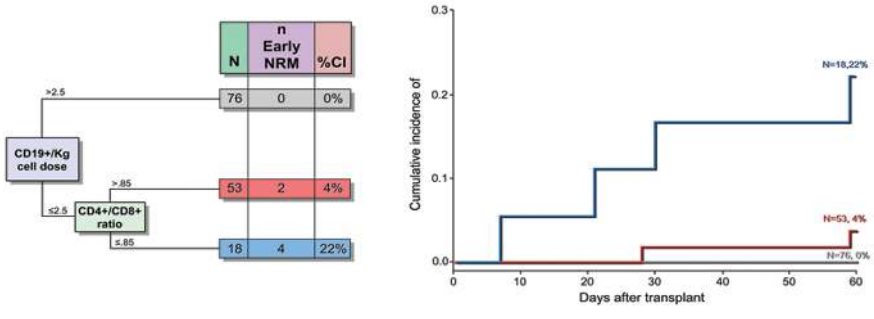

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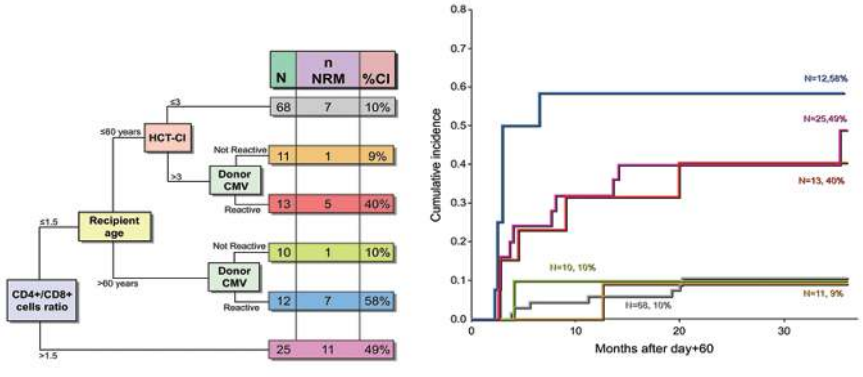

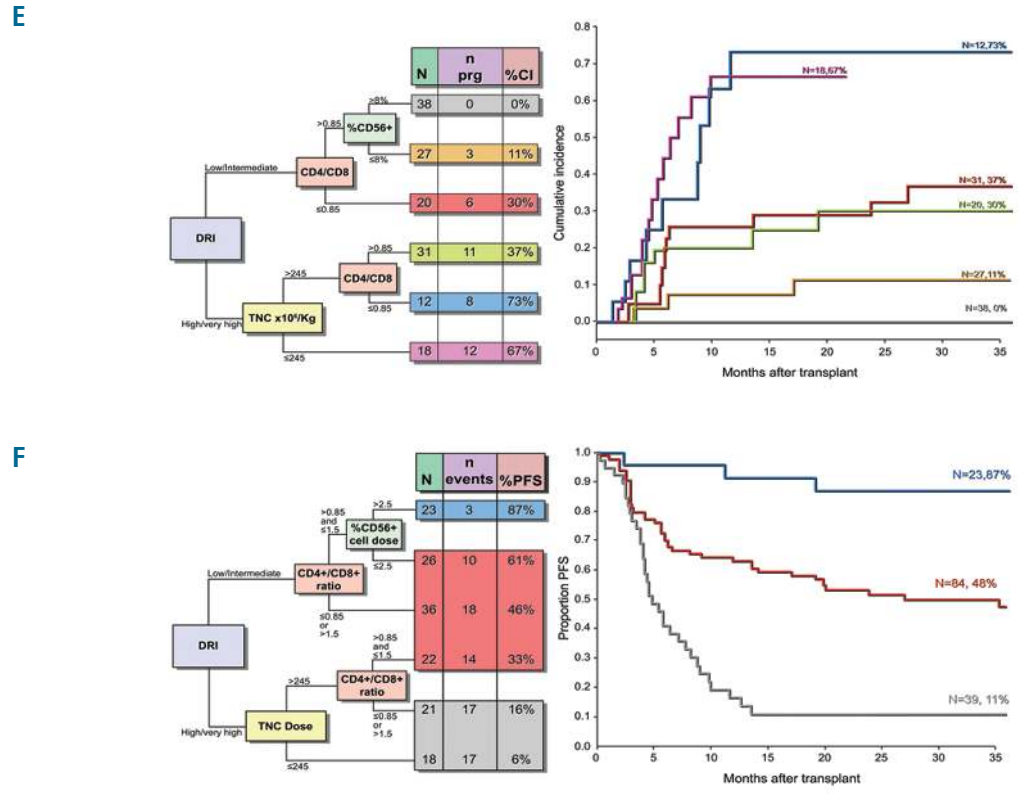

Figure 1. Distributions of CD4⁺ and CD8⁺ cell percentages and risk stratifications based on graft, donor and recipient-characteristics. (A) The distributions of %CD4⁺ and %CD8⁺ cells in unmanipulated bone marrow grafts vary according to CD4⁺/CD8⁺ cell ratio (CD4/CD8). Distributions of %CD4⁺ and %CD8⁺ cells are skewed in low (≤0.85) and high (>1.5) CD4/CD8 quartiles. CD8⁺ cells are predominant in grafts with a CD4/CD8 ≤0.85 and CD4⁺ cells are predominant in grafts with a CD4/CD8 >1.5. Data are illustrated in a box-and-whisker plot with the whiskers indicating the 25th and 75th percentiles. Lines within the box plots indicate the median %CD4⁺ and %CD8⁺ cells in each CD4/CD8 quartile. (B-F) Results of multivariate classification and regression tree (CART) analysis are shown for severe acute graft-*versus*-host disease (B), early non-relapse mortality (C), late non-relapse mortality (D), disease progression (E), and progression-free survival (F). Two figures are presented for each outcome depicting the risk classification algorithm (left figure) generated by CART analysis, and the corresponding cumulative incidence curves (right figure) for each subgroup represented in the risk stratification algorithm. N: number; aGVHD: acute graft-*versus*-host disease; %CI: percent cumulative incidence; NRM: non-relapse mortality; HCT-CI: Hematopoietic Cell Transplantation - Comorbidity Index; CMV: cytomegalovirus serostatus; DRI: Disease Risk Index; prg: progressive disease; TNC: total nucleated cells; PFS: progression-free survival.

(range, 0.4-3.1). As shown in Figure 1A, grafts with CD4/CD8 ≤0.85 had higher %CD8⁺ and grafts with a CD4/CD8 >1.5 had higher %CD4⁺. The %CD4⁺ and %CD8⁺ distributions were more balanced in grafts with a CD4/CD8 between 0.85 and 1.5.

Correlations between donor characteristics (age, gender, and cytomegalovirus status) and graft composition are presented in Table 1. Donor age >30 years was correlated with higher %CD4⁺ (median 41% *vs.* 38%, *P*=0.04) and CD4/CD8 >1.5 (24% *vs.* 10%, *P*=0.04), whereas donor age >50 years was correlated with lower %CD19⁺ (median 6% *vs.* 10%, *P*=0.0003) and lower infused CD34⁺ cell dose (median 2.6 *vs.* 2.1, *P*=0.04). Grafts from female donors had higher %CD4⁺ (median 42% *vs.* 39%, *P*=0.06) and were more likely to have a CD4/CD8 >0.85 (83% *vs.* 68%, *P*=0.04). Overall, cytomegalovirus seropositivity correlated with higher %CD8⁺ (median 37% *vs.* 33%, *P*=0.003), a CD4/CD8 ≤0.85 (median 31% *vs.* 15%, *P*=0.04), and an infused TNC dose >250 x 106/kg (81% *vs.* 64%, *P*=0.02).

Multivariable CART analyses have shown significant associations of recipient and donor characteristics and graft cellular composition with post-transplant outcomes, and generated risk groups with different post-transplant outcomes, as summarized in Table 2 and Figure 1B-F.

The incidence of severe acute GvHD ranged from 0% to 54% (Figure 1B). The highest risk of severe acute GvHD was associated with grafts from female donors, age >30 years with ≤6% CD56⁺ cells in the graft (n=11, cumulative incidence [CI] 54%).

The incidence of early non-relapse mortality (NRM), defined as <60 days after transplantation, was 0-22% (Figure 1C). Grafts with a low CD4/CD8 (<0.85) and low infused CD19⁺ cell dose (≤2.5x10⁶ CD19⁺ cells/kg) were associated with a higher early NRM rate. Notably, all cases of early NRM were due to infections.

The incidence of late NRM, defined as >60 days after transplantation, ranged from 9% to 58% (Figure 1D). The high-risk group for late NRM included three subgroups: (i) recipients of grafts with a CD4/CD8 >1.5; (ii) patients aged >60 years who received grafts with a CD4/CD8 ≤1.5 from cytomegalovirus-seropositive donors; and (iii) patients with comorbidity scores >3 who received grafts with a CD4/CD8 ≤1.5 from cytomegalovirus-seropositive donors. Late NRM was primarily related to GvHD.

The incidence of disease progression ranged from 0% to 73% (Figure 1E). Three risk groups for disease progression were defined based on DRI, TNC dose, and CD4/CD8. The group at highest risk of progression included patients with a high/very-high DRI whose

grafts had either a low TNC dose (relapse 67%) or a low (≤0.85) CD4/CD8 (relapse 73%).

The progression-free survival (PFS) rate ranged from 6% to 87% (Figure 1F). Three risk groups were defined based on DRI, TNC dose, CD4/CD8, and $CD56^+$ cell dose. Patients with low/intermediate DRI and grafts with a balanced (>0.85-1.5) CD4/CD8 and >2.5x10^6 $CD56^+$ cells/kg had the highest PFS rate (87%, hazard ratio [HR]=0.2, *P*=0.007). Patients (n=39) with high/very-high DRI and either a graft with low TNC/kg or a graft with an unbalanced (≤0.85 or >1.5) CD4/CD8 had the lowest PFS rate (11%, HR=2.9, *P*<0.001). The remaining patients (n=84, reference group) had an intermediate PFS rate of 48%.

To validate our findings, we tested the predictive value of CD4/CD8 in a subsequent cohort of 111

Table 2. Cellular subsets and donor and recipient characteristics as predictors of outcomes in multivariate classification and regression tree analysis.

Outcome	N. of patients	%CI	HR (95% CI)	*P*	Proposed Risk Stratification
Severe acute GvHD	146				
Female donor >30 y					
<6% $CD56^+$ cells	11	54%	Reference		High
≥6% $CD56^+$ cells	29	21%	0.3 (0.1-0.9)	0.03	Intermediate
Male donor any age or female donor ≤30 y					
CD4/CD8 >1.5	17	12%	0.2 (0.03-0.8)	0.03	Intermediate
CD4/CD8 ≤1.5, female donor	23	9%	0.1 (0.02-0.6)	0.01	Intermediate
CD4/CD8 ≤1.5, male donor	66	0%	NA	<0.01	Low
Early non-relapse mortality	147				
≤2.5x10^6 infused $CD19^+$ cells/kg					
CD4/CD8 ≤0.85	18	22%	Reference		High
CD4/CD8 >0.85	53	4%	0.1 (0.03-0.8)	0.03	Low
>2.5x10^6 infused $CD19^+$ cells/kg	76	0%	NA	<0.01	Low
Late non-relapse mortality	139				
CD4/CD8 >1.5	25	49%	0.5 (0.2-1.5)	0.3	High
CD4/CD8 ≤1.5					
Recipient >60 y, donor CMV R	12	58%	Reference		High
Recipient >60 y, donor CMV NR	10	10%	0.1 (0.01-0.9)	0.04	Low
Recipient ≤60 y, HCT-CI >3, donor CMV R	13	40%	0.5 (0.1-1.6)	0.2	High
Recipient ≤60 y, HCT-CI >3, donor CMV NR	11	9%	0.1 (0.01-0.7)	0.02	Low
Recipient ≤60 y, HCT-CI ≤3	68	10%	0.1 (0.03-0.3)	<0.001	Low
Disease progression	146				
High *vs.* high DRI					
≤245x10^6 TNC/kg	18	73%	Reference		High
>245x10^6 TNC/kg and CD4/CD8 ≤0.85 OR >1.5	12	67%	0.9 (0.4-2.1)	0.8	High
>245x10^6 TNC/kg and CD4/CD8 >0.85-1.5	31	37%	0.4 (0.2-0.9)	0.02	Intermediate
Intermediate or low DRI					
CD4/CD8 ≤0.85	20	30%	0.3 (0.1-0.8)	0.02	Intermediate
CD4/CD8 >0.85, ≤8% $CD56^+$ cells	27	11%	0.1 (0.03-0.4)	<0.01	Low
CD4/CD8 >0.85, >8% $CD56^+$ cells	38	0%	NA	<0.01	Low
Progression-free survival*	146				
High or very high DRI					
≤245x10^6 TNC/kg	18	6%	19 (5.5-66)	<0.01	High
>245x10^6 TNC/kg, CD4/CD8 ≤0.85 OR >1.5	21	16%	13 (3.7-43)	<0.01	High
>245x10^6 TNC/kg, CD4/CD8 >0.85-1.5	22	33%	6.6 (1.9-23)	0.003	Intermediate
Low or intermediate DRI					
CD4/CD8 ≤0.85 OR >1.5	36	46%	5.4 (1.6-18)	0.007	Intermediate
CD4/CD8 >0.85-1.5, ≤2.5x10^6 $CD56^+$ cells/kg	26	61%	3.5 (0.97-13)	0.054	Intermediate
CD4/CD8 >0.85-1.5, >2.5x10^6 CD56 cells/kg	23	87%	Reference		Low

%CI: percent cumulative incidence; HR: hazard ratio; 95% CI: 95% confidence interval; GvHD: graft-*versus*-host disease; y: years; CD4/CD8: $CD4^+/CD8^+$ cell ratio, NRM: non-relapse mortality; CMV: cytomegalovirus; R: reactive; NR: non-reactive; HCT-CI: Hematopoietic Cell Transplantation - Comorbidity Index; DRI: Disease Risk Index; TNC: total nucleated cells. *PFS proportion, rather than %CI, is provided.

patients treated in the same way as the training cohort. Demographic data are detailed in *Online Supplementary Material 2*. Results from the validation cohort were consistent with those from the training study: predominance of either CD4+ or CD8+ cells was associated with adverse outcomes, whereas a balanced distribution (0.9-1.1) was associated with superior PFS. CD4/CD8 ≤0.9 was significantly associated with higher relapse rate (HR=2.5, *P*=0.04), while CD4/CD8 >1.1 was associated with a significantly higher rate of NRM (HR=2.5, *P*=0.03) and lower rate of relapse (HR=0.4, *P*=0.03), in univariate analysis. These trends were independent of the DRI. Irrespective of the DRI, a CD4/CD8 between 0.9-1.1 was associated with superior PFS; however, the association did not reach statistical significance (HR=0.6, *P*=0.2).

In this study, we found that the composition of unprocessed bone marrow grafts had a major independent impact on the outcomes of patients who underwent haplo-HSCT with post-transplantation cyclophosphamide-based GvHD prophylaxis, and identified an optimal balanced CD4/CD8 associated with the longest PFS. Grafts with low or high CD4/CD8 were associated with inferior survival and different patterns of failure.

Previous studies of haplo-HSCT with granulocyte colony-stimulating factor-primed bone marrow and peripheral blood stem cells and antithymocyte globulin-based GvHD prophylaxis showed that a low CD4/CD8 is associated with adverse outcomes.[5,6] Our findings suggested that the predominance of either a CD4+ or CD8+ cell population, which translated into high and low (inverted) CD4/CD8 ratios, respectively, was associated with inferior outcomes. The predominance of CD8+ cells was associated with a high incidence of early NRM and disease progression, whereas the predominance of CD4+ cells was associated with a high incidence of severe acute GvHD and late NRM. These findings were confirmed in a bootstrap analysis and in a separate cohort of patients subsequently treated at our institution with the same conditioning and a fresh bone marrow graft. A better PFS was again noted in the validation cohort for grafts with a "balanced" CD4/CD8 ratio (0.9-1.1) (although this association did not reach statistical significance in the confirmatory group, likely due to lower number of patients and change in practice over time to avoid older female donors). The adverse effects associated with a predominance of either a CD4+ or CD8+ cell population could be interpreted in the context of the required cooperation between these cell subsets in mounting an effective immune response.[7-11] A direct anti-tumor effect for CD4+ cells, independently of CD8+ cells, has also been suggested.[7-9,12-14]

Our data also indicate that CD4/CD8 has significant synergistic effects with CD19+ cells in early NRM and a significant role for CD4/CD8 and natural killer cells in disease progression. Specifically, for early NRM, a low CD4/CD8 was associated with higher NRM only in recipients whose grafts had a low dose of infused CD19+ cells. Grafts with low doses of infused CD19+ cells and a CD4/CD8 ≤0.85 represented 12% of the graft pool and yet they accounted for 67% of the cases of early NRM.

In the case of disease progression, for recipients with a low or intermediate DRI, the antitumor effect associated with a CD4/CD8 >0.85 was amplified by concurrent high levels of natural killer cells in the graft. This is in line with recent evidence suggesting a synergistic antitumor effect between CD4+ and natural killer cells.[9]

In conclusion, we found that donor graft composition is a major independent determinant of transplant outcomes in patients receiving haploidentical bone marrow transplantation. A "balanced" graft with an optimal CD4/CD8 ratio close to 1 was associated with the best transplant outcomes. These findings have implications not only for donor selection but also for risk mitigation and suggest that customized grafts with optimal CD4/CD8 ratios, and CD19+ and CD56+ cell numbers should be explored in the future.

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Contributions: RMS conceived the study, performed statistical analysis, and wrote the manuscript. LV contributed with some data collection and manuscript writing. GR, JC, CM, LA contributed with data collection. GA, SA, AA, CMH, BO, KR, EJS, PK, IFK, UP, REC contributed with patient care, critical review of the manuscript. SOC contributed with study design, data collection, interpretation of results and manuscript writing. All authors reviewed and approved the submission.

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References

1. Ciurea SO, Zhang MJ, Bacigalupo AA, et al. Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. Blood. 2015; 126(8):1033-1040.
2. Kanate AS, Mussetti A, Kharfan-Dabaja MA, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors vs HLA-matched unrelated donors. Blood. 2016;127(7):938-947.
3. Bashey A, Zhang X, Sizemore CA, et al. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. J Clin Oncol. 2013; 31(10):1310-1316.
4. Gaballa S, Ge I, El Fakih R, et al. Results of a 2-arm, phase 2 clinical trial using post-transplantation cyclophosphamide for the prevention of graft-versus-host disease in haploidentical donor and mismatched unrelated donor hematopoietic stem cell transplantation. Cancer. 2016;122(21):3316-3326.
5. Luo XH, Chang YJ, Xu LP, Liu DH, Liu KY, Huang XJ. The impact of graft composition on clinical outcomes in unmanipulated HLA-mismatched/haploidentical hematopoietic SCT. Bone Marrow Transplant. 2009;43(1):29-36.
6. Xu LP, Luo XH, Chang YJ, et al. High CD4/CD8 ratio in allografts predicts adverse outcomes in unmanipulated HLA-mismatched/haploidentical hematopoietic stem cell transplantation for chronic myeloid leukemia. Ann Hematol. 2009;88(10):1015-1024.
7. Mumberg D, Monach PA, Wanderling S, et al. CD4(+) T cells eliminate MHC class II-negative cancer cells in vivo by indirect

effects of IFN-gamma. Proc Natl Acad Sci U S A. 1999;96 (15):8633-8638.
8. Muranski P, Restifo NP. Adoptive immunotherapy of cancer using CD4(+) T cells. Curr Opin Immunol. 2009;21(2):200-208.
9. Perez-Diez A, Joncker NT, Choi K, et al. CD4 cells can be more efficient at tumor rejection than CD8 cells. Blood. 2007;109(12):5346-5354.
10. Ferguson FG, Wikby A, Maxson P, Olsson J, Johansson B. Immune parameters in a longitudinal-study of a very old population of Swedish people - a comparison between survivors and nonsurvivors. J Gerontol A Biol Sci Med Sci. 1995;50(6):B378-B382.
11. Pawelec G, Ferguson FG, Wikby A. The SENIEUR protocol after 16 years. Mech Ageing Dev. 2001;122(2):132-134.
12. Corthay A, Skovseth DK, Lundin KU, et al. Primary antitumor immune response mediated by CD4(+) T cells. Immunity. 2005; 22(3):371-383.
13. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298(5594):850-854.
14. Hunder NN, Wallen H, Cao JH, et al. Treatment of metastatic melanoma with autologous CD4+T cells against NY-ESO-1. New Engl J Med. 2008;358(25):2698-2703.