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 | Median CD34+ cell dose, x10^6/kg (IQR) | Median TNC dose, x10^6/kg (IQR) | % CD4+ cells | % CD8+ cells | % CD4+ cells ≤0.8 | % CD4+ cells >1.5 | % CD19+ cells | % CD56+ cells |
|-----------------|----------------|----------------------------------------|-------------------------------|---------------|---------------|-------------------|-----------------|----------------|---------------|
| Age, years      |                |                                        |                               |               |               |                   |                 |                |               |
| >50             | 59             | 2.5                                    | 319 (17, 34)                  | 41 (37, 47)   | 35 (30, 42)   | 23 (19, 29)       | 30 (29, 32)     | 8 (6, 10)      | 9 (7, 11)     |
| ≤50             | 88             | 2.5                                    | 329 (236, 400)               | 38 (35, 44)   | 35 (30, 42)   | 29 (26, 32)       | 26 (26, 28)     | 9 (7, 11)      | 9 (7, 11)     |
| >50             | 27             | 2.1                                    | 315 (229, 404)               | 43 (38, 51)   | 36 (30, 45)   | 22 (20, 24)       | 30 (29, 32)     | 6 <0.01        | 9 (7, 11)     |
| ≤50             | 120            | 2.6*                                    | 321 (256, 404)               | 39 (35, 44)   | 35 (30, 42)   | 26 (25, 28)       | 16 (16, 18)     | 10 (9, 12)     | 9 (7, 11)     |
| Gender          |                |                                        |                               |               |               |                   |                 |                |               |
| Female          | 55             | 2.3*                                    | 315 (249, 410)               | 42 (37, 47)   | 35 (31, 41)   | 17 (14, 20)       | 19 (19, 21)     | 9 (7, 11)      | 8 (6, 12)     |
| Male            | 82             | 2.6                                    | 322 (249, 410)               | 39 (33, 44)   | 35 (30, 44)   | 35 (30, 35)       | 32 (31, 34)     | 9 (7, 11)      | 8 (6, 12)     |
| CMV serostatus  |                |                                        |                               |               |               |                   |                 |                |               |
| Reactive        | 94             | 2.5                                    | 326 (258, 406)               | 40 (36, 46)   | 37 (31, 44)   | 31 (28, 35)       | 30 (29, 32)     | 8 <0.01        | 8 (6, 10)     |
| Nonreactive     | 53             | 2.4*                                   | **295 (209, 375)             | 41 (35, 46)   | 33 (28, 37)   | 15 (15, 17)       | 19 (19, 21)     | 10 (8, 12)     | 10 (8, 12)    |

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

C

D

excludes outside values
(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 %CD4+ (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 (<2.5x10^6 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
grants 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

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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   |       |                               |
| ≥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             | 78%  | 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             | 40%  | 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: year; CD4/CD8: CD4/CD8 cell ratio; NR: 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.6,7 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.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.7 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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