Impact of Previously Unrecognized HLA Mismatches Using Ultrahigh Resolution Typing in Unrelated Donor Hematopoietic Cell Transplantation

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PURPOSE Ultrahigh resolution (UHR) HLA matching is reported to result in better outcomes following unrelated donor hematopoietic cell transplantation, improving survival and reducing post-transplant complications. However, most studies included relatively small numbers of patients. Here we report the findings from a large, multicenter validation study.

METHODS UHR HLA typing was available on 5,140 conventionally 10 out of 10 HLA-matched patients with malignant disease transplanted between 2008 and 2017.

RESULTS After UHR HLA typing, 82% of pairs remained 10 out of 10 UHR-matched; 12.3% of patients were 12 out of 12 UHR HLA-matched. Compared with 12 out of 12 UHR-matched patients, probabilities of grade 2-4 acute graft-versus-host disease (aGVHD) were significantly increased with UHR mismatches (overall $P = .0019$) and in those patients who were HLA-DPB1 T-cell epitope permissively mismatched or nonpermissively mismatched (overall $P = .0011$). In the T-cell–depleted subset, the degree of UHR HLA mismatch was only associated with increased transplant-related mortality (TRM) (overall $P = .0068$). In the T-cell–replete subset, UHR HLA matching was associated with a lower probability of aGVHD (overall $P = .0020$); 12 out of 12 UHR matching was associated with reduced TRM risk when compared with HLA-DPB1 T-cell epitope permissively mismatched patients, whereas nonpermissive mismatching resulted in a greater risk (overall $P = .0003$).

CONCLUSION This study did not confirm that UHR 12 out of 12 HLA matching increases the probability of overall survival but does demonstrate that aGVHD risk, and in certain settings TRM, is lowest in UHR HLA-matched pairs and thus warrants consideration when multiple 10 out of 10 HLA-matched donors of equivalent age are available.
HCT pairs from the United Kingdom predominantly receiving in vivo T-cell–depleting serotherapy, recipients receiving a 12 out of 12 ultrahigh resolution (UHR; including HLA-DPB1) HLA-matched graft (defined as matched at a genomic level for HLA class I genes and at an extended coding DNA sequence level for HLA class II) had significantly improved overall survival (OS) than those with any degree of mismatch. In addition, 10 out of 10 UHR-matched, permissively DPB1-mismatched transplant did not have significantly different OS compared with 12 out of 12 UHR-matched, whereas increasing UHR mismatching was associated with increased grade 2-4 acute graft-versus-host disease and in some cases transplant-related mortality.

The aims of this study were to determine whether UHR HLA matching in an independent cohort of MUD-HCT recipients was associated with transplant outcomes, to validate the previous findings of better OS and less aGVHD with UHR matching in a cohort of T-cell–depleted MUD-HCT patients, and to analyze a cohort of T-cell–replete MUD-HCTs.

METHODS

Study Design

UHR HLA typing and clinical data were available on a cohort of 5,140 first allogeneic MUD-HCT donor and recipient pairs in the Center for International Blood and Marrow Transplant Research (CIBMTR). Patients received a high-resolution, 10 out of 10 HLA-matched graft at ARD level between 2008 and 2017 for acute myelogenous leukemia, acute lymphatic leukemia, or myelodysplastic syndrome (Table 1). Recipients of umbilical cord blood grafts, ex vivo T-cell–depleted grafts, or post-transplant cyclophosphamide for GVHD prophylaxis were excluded. Subgroup analyses included those receiving in vivo T-cell–depleted (antithymocyte globulin or alemtuzumab, n = 2,039) and T-cell–replete transplants (n = 3,101). The National Marrow Donor Program Institutional Review Board approved this study. Informed written consent was obtained from all participants, in accordance with the Declaration of Helsinki.

HLA Typing

UHR HLA typing was performed by a commercial vendor using Pacific Biosciences' single-molecule real-time DNA sequencing method. Typing was completed for the six classical HLA loci (class I: HLA-A, HLA-B, and HLA-C; class II: HLA-DRB1, HLA-DQB1, and HLA-DPB1). The NGS typing methodology included all exons and the intervening introns for the classical HLA class I genes and exons 2 and 3 only for the class II genes.

Determining HLA Matching

Because of the timing of the typing, different ambiguous HLA typing results were obtained. All HLA typing data were reviewed, curated, and normalized to ensure consistency. Matching was determined according to criteria used in a previous study. Analyses that included the HLA-DPB1 TCE (DP-TCE) matching algorithm used the model proposed by Crivello et al. Sixteen pairs were removed from the analysis because of an inability to assign DP-TCE groups or where UHR matching identified uncharacterized null alleles resulting in ARD matches < 10 out of 10.

Models of UHR HLA Matching Analysis

Several models of HLA matching were tested to replicate those used previously: model 1, compared 12 out of 12 UHR HLA-matched individuals and those mismatched at any level, at any loci (12 out of 12 v ≤ 11 out of 12 matched); model 2, tested the degree of UHR HLA matching (12 out of 12 v 11 out of 12 v 10 out of 12 v ≤ 9

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### TABLE 1. Recipient and Donor Characteristics

| Variable                              | Overall, No. (%) | T-Cell–Depleted (ATG or Alemtuzumab), No. (%) | T-Cell–Replete, No. (%) |
|---------------------------------------|-----------------|-----------------------------------------------|------------------------|
| No. of patients                       | 5,140           | 2,039                                         | 3,101                  |
| **Disease at transplant**             |                 |                                               |                        |
| AML                                   | 2,573 (50)      | 1,047 (51)                                    | 1,526 (49)             |
| ALL                                   | 805 (16)        | 285 (14)                                      | 520 (17)               |
| MDS                                   | 1,762 (34)      | 707 (35)                                      | 1,055 (34)             |
| **Other**                             |                 |                                               |                        |
| **Disease status at transplant**      |                 |                                               |                        |
| Early                                 | 2,397 (47)      | 962 (47)                                      | 1,435 (46)             |
| Intermediate                          | 703 (14)        | 264 (13)                                      | 439 (14)               |
| Advanced                              | 1,971 (38)      | 781 (38)                                      | 1,190 (38)             |
| Missing                               | 69 (1)          | 32 (2)                                        | 37 (1)                 |
| **Recipient age at transplant, years**|                 |                                               |                        |
| 0-10                                  | 152 (3)         | 81 (4)                                        | 71 (2)                 |
| 11-18                                 | 135 (3)         | 64 (3)                                        | 71 (2)                 |
| 19-29                                 | 406 (8)         | 165 (8)                                       | 241 (8)                |
| 30-39                                 | 434 (8)         | 159 (8)                                       | 275 (9)                |
| 40-49                                 | 710 (14)        | 256 (13)                                      | 454 (15)               |
| 50-59                                 | 1,201 (23)      | 473 (23)                                      | 728 (23)               |
| 60+                                   | 2,102 (41)      | 841 (41)                                      | 1,261 (41)             |
| Median (range)                        | 57 (1-84)       | 57 (1-84)                                     | 57 (1-81)              |
| **Recipient sex**                     |                 |                                               |                        |
| Male                                  | 2,925 (57)      | 1,189 (58)                                    | 1,736 (56)             |
| Female                                | 2,215 (43)      | 850 (42)                                      | 1,365 (44)             |
| **Recipient race**                    |                 |                                               |                        |
| White                                 | 4,582 (89)      | 1,823 (89)                                    | 2,759 (89)             |
| Other                                 | 459 (9)         | 181 (9)                                       | 278 (9)                |
| Missing                               | 99 (2)          | 35 (2)                                        | 64 (2)                 |
| **Donor age group, years**            |                 |                                               |                        |
| 19-29                                 | 3,189 (62)      | 1,290 (63)                                    | 1,899 (61)             |
| 30-39                                 | 1,164 (23)      | 455 (22)                                      | 709 (23)               |
| 40-49                                 | 604 (12)        | 237 (12)                                      | 367 (12)               |
| 50-59                                 | 179 (3)         | 57 (3)                                        | 122 (4)                |
| 60+                                   | 4 (< 1)         | 0 (< 1)                                       | 4 (< 1)                |
| Median (range)                        | 27 (18-61)      | 27 (18-60)                                    | 28 (18-61)             |
| **Donor pregnancy**                   |                 |                                               |                        |
| No                                    | 898 (17)        | 325 (16)                                      | 573 (18)               |
| Yes                                   | 503 (10)        | 188 (9)                                       | 315 (10)               |
| Male                                  | 3,727 (73)      | 1,521 (75)                                    | 2,206 (71)             |
| Missing                               | 12 (< 1)        | 5 (< 1)                                       | 7 (< 1)                |
| **Karnofsky Performance Score**       |                 |                                               |                        |
| 10-80                                 | 2,031 (40)      | 773 (38)                                      | 1,258 (41)             |
| 90-100                                | 3,041 (59)      | 1,235 (61)                                    | 1,806 (58)             |
| Missing                               | 68 (1)          | 31 (2)                                        | 37 (1)                 |

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TABLE 1.Recipient and Donor Characteristics (continued)

| Variable | Overall, No. (%) | T-Cell–Depleted (ATG or Alemtuzumab), No. (%) | T-Cell–Replete, No. (%) |
|----------|------------------|-----------------------------------------------|------------------------|
| GVHD prophylaxis | | | |
| Tacrolimus + MMF + others | 742 (14) | 365 (18) | 377 (12) |
| Tacrolimus + MTX + others (except MMF) | 3,097 (60) | 1,228 (60) | 1,869 (60) |
| Tacrolimus + others (except MTX and MMF) | 400 (8) | 33 (2) | 367 (12) |
| Tacrolimus alone | 131 (3) | 119 (6) | 12 (< 1) |
| CSA + MMF + other(s) (except post-CY) | 338 (7) | 140 (7) | 198 (6) |
| CSA + MTX + other(s) (except MMF and post-CY) | 319 (6) | 101 (5) | 218 (7) |
| CSA + other(s) (except MTX, MMF, and post-CY) | 19 (< 1) | 12 (1) | 7 (< 1) |
| CSA alone | 21 (< 1) | 8 (< 1) | 13 (< 1) |
| Other GVHD prophylaxis | 73 (1) | 33 (2) | 40 (1) |
| Graft type | | | |
| Marrow | 895 (17) | 374 (18) | 521 (17) |
| PBSC | 4,245 (83) | 1,665 (82) | 2,580 (83) |
| HCT-CI | | | |
| 0 | 1,188 (23) | 508 (25) | 680 (22) |
| 1 | 670 (13) | 286 (14) | 384 (12) |
| 2 | 792 (15) | 318 (16) | 474 (15) |
| 3+ | 2,484 (48) | 925 (45) | 1,559 (50) |
| NA/12400 missing | 1 (< 1) | 0 (0) | 1 (< 1) |
| Missing | 5 (< 1) | 2 (< 1) | 3 (< 1) |
| Prior autologous transplant | | | |
| No | 5,054 (98) | 2,006 (98) | 3,048 (98) |
| Yes | 86 (2) | 33 (2) | 53 (2) |
| Conditioning regimen | | | |
| Myeloablative | 2,725 (53) | 1,032 (51) | 1,693 (55) |
| RIC/NMA | 2,399 (47) | 1,004 (49) | 1,395 (45) |
| Missing | 16 (< 1) | 3 (< 1) | 13 (< 1) |
| Donor-recipient sex match | | | |
| Male-male | 2,282 (44) | 932 (46) | 1,350 (44) |
| Male-female | 1,445 (28) | 589 (29) | 856 (28) |
| Female-male | 642 (12) | 256 (13) | 386 (12) |
| Female-female | 769 (15) | 261 (13) | 508 (16) |
| Unknown | 2 (< 1) | 1 (NA) | 1 (NA) |
| Donor-recipient race match | | | |
| Matched | 3,444 (67) | 1,366 (67) | 2,078 (67) |
| Mismatched | 341 (7) | 145 (7) | 196 (6) |
| Missing | 1,355 (26) | 528 (26) | 827 (27) |
| Donor-recipient CMV match | | | |
| Positive-positive | 1,356 (26) | 573 (28) | 783 (25) |
| Positive-negative | 473 (9) | 180 (9) | 293 (9) |
| Negative-positive | 1,853 (36) | 762 (37) | 1,091 (35) |
| Negative-negative | 1,427 (28) | 514 (25) | 913 (29) |
| Missing | 31 (< 1) | 10 (< 1) | 21 (1) |

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UHR HLA Typing Identifies aGVHD Risk

Statistical Methods

The primary study outcome was OS, defined as the time from HCT to death from any cause. Surviving patients were censored at the time of last follow-up. Secondary outcomes included aGVHD, chronic GVHD, relapse, transplant-related mortality (TRM), disease-free survival (DFS), and neutrophil engraftment. aGVHD and chronic GVHD were per CIBMTR definitions. TRM was death without recurrent malignancy. DFS was defined as the time to treatment failure because of death or relapse. HCT Comorbidity Index (HCT-CI) values were calculated using the model proposed by Sorror et al.24

Death was considered a competing risk event for all outcomes except OS, DFS, and TRM, whereas relapse was considered a competing risk for TRM. Patients were censored at time of second HCT or if alive at last follow-up.

Multivariate analyses for OS, aGVHD, relapse, DFS, TRM, and neutrophil engraftment were performed using Cox proportional hazards models. All variables were tested for the confirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted through stratification. A stepwise variable selection procedure was then used to select adjusted covariates and develop multivariable models for the primary and secondary outcomes. Seven UHR matching variables were tested separately for association with each outcome. For the association test of UHR matching in the T-cell–depleted cohort, a P value of < .05 was considered significant as this was a validation analysis of Mayor et al.19 For the main hypothesis of 12 out of 12 versus #11 out of 12 in the full cohort, P < .01 was considered significant. To adjust for multiple testing, a significance level of P < .01/6 = .0017 was used for all other UHR matching variables. All P values were raw and two-sided. Statistical analyses were done using SAS version 9.4 (Cary, NC).

RESULTS

Patient Characteristics

Median time of follow-up was 48 months (range 1 to 123; Table 1). Fifty percent of the patients had acute myelogenous leukemia, and 47% were transplanted with early disease stage. Fifty-three percent received myeloablative conditioning. Patients were predominantly Caucasian (89%), had a median age of 57 years, and mainly had young (19 to 29 years, 62%), male (73%) donors. The majority of patients received peripheral blood stem cell (83%). Nearly half of the patients had an HCT-CI of three or more (48%). The characteristics of the T-cell–depleted and T-cell–replete subgroups were well-balanced.

Impact of UHR Typing on HLA Matching Status

After UHR matching status was determined, only 82% of recipients remained a 10 out of 10 match, with the remainder being 9 out of 10 (17%) or less (1%; Table 2; Appendix Table A1, online only). When matching was extended to include HLA-DPB1, 634 (12%) patients received a 12 out of 12 match.

TABLE 1. Recipient and Donor Characteristics (continued)

| Variable | Overall, No. (%) | T-Cell–Depleted, No. (%) | T-Cell–Replete, No. (%) |
|----------|----------------|--------------------------|------------------------|
| Year of transplant | | | |
| 2008 | 71 (1) | 19 (1) | 52 (2) |
| 2009 | 199 (4) | 68 (3) | 131 (4) |
| 2010 | 433 (8) | 168 (8) | 265 (9) |
| 2011 | 481 (9) | 205 (10) | 276 (9) |
| 2012 | 570 (11) | 228 (11) | 342 (11) |
| 2013 | 553 (11) | 211 (10) | 342 (11) |
| 2014 | 695 (14) | 297 (15) | 398 (13) |
| 2015 | 1,171 (23) | 456 (22) | 715 (23) |
| 2016 | 784 (15) | 325 (16) | 459 (15) |
| 2017 | 183 (4) | 62 (3) | 121 (4) |
| Follow-up among survivors, months | | | |
| No. evaluable | 2,243 | 964 | 1,479 |
| Median (range) | 48 (91-123) | 48 (3-121) | 48 (1-123) |

Abbreviations: ALL, acute lymphatic leukemia; AML, acute myelogenous leukemia; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CSA, cyclosporin A; CY, cyclophosphamide; Eval, evaluable; GVHD, graft-versus-host disease; HCT-CI, Hematopoietic Cell Transplantation Comorbidity Index; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; NA, not applicable; NMA, non-myeloablative conditioning; PBSC, peripheral blood stem cell; RIC, reduced intensity conditioning.
UHR-matched graft, whereas 2,517 (49%), 1,643 (32%), and 346 (7%) received an 11 out of 12, 10 out of 12, or ≤ 9 out of 12 UHR-matched grafts, respectively. There were no significant differences in UHR matching status between the T-cell–depleted or T-cell–replete subgroups.

In recipients identified as 10 out of 10 UHR-matched (n = 4,202), 634 (15%) were found to be 12 out of 12 HLA-matched, whereas 2,011 were DP-TCE permissively mismatched (48%) and 1,557 were DP-TCE non-permissively mismatched (37%). As previously, no significant differences between the T-cell–depleted or T-cell–replete subgroups were observed.

**Analysis of the Full Cohort**

The 4-year OS for the complete cohort was 48% (95% CI, 47 to 49). Adjusting for significant clinical covariates, there were no significant associations between UHR matching and OS in the full cohort or when 12 out of 12 UHR-matched patients were compared with those with any other mismatch (model 1), nor when the degree of mismatch (model 2) was tested. There was no survival advantage for patients who were either 12 out of 12 UHR or HLA-DPB1 non-permissively mismatched (model 3) when compared with permissively mismatched patients.

The 4-year TRM for the complete cohort was 27% (95% CI, 26 to 28). Adjusting for patient and donor age, disease, and HCT-CI score, UHR HLA matching was not associated with risk of TRM in any of the models tested.

The probability of aGVHD at 100 days for the complete cohort was 43% (95% CI, 41 to 44). Adjusting for significant covariates, UHR HLA matching was associated with the probability of aGVHD (grades 2-4) in the overall cohort. Patients receiving a graft from a ≤ 11 out of 12 UHR-matched donor had a significantly higher risk of aGVHD as compared with those receiving a 12 out of 12 UHR match (model 1: hazard ratio (HR), 1.2; 95% CI, 1.05 to 1.36; P = .0061; Fig 1). This was further refined with UHR matching model 2, where increasing numbers of UHR mismatches were associated with increasing risk of aGVHD when compared with 12 out of 12 UHR-matched patients (11 out of 12 HR: 1.15; 95% CI, 1.01 to 1.32; 10 out of 12 HR: 1.26; 95% CI, 1.11 to 1.44; and ≤ 9 out of 12 HR: 1.24; 95% CI, 1.01 to 1.51; overall P = .0019; Fig 1).

Additionally, 10 out of 10 UHR DPB1-TCE permissively mismatched pairs (HR: 1.16; 95% CI, 1.01 to 1.34; P = .036) and DPB1-TCE non-permissively mismatched patients (HR: 1.26; 95% CI, 1.10 to 1.45; P = .0006; Fig 1) were associated with a higher risk of aGVHD compared with 12 out of 12 UHR-matched pairs (overall P = .0011).

**Analysis of the T-Cell–Depleted Subset**

No significant associations with OS were observed with any of the models tested in the T-cell–depleted subgroup (N = 2,039; data not shown). However, UHR mismatches were associated with TRM (Fig 2, Table 3; P = .0068 overall), with patients receiving a 10 out of 12 (HR: 1.46; 95% CI, 1.13 to 1.90; P = .0044) or ≤ 9 out of 12 UHR-

| TABLE 2. HLA Matching Status After UHR HLA Typing |
|-----------------------------------------------|
| **UHR HLA Matching Status**                      | **T-Cell–Depleted (ATG or Alemtuzumab), No. (%)** | **T-Cell–Replete, No. (%)** |
|-----------------------------------------------|
| **UHR matches of 10**                          |                                            |                             |
| 10 out of 10                                  | 1,663 (82)                                  | 2,539 (82)                 |
| 9 out of 10                                   | 340 (17)                                    | 510 (16)                   |
| 8 out of 10                                   | 31 (2)                                      | 46 (1)                     |
| 7 out of 10                                   | 5 (< 1)                                     | 6 (< 1)                    |
| **UHR matches of 12**                          |                                            |                             |
| 12 out of 12                                  | 245 (12)                                    | 389 (13)                   |
| 11 out of 12                                  | 1,015 (50)                                  | 1,502 (48)                 |
| 10 out of 12                                  | 630 (31)                                    | 1,013 (33)                 |
| 9 out of 12                                   | 130 (6)                                     | 175 (6)                    |
| 8 out of 12                                   | 16 (1)                                      | 20 (1)                     |
| 7 out of 12                                   | 3 (< 1)                                     | 2 (< 1)                    |
| **TCE match in UHR 10 out of 10**              |                                            |                             |
| Matched                                       | 245 (15)                                    | 389 (15)                   |
| Nonpermissive mismatch                        | 604 (36)                                    | 953 (38)                   |
| Permissive mismatch                           | 814 (49)                                    | 1,197 (47)                 |

NOTE. All pairs were identified as 10 out of 10 HLA-matched at an antigen recognition domain level prior to reanalysis. Abbreviations: ATG, anti-thymocyte globulin; TCE, T-cell epitope; UHR, ultrahigh resolution.
matched donor (HR: 1.74; 95% CI, 1.14 to 2.66; \( P = .0098 \)) at a significantly higher risk. No other UHR matching model had significant correlations with the probability of TRM, nor was UHR HLA matching in any model associated with any outcomes in this T-cell–depleted subset.

Analysis of the T-Cell–Replete Subset
No significant associations between any of the UHR HLA matching models and OS were identified in the T-cell–replete subset (data not shown). Compared with 12 out of 12 UHR HLA-matched pairs in model 3, 10 out of 12 DP-TCE nonpermissively mismatched pairs had increased risk for TRM (HR: 1.05; 95% CI, 0.83 to 1.32; \( P = .70 \); Table 3), whereas 10 out of 10 DP-TCE permissively mismatched patients had lower risk (HR: 0.81; 95% CI, 0.65 to 1.01; \( P = .066 \); overall \( P = .0003 \)).

The risk of aGVHD (grades 2-4) in the T-cell–replete subset was associated with increasing numbers of UHR mismatches. Compared with a 12 out of 12 UHR HLA match, the probability of aGVHD was higher (overall \( P = .0024 \); Table 3) with the use of 10 out of 12 (HR: 1.28; 95% CI, 1.09 to 1.51; \( P = .0024 \)) and \( \leq 9 \) out of 12 matched donors (HR: 1.31; 95% CI, 1.04 to 1.66; \( P = .024 \)).

Additional Outcome Analyses
UHR HLA matching was not associated with the risk of grade 3-4 aGVHD, DFS, relapse, and chronic GVHD, nor with neutrophil recovery for any of the models tested in any cohort.
Additional HLA Analyses

In the study by Mayor et al., a matching model that combined UHR HLA matching, the DP-TCE permissive matching algorithm, and cytomegalovirus serostatus matching was proposed. No significant associations were observed in any cohort using the proposed model. Additionally, analyses of noncoding variation found no significant associations with outcomes (Appendix Tables A2 and A3, online only).

DISCUSSION

The last 5 years has seen dramatic changes in the methodology routinely used to type HLA genes, particularly within the field of HCT, with increasing numbers of clinical laboratories, MUD registries, and commercial typing providers moving to NGS platforms. There are numerous reasons for this change including in some instances, cost, time, and throughput benefits. A key advantage of NGS protocols is rapid characterization of HLA genes in a timeframe amenable for clinical needs, reducing and often eliminating ambiguous typing results. With this readily available added information about HLA comes the opportunity to identify whether these additional data affect patient outcomes and determine the standard of matching using NGS.

The aim of this retrospective study was to investigate the impact of UHR HLA matching in a large independent data set of unrelated donor (UD) HCT patients from the CIBMTR. Both the full cohort as well as T-cell–depleted and T-cell–replete subgroups were tested. Unfortunately, this study did not confirm the primary hypothesis that UHR HLA matching was associated with improved OS in any of the cohorts tested. However, in the full cohort, UHR mismatching was associated with increased aGVHD risk in all matching models tested. In the T-cell–depleted subgroup, we partly validated the findings of the UK study, with a significant association between UHR HLA mismatching and the probability of TRM. In the T-cell–replete

| TABLE 3. Multivariate Analysis for Key Outcome Variables for the T-Cell–Depleted and T-Cell–Replete Cohorts |
|---------------------------------------------|-------------------------------|-----------------|----------------|-----------------|
| Cohort                        | Variable                  | No. of Cases | HR (95% CI) | P    |
|------------------------------|---------------------------|--------------|-------------|------|
| T-cell-depleted              | UHR matching model 1       |              |             |      |
| 12 out of 12                 | 239                       | 1.00         |             |      |
| < 12 out of 12               | 1,763                     | 1.33 (1.05 to 1.68) | .017 |
| UHR matching model 2         |                           |             |             |      |
| 12 out of 12                 | 239                       | 1.00         |             |      |
| 11 out of 12                 | 998                       | 1.18 (0.92 to 1.51) | .19  |
| 10 out of 12                 | 620                       | 1.46 (1.13 to 1.90) | .0044 |
| ≤ 9 out of 12                | 145                       | 1.74 (1.14 to 2.66) | .0098 |
| UHR matching model 3         |                           |             |             |      |
| 12 out of 12                 | 239                       | 1.00         |             |      |
| 10 out of 10 DP-TCE permissive | 801                       | 1.33 (1.04 to 1.71) | .022 |
| 10 out of 10 DP-TCE nonpermissive | 596                       | 1.23 (0.97 to 1.56) | .092 |
| T-cell-replete               | UHR matching model 1       |              |             |      |
| 12 out of 12                 | 381                       | 1.00         |             |      |
| < 12 out of 12               | 2,660                     | 0.90 (0.72 to 1.12) | .34  |
| UHR matching model 2         |                           |             |             |      |
| 12 out of 12                 | 381                       | 1.00         |             |      |
| 11 out of 12                 | 1,477                     | 0.87 (0.69 to 1.09) | .22  |
| 10 out of 12                 | 990                       | 0.99 (0.77 to 1.26) | .91  |
| ≤ 9 out of 12                | 193                       | 0.69 (0.47 to 1.02) | .066 |
| UHR matching model 3         |                           |             |             |      |
| 12 out of 12                 | 381                       | 1.00         |             |      |
| 10 out of 10 DP-TCE permissive | 1,176                     | 0.81 (0.65 to 1.01) | .066 |
| 10 out of 10 DP-TCE nonpermissive | 935                       | 1.05 (0.83 to 1.32) | .70  |

NOTE. Bold values denote significant P values.
Abbreviations: aGVHD, acute graft-versus-host disease; HR, hazard ratio; TCE, T-cell epitope; TRM, transplant-related mortality; UHR, ultrahigh resolution.
TABLE 4. Comparison of Cohort Characteristics Between This Study and That by Mayor et al.\(^{19}\)

| Variable                          | T-Cell–Depleted, No. (%) | T-Cell–Replete, No. (%) | UK Study, No. (%) |
|----------------------------------|--------------------------|-------------------------|------------------|
| **No. of patients**              | 2,039                    | 3,101                   | 891              |
| **Disease at transplant**        |                          |                         |                  |
| AML                              | 1,047 (51)               | 1,526 (49)              | 224 (25)         |
| ALL                              | 285 (14)                 | 520 (17)                | 164 (19)         |
| MDS                              | 707 (35)                 | 1,055 (34)              | 153 (17)         |
| Other                            |                          |                         | 350 (39)         |
| **Disease status at transplant** |                          |                         |                  |
| Early or good                    | 962 (47)                 | 1,435 (46)              | 406 (46)         |
| Intermediate                     | 264 (13)                 | 439 (14)                | 325 (36)         |
| Advanced or poor                 | 781 (38)                 | 1,190 (38)              | 135 (15)         |
| Missing                          | 32 (2)                   | 37 (1)                  | 25 (3)           |
| **Recipient age at transplant, years** |                     |                         |                  |
| 0-10                             | 81 (4)                   | 71 (2)                  | 83 (9)           |
| 11-18                            | 64 (3)                   | 71 (2)                  | 80 (9)           |
| 19-29                            | 165 (8)                  | 241 (8)                 | 130 (15)         |
| 30-39                            | 159 (8)                  | 275 (9)                 | 135 (15)         |
| 40-49                            | 256 (13)                 | 454 (15)                | 181 (20)         |
| 50-59                            | 473 (23)                 | 728 (23)                | 187 (21)         |
| 60+                              | 841 (41)                 | 1,261 (41)              | 95 (11)          |
| Median (range)                   | 57 (1-84)                | 57 (1-81)               | 41.4 (1.1-71.9)  |
| **Recipient sex**                |                          |                         |                  |
| Male                             | 1,189 (58)               | 1,736 (56)              | 551 (62)         |
| Female                           | 850 (42)                 | 1,365 (44)              | 340 (38)         |
| **Donor age group, years**       |                          |                         |                  |
| 19-29                            | 1,290 (63)               | 1,899 (61)              | 271 (30)         |
| 30-39                            | 455 (22)                 | 709 (23)                | 366 (41)         |
| 40-49                            | 237 (12)                 | 367 (12)                | 217 (24)         |
| 50-59                            | 57 (3)                   | 122 (4)                 | 29 (4)           |
| 60+                              | 0 (0)                    | 4 (< 1)                 | 0 (0)            |
| Missing                          |                          |                         | 8 (< 1)          |
| **Donor pregnancy**              |                          |                         |                  |
| No                               | 325 (16)                 | 573 (18)                | 71 (8)           |
| Yes                              | 188 (9)                  | 315 (10)                | 87 (10)          |
| Male                             | 1,521 (75)               | 2,206 (71)              | 723 (81)         |
| Missing                          | 5 (< 1)                  | 7 (< 1)                 | 10 (1)           |
| **Graft type**                   |                          |                         |                  |
| Marrow                           | 374 (18)                 | 521 (17)                | 402 (45)         |
| PBSC                             | 1,665 (82)               | 2,580 (83)              | 482 (54)         |
| Missing                          |                          |                         | 7 (< 1)          |
| **Prior autologous transplant**  |                          |                         |                  |
| No                               | 2,006 (98)               | 3,048 (98)              | 760 (85)         |
| Yes                              | 33 (2)                   | 53 (2)                  | 131 (15)         |

(continued on following page)
subgroup, increasing numbers of UHR mismatches were associated with increased risk of aGVHD. The probability of TRM was also higher in patients receiving 10 out of 10 UHR-matched, DP-TCE nonpermissively mismatched donors compared with DP-TCE permissively matched donors, but not 12 out of 12 UHR-matched pairs.

| Variable                        | T-Cell–Depleted, No. (%) | T-Cell–Replete, No. (%) | UK Study, No. (%) |
|---------------------------------|--------------------------|-------------------------|-------------------|
| Conditioning regimen            |                          |                         |                   |
| Myeloablative                   | 1,032 (51)               | 1,693 (55)              | 457 (51)          |
| RIC/NMA                         | 1,004 (49)               | 1,395 (45)              | 415 (47)          |
| Missing                         | 3 (< 1)                  | 13 (< 1)                | 19 (2)            |
| Donor-recipient sex match       |                          |                         |                   |
| Male-male                       | 932 (46)                 | 1,350 (44)              | 472 (53)          |
| Male-female                     | 589 (29)                 | 856 (28)                | 252 (28)          |
| Female-male                     | 256 (13)                 | 386 (12)                | 79 (9)            |
| Female-female                   | 261 (13)                 | 508 (16)                | 88 (10)           |
| Unknown                         | 1 (NA)                   | 1 (NA)                  | 0 (0)             |
| Donor-recipient CMV match       |                          |                         |                   |
| Positive-positive               | 573 (28)                 | 783 (25)                | 149 (17)          |
| Positive-negative               | 180 (9)                  | 293 (9)                 | 59 (7)            |
| Negative-positive               | 762 (37)                 | 1,091 (35)              | 179 (20)          |
| Negative-negative               | 514 (25)                 | 913 (29)                | 472 (53)          |
| Missing                         | 10 (< 1)                 | 21 (1)                  | 32 (3)            |
| Year of transplant              |                          |                         |                   |
| 1996                            | 0 (0)                    | 0 (0)                   | 1 (< 1)           |
| 1997                            | 0 (0)                    | 0 (0)                   | 18 (2)            |
| 1998                            | 0 (0)                    | 0 (0)                   | 37 (4)            |
| 1999                            | 0 (0)                    | 0 (0)                   | 41 (5)            |
| 2000                            | 0 (0)                    | 0 (0)                   | 62 (7)            |
| 2001                            | 0 (0)                    | 0 (0)                   | 76 (8)            |
| 2002                            | 0 (0)                    | 0 (0)                   | 83 (9)            |
| 2003                            | 0 (0)                    | 0 (0)                   | 88 (10)           |
| 2004                            | 0 (0)                    | 0 (0)                   | 45 (5)            |
| 2005                            | 0 (0)                    | 0 (0)                   | 57 (6)            |
| 2006                            | 0 (0)                    | 0 (0)                   | 73 (8)            |
| 2007                            | 0 (0)                    | 0 (0)                   | 88 (10)           |
| 2008                            | 19 (1)                   | 52 (2)                  | 71 (8)            |
| 2009                            | 68 (3)                   | 131 (4)                 | 63 (7)            |
| 2010                            | 168 (8)                  | 265 (9)                 | 66 (7)            |
| 2011                            | 205 (10)                 | 276 (9)                 | 22 (3)            |
| 2012                            | 228 (11)                 | 342 (11)                | 0 (0)             |
| 2013                            | 211 (10)                 | 342 (11)                | 0 (0)             |
| 2014                            | 297 (15)                 | 398 (13)                | 0 (0)             |
| 2015                            | 456 (22)                 | 715 (23)                | 0 (0)             |
| 2016                            | 325 (16)                 | 459 (15)                | 0 (0)             |
| 2017                            | 62 (3)                   | 121 (4)                 | 0 (0)             |

Abbreviations: ALL, acute lymphatic leukemia; AML, acute myelogenous leukemia; CMV, cytomegalovirus; MDS, myelodysplastic syndrome; NMA, non-myeloablative conditioning; PBSC, peripheral blood stem cell; RIC, reduced intensity conditioning.
This study confirmed that UHR HLA matching is associated with a decreased risk of aGVHD in the T-cell-replete subgroup. aGVHD remains a common, life-threatening condition that can affect patient quality of life after HCT.25 Thus, any means of reducing this risk, especially when achievable within the normal processes surrounding patient and donor testing prior to HCT, should be considered. Abrogation of the graft-versus-leukemia effect could be a concern, but we did not see any association between UHR HLA matching status and relapse or DFS.

A common assumption around NGS typing strategies that deliver UHR HLA typing is that the cost is prohibitive. In fact, today the cost and turnaround time of such typing is comparable with previously used methods, and continued demand for such tests will only continue to decrease costs. Although cost analyses were not within the scope of this study, it should be acknowledged that treatment of patients with aGVHD is also costly26 and not risk-free.

Identifying a 12 out of 12 UHR HLA-matched, or a 10 out of 10 UHR-matched, DP-TCE permissively mismatched donor for patients can be challenging and may not be a possible option. However, studies have shown that in similar populations from the United States, the probability of identifying a 10 out of 10, DP-TCE permissively mismatched donor was 69%, increasing to 80% if additional donors underwent typing, depending on the patient’s ethnic origin.27 The resolution of HLA typing used was lower than UHR, which will likely affect the probability of finding such a donor when UHR typing is used, but nonetheless, it suggests that identifying 12 out of 12 or 10 out of 10 DP-TCE permissively matched donors is a realistic possibility. The uptake of UHR typing methods by large UD registries will help facilitate UHR matching in clinical practice.28-30 The potential benefit of using UHR HLA typing for alternative donor sources is yet to be tested.

There were important differences between this study and that by Mayor et al.,19 which may have contributed to the differing results. Although both studies used Pacific Biosciences’ single-molecule real-time sequencing technologies, the extent of the gene coverage differed, with the previous study including all the defined 5’ and 3’ untranslated regions of the class I genes plus additional exons of HLA-DQB1 and HLA-DPB1 (exons 2-5 and 2-4, respectively). We determined that 45 out of 891 patients (5%) of the UK study cohort would have been classified as a different match level than the present analysis on the basis of the additional information obtained from the more extensive typing strategy. If extrapolated to this study cohort, a potential 250 of the 5,140 patients studied may have been classified as less well-matched if the more extensive typing methodology was used. Although it is unlikely that differences in the untranslated regions have functional consequences, they could be suggestive of lesser compatibility over the extended MHC region, resulting in more genetic disparity between patients and donors, and the potential for increased complications post-transplant.17 Further studies at a comparable level of typing will be needed to determine whether this additional information is clinically important.

In addition to the typing differences, the characteristics of the current cohort were notably different (Table 4). Of note, the present study was limited to 10 out of 10 ARD HLA-matched pairs, whereas the previous study included ARD mismatches. Additionally, the years of transplant differed, overlapping by only 4 years (2008-2011). Transplant practice changes over time, which is reflected in additional differences between the two studies (change in disease, use of peripheral blood, older patients, and younger UDs recently). Finally, this study included more advanced disease stage (38% vs 15% previously). Although we attempted to mitigate the effects of these differences in the statistical analysis, this variation may have contributed to a different risk profile than that of the UK study. Indeed, HLA mismatching UD-HCT patients at high resolution6 and transplanting patients in older eras31,32 are associated with detrimental outcomes, whereas the adverse effect of HLA mismatching diminishes in patients with advanced disease.7,33 Patients transplanted in more recent years may benefit from changes in practice to the extent that other risk factors such as HLA matching become less marked.

Further evidence to support this observation is the surprising lack of association between the DP-TCE matching model and outcomes, which is in contrast to several previous publications. An earlier study from the CIBMTR in a similar cohort of patients reported that either permissive or nonpermissive DPB1 mismatching was associated with increased probability of aGVHD, while nonpermissive DP-TCE mismatching was associated with reduced OS.7 Of note, the cohort studied by Pidala et al resembled the UK UHR cohort; transplants between 1999 and 2011, more bone marrow grafts, more myeloablative conditioning, similar proportions of advanced disease stage, and inclusion of patients with chronic myeloid leukemia. Further studies in more recent cohorts are needed to confirm whether factors previously thought to affect outcome remain as relevant in the current era.

In conclusion, this study did not confirm previous findings that UHR HLA typing was associated with OS in any of the models tested. However, when multiple 10 out of 10 HLA-matched donors of equivalent age are available, the consideration of UHR HLA typing data may identify donors associated with a reduced risk of aGVHD, and in some cases TRM, even if OS does not differ.
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AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Impact of Previously Unrecognized HLA Mismatches Using Ultrahigh Resolution Typing in Unrelated Donor Hematopoietic Cell Transplantation

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### TABLE A1. Location of Variation Observed in the 5,140 Pairs Previously Characterized as 10 out of 10 HLA High-Resolution–Matched After Ultrahigh Resolution HLA Typing

| Parameter       | HLA-A | HLA-B | HLA-C | HLA-DRB1 | HLA-DQB1 | HLA-DPB1 |
|-----------------|-------|-------|-------|----------|----------|----------|
| Matching level  |       |       |       |          |          |          |
| Matched         | 4,681 | 4,941 | 4,846 | 5,106    | 5,101    | 782      |
| One mismatch    | 450   | 199   | 292   | 33       | 39       | 2,867    |
| Two mismatches  | 9     | 0     | 2     | 1        | 0        | 1,491    |
| Location of mismatches |     |       |       |          |          |          |
| Protein         | 15    | 21    | 22    | 26       | 21       | 5,839    |
| Synonymous      | 84    | 39    | 32    | 9        | 18       | 10       |
| Noncoding (intron and UTR) | 369  | 139   | 242   | —        | —        | —        |
| Total variants  | 468   | 199   | 296   | 35       | 39       | 5,849    |

NOTE. Pairs may have had more than one variant; thus, total pairs with coding and noncoding variants do not correlate with the total mismatched pairs. Abbreviation: UTR, untranslated regions.
## TABLE A2. Multivariate Analysis for 12 out of 12 Versus 12 out of 12 CDS-Matched Versus CDS-Mismatched Pairs in the Full Cohort

| Outcome Variable | Full Cohort |
|------------------|-------------|
|                 | No. of Cases | HR (95% CI) | P   |
| OS               | Overall      |             | .7605 |
|                  | 12 out of 12 | 627         | 1.00  |
|                  | 12 out of 12 CDS-matched | 104 | 0.90 (0.66 to 1.23) | .5223 |
|                  | Mismatched   | 4,379       | 1.01 (0.90 to 1.13) | .8838 |
| DFS              | Overall      |             | .7009 |
|                  | 12 out of 12 | 625         | 1.00  |
|                  | 12 out of 12 CDS-matched | 102 | 0.91 (0.70 to 1.19) | .4958 |
|                  | Mismatched   | 4,332       | 0.97 (0.89 to 1.07) | .5781 |
| TRM              | Overall      |             | .8371 |
|                  | 12 out of 12 | 620         | 1.00  |
|                  | 12 out of 12 CDS-matched | 102 | 0.93 (0.59 to 1.46) | .7433 |
|                  | Mismatched   | 4,321       | 1.02 (0.87 to 1.20) | .7789 |
| Relapse          | Overall      |             | .4597 |
|                  | 12 out of 12 | 621         | 1.00  |
|                  | 12 out of 12 CDS-matched | 103 | 0.87 (0.62 to 1.23) | .4424 |
|                  | Mismatched   | 4,316       | 0.93 (0.80 to 1.07) | .2939 |
| aGVHD (grades 2-4) | Overall   |             | .0183 |
|                  | 12 out of 12 | 590         | 1.00  |
|                  | 12 out of 12 CDS-matched | 93  | 1.01 (0.73 to 1.41) | .9397 |
|                  | Mismatched   | 4,135       | 1.20 (1.06 to 1.37) | .0055 |
| cGVHD            | Overall      |             | .7661 |
|                  | 12 out of 12 | 624         | 1.00  |
|                  | 12 out of 12 CDS-matched | 105 | 1.03 (0.84 to 1.27) | .7676 |
|                  | Mismatched   | 4,253       | 0.97 (0.86 to 1.08) | .5502 |
| ANC              | Overall      |             | .6957 |
|                  | 12 out of 12 | 628         | 1.00  |
|                  | 12 out of 12 CDS-matched | 105 | 1.07 (0.90 to 1.28) | .4490 |
|                  | Mismatched   | 4,367       | 1.03 (0.95 to 1.12) | .4728 |

Abbreviations: aGVHD, acute graft-versus-host disease; ANC, absolute neutrophil count; CDS, coding DNA sequence; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HR, hazard ratio; OS, overall survival; TRM, transplant-related mortality.
| Outcome  | Variable | T-Cell–Depleted | T-Cell–Replete |
|----------|----------|-----------------|-----------------|
|          | No. of Cases | HR (95% CI) | P  | No. of Cases | HR (95% CI) | P  |
| OS       | Overall   | .0969          | .7538          | .6338          | .4492          |
|          | 12 out of 12 | 243            | 1.00          | 384            | 1.00          |
|          | 12 out of 12 CDS-matched | 33            | 0.80 (0.53 to 1.21) | .2893          | 71            | 0.97 (0.67 to 1.42) | .8914          |
|          | Mismatched | 1,752          | 1.15 (0.98 to 1.36) | .0837          | 2,627         | 0.95 (0.82 to 1.09) | .4552          |
| DFS      | Overall   | .0116          | .3771          | .3883          | .6212          |
|          | 12 out of 12 | 239            | 1.00          | 381            | 1.00          |
|          | 12 out of 12 CDS-matched | 33            | 0.67 (0.29 to 1.57) | .3536          | 69            | 1.04 (0.61 to 1.76) | .8971          |
|          | Mismatched | 1,730          | 1.34 (1.06 to 1.70) | .0131          | 2,591         | 0.89 (0.72 to 1.11) | .3168          |
| TRM      | Overall   | .3883          | .6212          | .7864          | .3181          |
|          | 12 out of 12 | 240            | 1.00          | 381            | 1.00          |
|          | 12 out of 12 CDS-matched | 34            | 1.01 (0.58 to 1.73) | .9837          | 69            | 0.81 (0.52 to 1.25) | .3362          |
|          | Mismatched | 1,724          | 0.88 (0.73 to 1.06) | .1834          | 2,592         | 0.97 (0.77 to 1.21) | .7627          |
| Relapse  | Overall   | .0985          | .0702          | .7657          | .8392          |
|          | 12 out of 12 | 230            | 1.00          | 360            | 1.00          |
|          | 12 out of 12 CDS-matched | 31            | 0.82 (0.46 to 1.45) | .4904          | 62            | 1.09 (0.72 to 1.66) | .6775          |
|          | Mismatched | 1,675          | 1.18 (0.97 to 1.44) | .0908          | 2,460         | 1.21 (1.03 to 1.43) | .0212          |
| aGVHD (grades 2-4) | Overall   | .0985          | .0702          | .7864          | .3181          |
|          | 12 out of 12 | 230            | 1.00          | 360            | 1.00          |
|          | 12 out of 12 CDS-matched | 31            | 0.82 (0.46 to 1.45) | .4904          | 62            | 1.09 (0.72 to 1.66) | .6775          |
|          | Mismatched | 1,675          | 1.18 (0.97 to 1.44) | .0908          | 2,460         | 1.21 (1.03 to 1.43) | .0212          |
| cGVHD    | Overall   | .7864          | .3181          | .7657          | .8392          |
|          | 12 out of 12 | 240            | 1.00          | 384            | 1.00          |
|          | 12 out of 12 CDS-matched | 34            | 0.85 (0.47 to 1.53) | .5823          | 71            | 1.10 (0.89 to 1.36) | .3839          |
|          | Mismatched | 1,690          | 1.03 (0.82 to 1.31) | .7789          | 2,563         | 0.93 (0.80 to 1.08) | .3485          |
| ANC      | Overall   | .6057          | .9592          | .7657          | .8392          |
|          | 12 out of 12 | 244            | 1.00          | 384            | 1.00          |
|          | 12 out of 12 CDS-matched | 34            | 1.20 (0.84 to 1.71) | .3232          | 71            | 1.06 (0.86 to 1.32) | .5801          |
|          | Mismatched | 1,747          | 1.03 (0.95 to 1.12) | .6276          | 2,620         | 1.02 (0.93 to 1.13) | .6243          |

Abbreviations: aGVHD, acute graft-versus-host disease; ANC, absolute neutrophil count; CDS, coding DNA sequence; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HR, hazard ratio; OS, overall survival; TRM, transplant-related mortality.