

# HLAMatchmaker-Defined Triplet Matching Is Not Associated with Better Survival Rates of Patients with Class I HLA Allele Mismatched Hematopoietic Cell Transplants from Unrelated Donors

Rene Duquesnoy,<sup>1</sup> Stephen Spellman,<sup>2</sup> Michael Haagenson,<sup>3</sup> Tao Wang,<sup>4</sup> Mary M. Horowitz,<sup>4</sup> Machteld Oudshoorn<sup>5</sup>

<sup>1</sup>University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; <sup>2</sup>National Marrow Donor Program, Minneapolis, Minnesota; <sup>3</sup>Center for International Blood & Marrow Transplant Research, Minneapolis, Minnesota; <sup>4</sup>Center for International Blood & Marrow Transplant Research, Milwaukee, Wisconsin; and <sup>5</sup>Leiden University Medical Center, Leiden, The Netherlands

Correspondence and reprint requests: René J. Duquesnoy, PhD, Professor of Pathology and Surgery, Biomedical Science Tower, Room W1552, University of Pittsburgh Medical Center, Pittsburgh, PA 15261 (e-mail: [Duquesnoyr@upmc.edu](mailto:Duquesnoyr@upmc.edu)).

Received April 24, 2008; accepted July 2, 2008

## ABSTRACT

This report addresses the concept that permissible HLA mismatching, that is, mismatches that do not generate an allogeneic response, in hematopoietic stem cell transplantation (HCT) can be determined with structural similarity of polymorphic regions. We have applied the triplet version of a structural algorithm called HLAMatchmaker, which considers short sequences involving polymorphic amino acid residues on the molecular surface as key elements of immunogenic epitopes. The triplet matching effect was analyzed in a National Marrow Donor Program dataset consisting of 744 unrelated hematopoietic cell transplantation cases with 1 HLA-A, -B, or -C mismatch and 1690 fully HLA-A, -B, -C, -DR, or -DQ allele matched cases. In multivariate models adjusting for other significant clinical risk factors, the degree of triplet mismatching did not significantly correlate with patient survival, engraftment, or acute graft-versus-host disease (aGVHD). Other structurally based strategies should be pursued to identify permissible HLA mismatches in HCT.

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## KEY WORDS

HLAMatchmaker • Unrelated donor • Transplantation • Amino acid triplet

## INTRODUCTION

Although the outcome of unrelated donor hematopoietic cell transplants (HCT) can be optimized by matching for HLA-A, -B, -C, and -DRB1 alleles, many patients have no access to such matched donors. A recent analysis of the National Marrow Donor Program (NMDP) experience has shown that about one-quarter of patients received 1 allele mismatched HCT primarily involving the HLA-A, -B, and -C loci [1]. Patients with such transplants have, however, less successful engraftment, more acute graft-versus-host disease (aGVHD) and lower survival rates [1-4]. This problem might be overcome with HCT from donors with “permissible” mismatches that do not in-

crease patient morbidity and mortality. Permissive mismatches can be defined as HLA allele differences between the donor and recipient that do not elicit an allogeneic GVHD or rejection response.

At least 2 studies have considered structural similarity among HLA allelic products as a means of predicting improved outcome. In a previous analysis of NMDP data, HLA mismatching within a serologic crossreactive group (CREG) at HLA-A or -B was not associated with a survival benefit in comparison to mismatches outside a CREG [5]. The findings suggest that serologically defined similarities between mismatched HLA class I antigens are not indicative of permissive mismatches. A second approach to define

permissive mismatching used an algorithm, Histocheck, that applies the so-called distance index of Risler [6] to assess functional similarities between amino acid substitutions on disparate HLA molecules [7]. Similarity, as measured by a low Histocheck score, offered no benefit to patients with bone marrow transplants from class I mismatched donors [8]. In addition, a recent report by Kawase et al. [9] suggested that certain amino acid positions in HLA class I mismatches contribute to the severity of GVHD. At present, the criteria for HLA mismatch permissibility are not readily defined [10].

HLAMatchmaker is a matching algorithm that considers the structural basis of epitopes on HLA antigens. Each class I HLA antigen is viewed as a string of short sequences (triplets) involving polymorphic amino acid residues on the molecular surface. These residues are hypothesized to comprise key elements of epitopes that can induce HLA-specific antibodies [11,12]. Donor-recipient compatibility is assessed by determining which triplets in corresponding sequence positions are different. The degree of structural compatibility of an HLA mismatch can vary between 0 or few triplets to >15 triplets. The triplet matching concept has clinical relevance as suggested by an analysis of 2 large kidney transplant databases showing that HLA-A, -B mismatched kidneys that are compatible at the triplet level exhibit almost identical graft survival rates as the 0 HLA-A, -B antigen mismatches defined by conventional criteria [13]. Moreover, the degree of humoral sensitization correlates with the number of mismatched triplets on immunizing HLA antigens [14-16]. HLAMatchmaker has also been used in refining and expanding platelet donor selection for refractory, thrombocytopenic patients [17,18].

We have addressed the hypothesis that matching at the structural level using HLAMatchmaker permits the identification of permissible mismatches for HCT. This report describes our analysis of the effect of class I HLA mismatches with minimal mismatched triplets on HCT outcome.

## MATERIALS AND METHODS

### NMDP Database and Patient Characteristics

Patients reported to the NMDP who were transplanted between 1988 and 2003 and fully HLA-typed through the NMDP's ongoing retrospective high resolution typing project were included in this analysis (Table 1). Eligible diagnoses included acute lymphoblastic leukemia (ALL, N = 581), acute myeloid leukemia (AML, N = 676), chronic myeloid leukemia (CML, N = 954), and myelodysplastic syndrome (MDS, N = 223). Early-stage disease (N = 1046) was defined as AML and ALL in the first complete remission, CML in the first chronic phase, and MDS subtype refractory anemia. Intermediate-stage disease (N = 937) was AML or ALL in the second or subse-

quent complete remission or in the first relapse, and CML in the accelerated phase or second chronic phase. Advanced phase disease (N = 448) was AML in the second or higher relapse or primary induction failure, CML in the blast phase, MDS subtypes refractory anemia with excess blasts or in transformation, or MDS, not otherwise classified. All patients received standard myeloablative conditioning regimens.

Patients undergoing conditioning regimens of lower intensity, second or subsequent transplantation, or surviving patients who did not provide signed, informed consent to allow analysis of their clinical data or HLA typing of stored NMDP Research Repository samples were excluded. All surviving recipients included in this analysis were retrospectively contacted, and provided informed consent for participation in the NMDP research program. Informed consent was waived by the NMDP institutional review board for all deceased recipients. To adjust for the potential bias introduced by exclusion of nonconsenting surviving patients, a modeling process randomly excluded the same percentage of deceased patients using a biased coin randomization with exclusion probabilities based on characteristics associated with not providing consent for use of the data in survivors [1].

### HLA Matching at the Triplet Level

High-resolution typing was performed retrospectively through the NMDP Donor/Recipient Pair Project for HLA-A, -B, -C, -DRB1, and -DQB1, as previously described [13]. To evaluate the role of triplet mismatching at the HLA class I loci (HLA-A, -B, and -C) the study population was restricted to cases either fully matched for HLA-A, -B, -C, -DRB1, and -DQB1 alleles (10 of 10 matches) or with a single mismatch at HLA class I (9 of 10 allele/antigen matches). A total of 2434 donor-recipient pairs were included in the analysis, 1690 of which were 10 of 10 HLA matches. There were 744 donor-recipient pairs that were 9 of 10 HLA matched with 1 class I mismatch: 242 (33%) for HLA-A, 97 (13%) for HLA-B, and 405 (54%) for HLA-C (Table 2).

The triplet version of HLAMatchmaker was used to assess the degree of structural compatibility between 1-allele mismatches [11]. Table 2 shows the distribution of the numbers of mismatched triplets in the host-versus-graft (HVG) and graft-versus-host (GVH) direction among the allele mismatches for HLA-A, -B, and -C. The 0-triplet mismatch group comprised relatively more HLA-B allele mismatches than the other triplet mismatch groups.

### Clinical Endpoints

The primary endpoint of this study was disease-free patient survival; engraftment and aGVHD were secondary endpoints. Two parameters were used to

**Table 1.** Patient Characteristics of Different Mismatched Groups in the GVH Direction

	0 Allele MM	0 Triplet MM	1 Triplet MM	2-3 Triplet MM	4-5 Triplet MM	6+ Triplet MM
Number of patients	1690	241	140	143	102	118
Number of centers	105	72	59	54	44	47
Median age (years) (range)	35(<1-65)	32(<1-60)	35(1-58)	30(<1-57)	32(1-59)	28(2-65)
Male gender	960 (57%)	124 (51%)	73 (52%)	80 (56%)	56 (55%)	64 (54%)
≥90 Karnofsky score	1225 (75%)	168 (71%)	101(74%)	100 (72%)	79 (81%)	86 (74%)
<b>Disease at transplant</b>						
<b>AML</b>	452 (27%)	72 (30%)	41 (29%)	51 (36%)	27 (27%)	33 (28%)
<b>ALL</b>	382 (23%)	64 (26%)	30 (22%)	40 (28%)	33 (32%)	32 (27%)
<b>CML</b>	695 (41%)	89 (37%)	62 (44%)	38 (26%)	32 (31%)	38 (32%)
<b>MDS</b>	161 (9%)	16 (7%)	7 (5%)	14 (10%)	10 (10%)	15 (13%)
<b>Disease status</b>						
<b>Early</b>	761 (45%)	96 (40%)	64 (46%)	41 (29%)	36 (35%)	48 (41%)
<b>Intermediate</b>	631 (37%)	104 (43%)	54 (38%)	72 (50%)	34 (33%)	42 (36%)
<b>Advanced</b>	296 (18%)	41 (17%)	22 (16%)	30 (21%)	32 (32%)	27 (23%)
<b>Graft type</b>						
<b>Marrow</b>	1561 (92)	223 (93)	126 (90)	128 (90)	97 (95)	109 (92)
<b>Peripheral blood</b>	129 (8)	18 (7)	14 (10)	15 (10)	5 (5)	9 (8)
<b>Donor/recipient CMV match</b>						
<b>Negative/negative</b>	619 (37)	73 (30)	51 (37)	53 (37)	31 (30)	44 (37)
<b>Negative/positive</b>	463 (27)	71 (30)	39 (28)	36 (25)	29 (28)	32 (27)
<b>Positive/negative</b>	277 (16)	44 (18)	24 (17)	25 (17)	17 (17)	15 (13)
<b>Positive/positive</b>	280 (17)	49 (20)	24 (17)	28 (20)	22 (22)	23 (20)
<b>Unknown</b>	51 (3)	4 (2)	2 (1)	1 (1)	3 (3)	4 (3)
<b>Donor/recipient sex match</b>						
<b>Male/Mmale</b>	665 (39)	82 (34)	44 (31)	46 (32)	30 (29)	44 (37)
<b>Male/female</b>	407 (24)	61 (25)	35 (25)	29 (20)	29 (28)	33 (28)
<b>Female/male</b>	295 (18)	42 (18)	29 (21)	34 (24)	26 (26)	20 (17)
<b>Female/female</b>	323 (19)	56 (23)	32 (23)	34 (24)	17 (17)	21 (18)
<b>GVHD prophylaxis</b>						
<b>FK506 ± other</b>	339 (20)	58 (24)	33 (23)	29 (20)	19 (19)	17 (14)
<b>CSA + MTX ± other</b>	984 (58)	133 (55)	77 (55)	64 (45)	55 (54)	66 (56)
<b>CSA± other (no MTX)</b>	63 (4)	4 (2)	4 (3)	4 (3)	1 (1)	4 (3)
<b>T cell depletion</b>	285 (17)	44 (18)	26 (19)	43 (30)	27 (26)	30 (26)
<b>Other</b>	19 (1)	2 (1)	0	3 (2)	0	1 (1)

MM indicates mismatches; CSA, cyclosporine; MTX, methotrexate.

assess successful engraftment: blood neutrophil counts of  $\geq 500/\text{mm}^3$  for 3 consecutive days and blood platelet counts reaching  $\geq 20,000 \times 10^9/\text{L}$ . The incidence of grades II-IV and III-IV aGVHD disease was determined during the first 100 days posttransplant. Events were summarized by the cumulative incidence estimate with death as a competing risk.

### Statistical Methods

The statistical analyses of transplant outcome parameters comparing matching in HVG and GVH directions were similar to those in a previous report on CREG matching [5]. To compare pretransplant characteristics for discrete factors, the number of cases and their respective percentages were calculated and chi-square testing was done to compare the HLA matched, triplet matched, and triplet mismatched groups. For continuous factors, the median and ranges were calculated and the Kruskal-Wallis test was used to analyze differences between HLA matched, triplet matched, and triplet mismatched groups. Probabilities of disease-free survival (DFS) were calculated using the Kaplan-Meier estimator with variance estimated

by Greenwood's formula. Comparisons of survival curves were done with the log-rank test.

Multivariate analyses were performed using the proportional hazards model to compare the HLA matched, triplet matched, and triplet mismatched groups. Models were fit to determine which risk factors may be related to a given outcome. All variables were tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted using time-dependent variables or stratification before the stepwise model building approach was used in developing models for the primary and secondary outcomes. Five patients were excluded from the multivariate models because of missing clinical and/or outcome data.

## RESULTS

### Engraftment

Figure 1a and b shows the unadjusted cumulative percentages of patients with successful neutrophil engraftment for the 10 of 10 matched and HVG direction triplet mismatched groups. There was no significant

**Table 2.** Distribution of Triplet Mismatch Groups among HLA Class I Mismatched Donor-Recipient Pairs

Mismatch group	HLA-A mismatches	HLA-B mismatches	HLA-C mismatches	Total
	242 (33%)	97 (13%)	405 (54%)	744
<b>HVG direction</b>				
0 Triplets	67 (29%)	76 (33%)	86 (38%)	229
1 Triplet	45 (32%)	9 (6%)	89 (62%)	143
2-3 Triplets	32 (22%)	5 (3%)	108 (75%)	145
4-5 Triplets	37 (32%)	4 (4%)	73 (64%)	114
6+ Triplets	61 (54%)	3 (3%)	49 (43%)	113
<b>GVH direction</b>				
0 Triplets	73 (30%)	73 (30%)	95 (40%)	241
1 Triplet	44 (31%)	14 (10%)	82 (59%)	140
2-3 Triplets	37 (26%)	6 (4%)	100 (70%)	143
4-5 Triplets	36 (35%)	1 (1%)	65 (64%)	102
6+ Triplets	52 (44%)	3 (3%)	63 (53%)	118

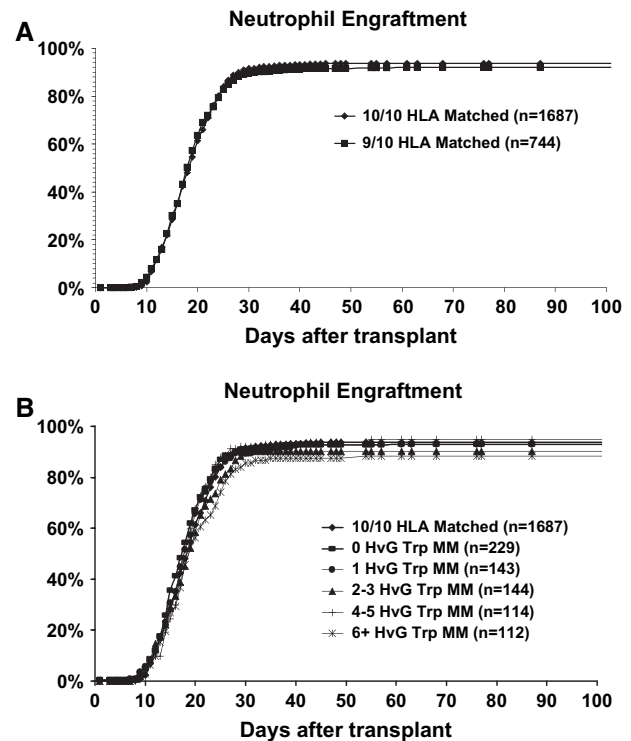
GVH indicates graft-versus-host; HVG, host-versus-graft.

difference in neutrophil engraftment rates at 28 days between the 10 of 10 and 9 of 10 allele matched groups ( $P = .33$ ). On day 60 posttransplant, the cumulative incidence of neutrophil engraftment was 94% for the 10 of 10 group, 93% for the 0-triplet mismatches, 93% for the 1-triplet mismatches, 90% for the 2-3 triplet mismatches, 95% for the 4-5 triplet mismatches, and 88% for the  $\geq 6$  triplet mismatches. The differences between the groups were not statistically significant.

Figure 2a and b shows the unadjusted cumulative percentages of patients with blood platelet counts reaching  $\geq 20,000/\text{mm}^3$  for the 10 of 10 matched and HVG direction triplet mismatched groups. The 9 of 10 matches had significantly lower success rates than the 10 of 10 matches (after 100 days: 65% versus 72%,  $P = .001$ ). At 100 days, the engraftment rates were 67% for the 0-triplet mismatches, 63% for the 1-triplet mismatches, 68% for the 2-3 triplet mismatches, 61% for the 4-5 triplet mismatches, and 61% for the  $\geq 6$  triplet mismatches. The platelet engraftment differences observed between the triplet mismatched groups were not statistically significant.

### Acute GVHD

The cumulative incidence of grade II-IV aGVHD was higher for the 9 of 10 matches than the 10 of 10 matches, 52% versus 49% after 100 days, but this difference was not statistically significant ( $P = .15$ ) (Figure 3a). However, in a multivariate analysis adjusting for other risk factors, the 9 of 10 matches had a significantly higher risk for grade II-IV ( $P = .008$ ) and grade III-IV ( $P < .001$ ) GVHD than the 10 of 10 matches (Table 3). Among the 9 of 10 matches, the 0-triplet mismatches in the GVH direction had a similar incidence of grade II-IV aGVHD as the 10 of 10 matches: 47% versus 49% after 100 days (Figure 3b). The other triplet mismatches had a higher incidence of aGVHD disease, namely 1 triplet (54%),



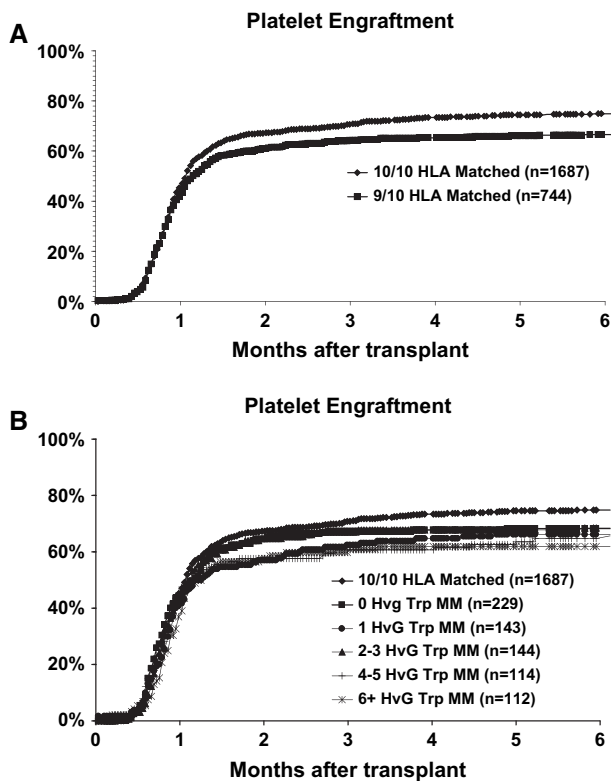
**Figure 1.** Matching effect on neutrophil engraftment. (A) The 9 of 10 matches have slightly lower rates than 10 of 10 matches (at 28 days  $P = .33$ , NS). (B) Effect of triplet mismatching in the HVG direction. Although the 6+ triplet mismatch group had the lowest neutrophil engraftment rate, the differences between the different match groups were not statistically significant.

2-3 triplets (58%), 4-5 triplets (53%), and  $\geq 6$  triplets (53%), but were not statistically different.

A multivariate analysis showed that the risk of grade II-IV GVH disease for the 0, 1, 4-5, and  $\geq 6$  triplet mismatches and the risk of grade III-IV GVH disease for the 0 and  $\geq 6$  triplet mismatches was not statistically different when compared to the 10 of 10 matched cohort (Table 3). However, when the individual triplet mismatch groups were compared, none of the groups were significantly different from each other ( $P = .16$  and  $P = .13$  for grades II-IV and III-IV, respectively). Altogether, this multivariate analysis suggests that the degree of triplet mismatching among the 9 of 10 matches does not correlate with the risk for aGVHD.

### DFS

DFS was significantly lower for the 9 of 10 matches than the 10 of 10 matches (Figure 4a). Two-year DFS was 32% and 40% for 9 of 10 and 10 of 10 groups, respectively ( $P < .001$ ). Triplet matching in the GVH direction within the 9 of 10 matches did not improve DFS (Figure 4b). The 2-year unadjusted DFS rates were 31% for the 0-triplet mismatches, 36% for the 1-triplet mismatches, 34% for the 2-3 triplet mismatches, 29% for the 4-5 triplet mismatches, and



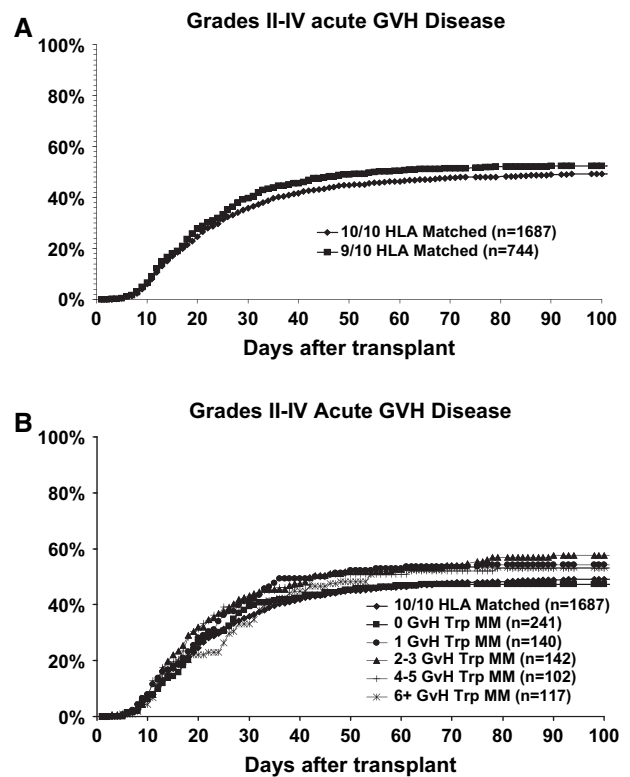
**Figure 2.** Matching effect on platelet engraftment. (A) The 9 of 10 match group had a significantly lower engraftment rate than the 10 of 10 matches ( $P = .001$  after 100 days). (B) Effect of triplet matching; the 4-5 and 6 triplet mismatch groups had the lowest platelet engraftment rates but the differences between the triplet groups were not statistically significant.

29% for the  $\geq 6$  triplet mismatches. A similar analysis showed that triplet mismatching in the HvG direction did not benefit DFS (data not shown).

Multivariate analysis showed that the overall risk of treatment failure (relapse or death, the inverse of DFS) ( $P = .008$ ) was significantly higher after 9 of 10 than after 10 of 10 matched transplants (Table 4). The evaluation of individual triplet groups revealed that the difference in DFS between some triplet mismatch groups and the 10 of 10 matched group did not reach statistical significance ( $P > .01$ ), that is, 1, 2-3, and  $\geq 6$  triplet mismatches. However, when the individual triplet mismatch groups were compared, none were significantly different from each other ( $P = .62$ ). Altogether, this multivariate analysis suggests that the degree of triplet mismatching among the 9 of 10 matches does not influence the likelihood of DFS.

## DISCUSSION

This study confirms previous reports that a single class I HLA mismatch has an adverse effect on HCT outcome as manifested by less engraftment, more aGVHD and lower patient survival [1,3,4]. The goal of this analysis was to identify structurally similar per-



**Figure 3.** HLA matching effect on acute grade II-IV GVHD. (A) The 9 of 10 match group had a statistically insignificant higher incidence than the 10 of 10 matches; 52% versus 49% after 100 days,  $P = .15$ . (B) Effect of triplet mismatching in the GVH direction. There was no statistically significant correlation between the number of mismatched triplets and the incidence of aGVHD.

missive class I mismatches that result in the same outcome as the fully matched transplants or, at least, with better outcomes than class I mismatches with higher structural dissimilarity. However, the results of this analysis suggest that matching for HLA Matchmaker assigned triplets does not benefit HCT outcome.

HLA Matchmaker considers compatibility at the humoral immune level, that is, a structural identification of antibody accessible epitopes exposed on the HLA molecular surface. The original version uses triplets, that is, linear sequences of 3 amino acids, at least 1 of which is an antibody-accessible polymorphic residue [11]. The triplet version does not address compatibility at the cellular immune level involving T cell activation influenced by structural polymorphisms in the peptide-binding groove and on the HLA molecular surface. Alloreactive T cells play a dominant role in GVHD and their T cell receptors recognize peptides presented by HLA molecules. One might expect that 0-triplet mismatches would be structurally more similar to the corresponding self-alleles of the patient and may therefore bind the same peptide repertoire unless the peptide-binding groove has significant amino acid differences.

The literature has many reports describing alloreactive T cells that are specific for serologic HLA antigens

**Table 3.** Multivariate Analysis Comparing aGVHD for 10 of 10 Matches with the 9 of 10 Match Groups with Triplet Mismatches in the GVH Direction

Match group	N	Relative risk (95% confidence interval)	P-value
<b>Grade II-IV GVHD</b>			
10 of 10 matches	168	1.00	
0 Triplet mismatches	241	1.04 (0.85-1.26)	.72
1 Triplet mismatches	140	1.16 (0.92-1.46)	.22
2-3 Triplet mismatches	142	1.46 (1.16-1.84)	.001
4-5 Triplet mismatches	102	1.36 (1.03-1.79)	.031
≥6 Triplet mismatches	117	1.17 (0.91-1.50)	.23
<b>Grade III-IV GVHD</b>			
10 of 10 matches	168	1.00	
0 Triplet mismatches	241	1.23 (0.97-1.57)	.10
1 Triplet mismatches	140	1.46 (1.09-1.95)	.01
2-3 Triplet mismatches	142	1.93 (1.47-2.54)	.0001
4-5 Triplet mismatches	102	1.60 (1.14-2.250)	.007
≥6 Triplet mismatches	117	1.39 (1.00-1.92)	.048

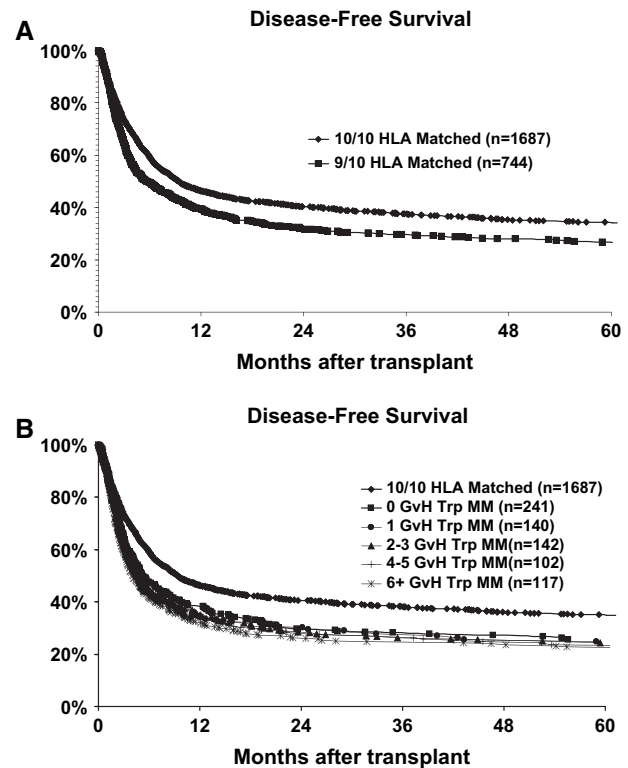
Adjusted factors: disease, GVHD prophylaxis, graft type, Karnofsky score, year of transplant, recipient gender. The overall comparison of 10 of 10 matches versus all triplet groups was significantly different (chi-square test,  $P = .008$  and  $P < .0001$  for grades II-IV and III-IV, respectively). The overall comparison of individual triplet mismatch groups found that none were significantly different from each other (chi-square test,  $P = .16$  and  $P = .13$  for grades II-IV and III-IV, respectively).

aGVHD indicates acute graft-versus-host disease; GVH, graft-versus-host.

that can be structurally defined by triplets. For instance, alloreactive T cell clones have been described that are specific for HLA-A2, -B7, or -Bw4 [19-23]; these antigens carry unique triplets: 66RKV, 177DK, and 82ALR, respectively (the numbers indicate the HLA protein sequence position and amino acids are shown with the standard 1-letter code). Although triplet matching improves outcome in renal and corneal transplantation [13,24] and reduces humoral sensitization [14-16], we must conclude that triplet matching is not useful in the selection of mismatched donors for HCT.

It should be pointed out that HLAMatchmaker considers a considerable proportion of polymorphic residues involved with peptide binding. The peptide-binding sites of class I HLA molecules comprise 32-36 amino acid residues [25-28], 25 of them are polymorphic. HLAMatchmaker includes 18 of these polymorphic residues to define triplets accessible to antibodies; polymorphisms in nonexposed sequence positions 24, 73, 95, 97, 99, 113, and 114 are not considered. Certain positions in the peptide-binding groove have been reported to be relevant for HCT. For instance, amino acid differences in sequence positions 116 and 156 have been shown to increase the risk of GVHD and patient mortality after HCT [29-31]. This structural matching algorithm includes triplets in positions 116 and 156.

At this time, the application of a structurally based HLA mismatch permissibility algorithm for HCT re-

**Figure 4.** HLA matching effect on patient DFS. (A) The 9 of 10 matches had significantly lower survival rates than the 10 of 10 matches ( $P = .001$ ). (B) Triplet matching did not increase patient survival rates.

mains elusive. Because HCT outcome is primarily affected by cellular immune mechanisms mediated by various types of T-lymphocytes and natural killer (NK) cells, the algorithm must take in account all amino acid polymorphisms of HLA. This includes the residues in the peptide-binding groove that are important in determining the repertoire of HLA bound peptides [28,32,33], the amino acids on the  $\alpha 1$  and  $\alpha 2$  helices that contact the TCR of alloreactive lymphocytes [34-37] and the HLA polymorphisms that

**Table 4.** Multivariate Analysis Comparing DFS for 10 of 10 and 9 of 10 Matches with Triplet Mismatches in the GVH Direction

Match group	N	Relative risk (95% confidence interval)	P-value
10 of 10 matches	1687	1.00	
0 Triplet mismatches	241	1.26 (1.07-1.48)	.005
1 Triplet mismatches	140	1.13 (0.91-1.39)	.263
2-3 Triplet mismatches	142	1.17 (0.95-1.43)	.147
4-5 Triplet mismatches	102	1.39 (1.10-1.75)	.005
≥6 Triplet mismatches	117	1.14 (0.91-1.42)	.264

Adjusted factors: disease, disease stage, recipient age, GVHD prophylaxis, Karnofsky score, recipient gender. The overall comparison of 10 of 10 matches versus all triplet groups was significantly different (chi-square test,  $P = .008$ ). The overall comparison of individual triplet mismatch groups found that none were significantly different from each other (chi-square test,  $P = .62$ ).

GVH indicates graft-versus-host; DFS, disease-free survival.

affect NK function [38-41]. With this complexity, the development of a permissible mismatch strategy represents a considerable challenge.

#### ACKNOWLEDGMENTS

The authors would like to thank Dr. Carolyn K. Hurley for providing advice and a critical review of the manuscript. This study was supported by Grant RO1-AI-55933 from the National Institutes of Health to Dr. Duquesnoy and funding from the National Marrow Donor Program and the Department of the Navy, Office of Naval Research Grants #N00014-99-2-0006 and #N00014-05-1-0859 to the National Marrow Donor Program. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Office of Naval Research or the National Marrow Donor Program.

#### REFERENCES

- Lee S, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576.
- Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. *N Engl J Med*. 1998;339:1177.
- Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood*. 2004;104:1923.
- Petersdorf E, Hansen J, Martin P, et al. Major histocompatibility complex class I alleles and antigens in hematopoietic cell transplantation. *N Engl J Med*. 2001;345:1794.
- Wade JA, Hurley CK, Takemoto SK, et al. HLA mismatching within or outside of cross reactive groups (CREG) is associated with similar outcomes after unrelated donor bone marrow transplant. *Blood*. 2007;109:4064.
- Risler JL, Delorme MO, Delacroix H, Henaut A. Amino acid substitutions in structurally related proteins. A pattern recognition approach. Determination of a new and efficient scoring matrix. *J Mol Biol*. 1998;204:1019.
- Elsner H-A, Blasczyk R. Sequence similarity matching: proposal of a structure-based rating system for bone marrow transplantation. *Eur J Immunogenet*. 2002;29:229-236.
- Shaw BE, Barber LD, Madrigal JA, Cleaver S, Marsh SGE. Scoring for HLA matching? A clinical test of HistoCheck. *Bone Marrow Transplant*. 2004;34:367-368.
- Kawase T, Morishima Y, Matsuo K, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. *Blood*. 2007;110:2235-2241.
- Petersdorf EW, Gooley T, Malkki M, Horowitz M, The International Histocompatibility Working Group in Hematopoietic Cell T. Clinical significance of donor-recipient HLA matching on survival after myeloablative hematopoietic cell transplantation from unrelated donors. *Tissue Antigens*. 2007; 69(Suppl 1):25.
- Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Hum Immunol*. 2002;63:339.
- Duquesnoy RJ, Marrari M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. II. Verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes. *Hum Immunol*. 2002;63:353.
- Duquesnoy RJ, Takemoto S, De Lange P, Doxiadis IIN, Schreuder GMT, Claas FHJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination III. Effect of matching at the HLA-A, B amino acid triplet level on kidney transplant survival. *Transplantation*. 2003;75:884.
- Dankers MKA, Witvliet MD, Roelen DL, et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched HLA antigens. *Transplantation*. 2004;128:1236-1239.
- Mihaylova A, Baltadjieva D, Boneva P, et al. Clinical relevance of Anti-HLA antibodies detected by flow-cytometry bead-based assays—single-center experience. *Hum Immunol*. 2006;67:787.
- Goodman R, Taylor C, O'Rourke C, Lynch A, Bradley A, Key K. Utility of HLAMatchmaker and single-antigen HLA-antibody detection beads for identification of acceptable mismatches in highly sensitised patients awaiting kidney transplantation. *Transplantation*. 2006;81:1331.
- Nambiar A, Duquesnoy RJ, Adams S, et al. HLAMatchmaker-driven analysis of response to HLA matched platelet transfusions in alloimmunized patients. *Blood*. 2006;107:1680.
- Duquesnoy RJ. "Match and Treat," an effective strategy for transplanting highly sensitized pediatric transplant candidates? (Editorial). *Pediatr Transpl*. 2007;11:3.
- Wallace LE, Houghton MA, Rickinson AB, et al. Allospecific T cell recognition of HLA-A2 antigens: evidence for group-specific and subgroup-specific epitopes. *Immunogenetics*. 1985; 21:201.
- Elliott TJ, Eisen HN. Cytotoxic T lymphocytes recognize a reconstituted class I histocompatibility antigen (HLA-A2) as an allogeneic target molecule. *Proc Natl Acad Sci USA*. 1990; 87:5213.
- van Severter GA, Huis B, Melief CJ, et al. Fine specificity of human HLA-B7-specific cytotoxic T-lymphocyte clones. I. Identification of HLA-B7 subtypes and histotopes of the HLA-B7 cross-reacting group. *Hum Immunol*. 1986;16:375.
- Smith KD, Epperson DF, Lutz CT. Alloreactive cytotoxic T-lymphocyte-defined HLA-B7 subtypes differ in peptide antigen presentation. *Immunogenetics*. 1996;43(1-2):27.
- Hiraiwa M, Yamamoto J, Matsumoto K, et al. T cell can recognize the allospecificities formed by the substitution of amino acids associated with HLA-Bw4/Bw6 public epitopes. *Hum Immunol*. 1991;32:41.
- Boehringer D, Reinhard T, Duquesnoy R, et al. Beneficial effect of matching at the HLA-A and B amino-acid triplet level on rejection free survival in penetrating keratoplasty. *Transplantation*. 2004;77:417.
- Saper M, Bjorkman P, Wiley DC. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol*. 1991;219:277.
- Smith KJ, Reid SW, Harlos K, et al. Bound water structure and polymorphic amino acids act together to allow binding of different peptides to MHC class I HLA-B53. *Immunity*. 1996;4:215.
- Fan QOR, Garboczi DN, Winter CC, Wagtmann N, Long EO, Wiley DC. Direct binding of a soluble natural killer cell inhib-

- itory receptor to a soluble human leukocyte antigen-Cw4 class I major histocompatibility complex molecule. *Proc Natl Acad Sci USA*. 1996;93:7178.
28. Doytchinova IA, Guan P, Flower DR. Identifying human MHC supertypes using bioinformatic methods. *J Immunol*. 2004;172:4314.
  29. Ferrara GB, Bacigalupo A, Lamparelli T, et al. Bone marrow transplantation from unrelated donors: the impact of mismatches with substitutions at position 116 of the human leukocyte antigen class I heavy chain. *Blood*. 2001;98:3150.
  30. Clayberger C, Rosen M, Parham P, Krensky AM. Recognition of an HLA public determinant (Bw4) by human allogeneic cytotoxic T lymphocytes. *J Immunol*. 1990;144:4172.
  31. Fleischhauer K, Kernan NA, O'Reilly RJ, et al. Bone marrow allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44. *N Engl J Med*. 1990;323:1818.
  32. Falk KO, Rotzschke O, Stefanovic S, Jung G, Rammensee HG. Allele specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature*. 1991;351:390.
  33. Sette A, Sidney J. Nine major HLA class I supertypes account for the vast preponderance of HLA-A and HLA-B polymorphism. *Immunogenetics*. 1999;50:201.
  34. Noun G, Reboul M, Abastado JP, et al. Strong alloantigenicity of the alpha-helices residues of the MHC class I molecule. *J Immunol*. 1998;161:148.
  35. Pullen JK, Tallquist MD, Melvold RW, et al. Recognition of a single amino acid change on the surface of a major transplantation antigen is in the context of self peptide. *J Immunol*. 1994;152:3445.
  36. Lombardi G, Matsui M, Moots R, et al. Limited regions of the alpha 2-domain alpha-helix control anti-A2 allorecognition: an analysis using a panel of A2 mutants. *Immunogenetics*. 1991;34:149.
  37. Smith KD, Lutz CT. Alloreactive T cell recognition of MHC Class I molecules. The T cell receptor interacts with limited regions of the MHC Class I long alpha helices. *J Immunol*. 1997;158:2805.
  38. Gagne K, Brizard G, Gueglio B, et al. Relevance of KIR gene polymorphisms in bone marrow transplantation outcome. *Hum Immunol*. 2002;63:271.
  39. Shilling HG, Young N, Guethlein LA, et al. Genetic control of human NK cell repertoire. *J Immunol*. 2002;169:239.
  40. Charron D. Immunogenomics of hematopoietic stem cell transplantation. *Transfus Clin Biol*. 2003;10:156.
  41. Hsu KC, Pinto-Agnello C, Gooley T, et al. Hematopoietic stem cell transplantation: killer immunoglobulin-like receptor component. *Tissue Antigens*. 2007;69(Suppl 1):42.