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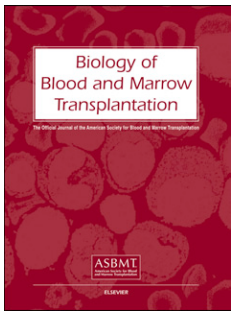

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PII: S1083-8791(12)00286-8

DOI: 10.1016/j.bbmt.2012.07.010

Reference: YBBMT 52842

To appear in: *Biology of Blood and Marrow Transplantation*

Received Date: 9 May 2012

Accepted Date: 10 July 2012

Please cite this article as: Rocha V, Spellman S, Zhang MJ, Ruggeri A, Purtill D, Brady C, Baxter-Lowe LA, Baudoux E, Bergamaschi P, Chow R, Freed B, Koegler G, Kurtzberg J, Larghero J, Lecchi L, Nagler A, Navarette C, Prasad V, Pouthier F, Price T, Ratanatharathorn V, van Rood JJ, Horowitz MM, Gluckman E, Eapen M, Effect of HLA-matching recipients to donor non-inherited maternal antigens on outcomes after mismatched umbilical cord blood transplantation for hematologic malignancy, *Biology of Blood and Marrow Transplantation* (2012), doi: 10.1016/j.bbmt.2012.07.010.

Effect of HLA-matching recipients to donor non-inherited maternal antigens on outcomes after mismatched umbilical cord blood transplantation for hematologic malignancy

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Short title: Effect of matching at NIMA and UCB transplantation

Key words: cord blood transplant, non-inherited maternal antigen, permissive mismatch

ABSTRACT

Transplant-related mortality (TRM) after HLA-mismatched umbilical cord blood (UCB) transplantation is high. In-utero exposure to the non-inherited maternal antigen (NIMA) is recognized by the fetus, which induces T regulator cells to that haplotype. It is plausible UCB transplantations where recipients are matched to donor NIMAs may alleviate some of the excess mortality associated with this treatment. To explore this further, we used marginal matched-pair Cox regression analysis to compare outcomes after 48 UCB transplantations that were NIMA-matched (i.e., the NIMA of the donor UCB unit was matched to the patient) to 116 transplantations that were not NIMA-matched. All patients had hematologic malignancies and received a single UCB unit. Cases and controls were matched on age, disease, disease status, transplant-conditioning regimen, HLA-match and infused cell dose. TRM was lower after NIMA-matched compared to NIMA-mismatched transplantations (relative risk=0.48, p=0.05; 18% vs. 32% at 5 years after transplantation). Consequently, overall survival was higher after NIMA-matched transplantations. The 5-year probabilities of overall survival after NIMA-matched and NIMA-mismatched transplantations were 55% and 38%, respectively (p=0.04). When faced with the choice of multiple HLA-mismatched UCB units containing adequate cell dose, selecting a NIMA-matched UCB unit may improve survival after mismatched UCB transplantation.

INTRODUCTION

Umbilical cord blood (UCB) is an acceptable graft choice when considering unrelated donor transplantation for patients with hematologic malignancies. In the United States, UCB grafts are used for about 20% of unrelated donor transplantations for hematologic malignancies and in Europe, for about 12%. We and others have shown similar leukemia-free survival despite higher transplant-related mortality (TRM) after transplantation of UCB compared to transplantation of HLA-matched bone marrow or peripheral blood progenitor cells from unrelated adult donors in children and adults with leukemia.[1,2] High TRM after UCB transplantation remains a significant limitation and can be attributed to multiple factors. Some of the excess TRM after UCB transplantation results from infusion of units containing a relatively low total nucleated cell (TNC) dose. The accepted standard now is to use a UCB unit that contains a minimum pre-cryopreserved TNC of 3 x 10^7/kilogram patient body weight and others recommend an incremental increase in TNC to overcome the HLA-barrier.[3,4] When such a unit is lacking, the co-infusion of two un-manipulated UCB units is used to deliver higher TNC doses.[4,5] Infusion of expanded hematopoietic progenitor cells with a single UCB unit is also employed to deliver higher TNC doses.[6,7] Avoiding UCB units to which donor specific anti-HLA antibodies are present in the recipient lowers graft failure and mortality risks.[8-10] The importance of better donor-recipient HLA-matching for unrelated adult donor transplantation is clear.[11] Best results are obtained with an unrelated adult donor allele-matched to the recipient at HLA-A, -B, -C and –DRB1. Matching the UCB unit to the recipient at the HLA-C locus is associated with lower TRM.[12] The role of allelic

HLA-matching at HLA-A, -B and –C remains to be determined in the setting of UCB transplantation.

Two independent clinical studies done a decade apart,[13,14] observed tolerance to non-inherited maternal antigens (NIMA) in renal transplant recipients implying that fetal exposure to NIMAs may promote lasting tolerance in humans. As the fetal immune system develops, T cells develop tolerance to self-antigens and recognize and react against foreign antigens. The placental circulation permits crossing of maternal cells to the fetus and vice-versa. In a recent report, Mold and colleagues showed that the human fetal immune system generate regulatory T cells ($CD4+CD25^{high}FoxP3+$ T_{regs}) that suppress fetal immune responses to maternal antigens and that this tolerance persists at least until early adulthood.[15] In a recent report from the New York Blood Center[16] HLA-mismatched UCB transplantations where the mismatched antigen in the recipient matched the NIMA of the UCB donor (NIMA-matched transplant) were associated with higher neutrophil recovery and lower mortality rates. Yet, in another recent report from the same group,[17] NIMA-matched transplants were not associated with transplant-related or overall mortality even though both analyses were performed on largely the same cohort of donor-recipient pairs. As the majority of UCB transplants are mismatched and TRM a barrier to successful outcome, the current analysis was undertaken in an independent cohort of patients to determine whether matching the recipient to the UCB's non-inherited maternal antigen (i.e., NIMA) as reported by van Rood and colleagues[16] would indeed lower some of the excess mortality associated with mismatched UCB transplants.

METHODS

Data Collection

The study includes patients reported to Eurocord-European Group for Blood and Marrow Transplantation and the Center for International Blood and Marrow Transplant Research. To be eligible, UCB unit HLA-typing, UCB donor's maternal HLA-typing or maternal sample and recipient HLA-typing had to be available. Seven Netcord Banks in Europe, and ten Cord Blood Banks in the National Marrow Donor Program network, in the U.S., provided UCB units. Data for transplantations in Europe were obtained from the Eurocord and in the United States, from the Center for International Blood and Marrow Transplant Research. All patients received a single unrelated umbilical cord unit for treatment of leukemia, lymphoma or myelodysplastic syndrome (MDS). Patients who received UCB units that were matched at HLA-A, -B and –DRB1, co-infusion of two units or expanded units were excluded. All patients (or their guardians) provided written consent for research. The Institutional Review Boards of the Medical College of Wisconsin, the Eurocord-Netcord scientific committee and the National Marrow Donor Program approved this study.

HLA typing and match assignment

Donor, donor maternal and recipient HLA-typing considered matching at HLA-A, -B and –DRB1. Donor-recipient match grades were assigned considering HLA-A and -B at intermediate resolution (antigen level) and -DRB1 at high resolution (allele-level). For transplantations facilitated by the Netcord banks, maternal HLA typings were available from the banks. For transplantations facilitated by the National Marrow Donor Program, maternal HLA typings were obtained from the banks when available or HLA typing of

banked maternal samples were performed at a centralized laboratory using DNA-based methods. Maternal HLA typing was scored at intermediate resolution (antigen-level) for HLA-A and –B and at high resolution (allele-level) for HLA-DRB1. Assignment of transplantations as NIMA-matched or NIMA-mismatched, were ascertained by reviewing recipient, donor and donor maternal HLA typing at HLA-A, -B and –DRB1. Transplants were assigned as NIMA-matched when the mismatched antigen of the recipient was matched to the non-inherited maternal antigen of the UCB donor (NIMA-matched transplant). Transplants were assigned as NIMA-mismatched when the mismatched antigen of the recipient was not matched to the non-inherited maternal antigen of the UCB donor. Examples of NIMA-match and mismatch are shown in Table 1.

Outcomes

TRM was defined as the time from transplantation to death not related to disease recurrence or progression and overall mortality defined as death from any cause. Neutrophil recovery was defined as achieving an absolute neutrophil count $\geq 0.5 \times 10^9$/L for three consecutive measurements on different days; grade 2-4 acute[18] and chronic[19] graft-versus-host disease (GVHD) based on reports using standard criteria from each transplant center; disease recurrence based on morphological evaluation supported by reappearance of abnormalities in cytogenetic or molecular analyses.

Statistical methods

The probabilities of TRM, recurrent disease, neutrophil recovery and acute and chronic GVHD were calculated using the cumulative incidence estimator to accommodate competing risks.[20] The probability of overall survival was calculated using the Kaplan-

Meier estimator.[20] 95% confidence intervals (CI) were calculated with log transformation.

To assess the association between clinical outcomes and NIMA matching, cases (NIMA-matched) were matched to controls (NIMA-mismatched). A matched-pair analysis was considered appropriate given the relatively low frequency of NIMA-matched transplantations (8.5%). Prior to matching cases to controls, we built multivariate Cox regression model for TRM using patients that met the study eligibility (n=508).[21] Results are expressed as relative risk (RR). The characteristics of this cohort are shown in Supplemental Table 1; 52 donor-recipient pairs were NIMA-matched and 456, NIMA-mismatched. We aimed to identify variables, other than NIMA matching, with a significant effect on TRM: patient age, donor-recipient HLA-match, disease status at transplantation and transplant conditioning regimen were significantly associated with TRM (Supplemental Table 2). Cases were matched to controls for patient age, HLA-match, disease status and conditioning regimen and two other variables (disease type and total nucleated cell dose [TNC] $\leq 3 \times 10^7$/kg vs. $>3 \times 10^7$/kg), known to be frequently associated with UCB transplantation outcomes.

The final study population included 48 NIMA-matched and 116 NIMA-mismatched transplant recipients. Nineteen cases were matched to 76 controls (1:4), 1 case was matched to 3 controls (1:3), 9 cases were matched to 18 controls (1:2) and 19 cases were matched to 19 controls (1:1). To assess the association between clinical outcomes and NIMA match status, using matched-pairs we built marginal Cox regression models for neutrophil recovery, acute and chronic GVHD, TRM disease recurrence and overall mortality.[21] Models were built with the forward stepwise selection procedure and

confirmed with the use of backward selection procedure. All variables met the proportional hazards assumption. P-values are two-sided and values of 0.05 or less were considered statistically significant. Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

The frequencies of NIMA-matched and NIMA-mismatched antigens in U.S. transplantations were evaluated by comparing the average antigen (HLA-A, -B) and allele (HLA-DRB1) frequencies within the overall population. HLA frequencies for the U.S. donor population provided by the NMDP were used as a reference. NIMA-matched and mismatched antigens/alleles were aggregated by locus and average frequency compared between the NIMA-matched and mismatched groups by two-sided T-test. Analyses were performed for the overall population and the Caucasian subset.

RESULTS

Patient, disease and transplant characteristics

Characteristics of cases and controls are shown in Table 2. Seventy-five percent of patients were 16 years or younger at transplantation, 52%, male sex and 46% were CMV seropositive. Acute leukemia was the most common indication for transplantation and 74% of transplantations occurred in remission. TBI-containing myeloablative conditioning regimens were used for 82% of transplantations and cyclosporine alone or in combination with steroids, methotrexate or mycophenolate mofetil for GVHD prophylaxis for 86%. Thirty-five percent of transplantations were mismatched at one and the remainder at two HLA-loci. All transplantation occurred in 2002 – 2009. Fifty percent of NIMA-matched and 42% of NIMA-mismatched transplants occurred between 2002 and 2005 and the remainder between 2006 and 2009. Seventy-five percent of

recipients received TNC >3 x 10^7/kg. The median follow-up of surviving patients was 42 months (range 3 – 103) after NIMA-matched and 36 months (3 – 93) after NIMA-mismatched transplantations.

Neutrophil recovery

Neutrophil recovery after NIMA-matched and NIMA-mismatched transplantations was not different (RR 1.18 95% CI 0.80 – 1.74, p=0.42). The median time to recovery was 20 and 23 days after transplantation of NIMA-matched and NIMA-mismatched units. The corresponding day-28 probabilities of recovery were 71% (95% CI 57% – 81%) and 59% (95% CI 50% – 67%).

Acute and chronic graft-versus-host disease

Risks of grade 2-4 acute GVHD (RR 0.94 95% CI 0.56 – 1.59, p=0.82) and chronic GVHD (RR 0.85 95% CI 0.44 – 1.63, p=0.61) were not different after NIMA-matched and NIMA-mismatched transplantations. The day-100 probabilities of grade 2-4 acute GVHD after NIMA-matched and NIMA-mismatched transplantations were 40% (95% CI 26% – 53%) and 46% (95% CI 37% – 54%), respectively. The corresponding 5-year probabilities of chronic GVHD were 26% (95% CI 15% – 39%) and 27% (95% CI 19% – 35%).

Transplant-related and overall mortality

The risk of TRM was lower after NIMA-matched compared to NIMA-mismatched transplantations (RR 0.48 95% CI 0.23 – 1.01, p=0.05; Figure 1A). Similarly, overall mortality risks were also lower after NIMA-matched compared to NIMA-mismatched transplantations (RR 0.61 95% CI 0.38 – 0.98, p=0.04; Figure 1B). Data on infections that occurred in the first 100 days after transplantation were available for 113 of 164

(69%) transplants. The day-30 cumulative incidence of infections was 15% and 27% after NIMA-matched and NIMA-mismatched transplants (p=0.24). The corresponding day-100 cumulative incidence at day-100 was 48% and 50%. Six of 20 (30%) deaths after NIMA-matched transplantations were attributed to TRM. Most of these deaths (4 of 6) occurred within 6 months after transplantation (n=2 multi-organ failure, n=1 infection, n=1 hemorrhage). Two deaths occurred beyond 6 months, one from infection and the other from chronic GVHD. Thirty-one of 66 (47%) deaths after NIMA-mismatched transplantations were attributed to TRM. Twenty-three of the 31 deaths occurred within 6 months after transplantation (n=6 multi-organ failure, n=8 infection, n=4 adult respiratory distress syndrome/interstitial pneumonitis, n=2 diffuse alveolar hemorrhage, n=1 Epstein Barr virus post-transplant lymphoproliferative disease; cause of death was not reported for two patients). Eight deaths occurred beyond 6 months (n=2 chronic GVHD, n=3 infection, n=2 multi-organ failure; cause of death was not reported for 1 patient).

Relapse

Relapse risks after NIMA-matched and NIMA-mismatched transplants were not different (RR 0.82 95% CI 0.47 – 1.43, p=0.47). The 5-year probabilities of relapse were 31% (95% CI 18% – 44%) after NIMA-matched transplantations and 33% (95% CI 24% – 42%) after NIMA-mismatched transplantations.

The influence of antigen frequency on NIMA matching

The frequencies of NIMA-matched and NIMA-mismatched antigens/alleles were evaluated for the U.S cohort (N=429). Transplantations in Europe were excluded because the race of cord blood units and recipients were not always available. The

antigen (HLA-A, B) and allele (HLA-DRB1) frequencies observed on the NMDP's donor registry served as the reference for the population and were adjusted based on subject race. Overall, NIMA-matched antigens/alleles had higher population based frequencies than NIMA-mismatched antigens/alleles (NIMA-matched 0.110 vs. NIMA-mismatched 0.052, $p<0.001$). The NIMA matches were all associated with relatively common HLA antigens (frequencies >0.058) while the NIMA mismatched antigens were observed across common and uncommon HLA antigens. The most frequent NIMA-match (N=6, 22%) was at HLA-A*02, which is also the most common antigen in the U.S. Caucasian population (frequency = 0.308).[22] To ensure that HLA-A*02 was not inordinately influencing these results, we repeated the analysis restricting the population to non-HLA-A*02 mismatches. Consistent with the main analysis, non-HLA-A*02 NIMA-matched antigens had higher frequencies compared to NIMA-mismatched antigens (NIMA-matched 0.107 vs. NIMA-mismatched 0.054, $p=0.008$).

DISCUSSION

Our primary objective was to assess the effect of tolerance to NIMA and its effect on mortality after HLA-mismatched UCB transplantation. Tolerance to NIMA in renal transplantation is well documented.[13,14] In contrast, tolerance to NIMA and its effect on survival after mismatched UCB transplant is by no means conclusive.[16,17] To circumvent the relatively small sample of NIMA-matched transplantations we adopted a matched-pair analysis, matching recipients for factors that influence TRM and overall survival, which allowed us to perform a carefully controlled analysis. We observed marginally lower TRM and overall mortality after NIMA-matched compared to NIMA-mismatched transplantations. This is consistent with the earlier report on the impact of NIMAs in

UCB transplantation.[16] We hypothesize, allowing for permissive mismatching between UCB unit and the recipient lowered some of the excess mortality associated with HLA-mismatched UCB transplantation. But the exact mechanism by which mortality is reduced is not easily explained. Higher survival after NIMA-matched transplants is likely to have been mediated by multiple factors such as better hematopoietic recovery, lower acute GVHD, lower rate of infections in the early post-transplant period and better immune reconstitution which together contributed to the observed survival advantage.

Unlike reports after haplo-identical transplantations,[23] we failed to see significant differences in acute or chronic GVHD rates after NIMA-matched and NIMA-mismatched UCB transplantations. Acute and chronic GVHD rates after UCB transplants are substantially lower than after haplo-identical transplants; as only about 10% of mismatched UCB transplants are NIMA-matched, several hundreds of patients are needed before we can conclude whether there are differences in GVHD rates after NIMA-matched and NIMA–mismatched transplantations.

Better HLA-matching of donors and recipients is associated with better hematopoietic recovery and consequently lower early mortality. In the current analysis, there is a 12% difference in the probability of neutrophil recovery after NIMA-matched and NIMA-mismatched transplants. Our inability to detect a statistically significant difference can be explained by the small sample size and the ensuing wide confidence intervals of probability estimates. Further, the use of UCB units with relatively high TNC (>3 x 10^7/kg) may have also lessened the importance of NIMA-matching for neutrophil recovery as has been shown to be the case with HLA-mismatched UCB transplants.[3,24] Consistent with our findings, reports on tolerance to NIMAs after haplo-identical

transplantations where very high cell doses are used have also failed to show an association between NIMA-matching and neutrophil recovery.[23,25] It is noteworthy that most deaths from transplant-related complications occurred within six months from transplantation. There may be differences in immune reconstitution after NIMA-matched and NIMA-mismatched transplants, a hypothesis we cannot test in this population. We used data reported to transplant registries and data on immune reconstitution are not available.

NIMA-matched transplants account for less than 10% of UCB transplants. Incorporating NIMA-matching in an algorithm for UCB unit selection is complex and logistically challenging. As maternal HLA-typing is not listed for banked UCB units, NIMA-matched transplants are more likely to occur randomly than by choice. However, about a third of the cord banks in Netcord / National Marrow Donor Program routinely perform UCB unit maternal HLA typing and listing maternal and UCB unit HLA typing will allow physicians select UCB units that are NIMA-matched to the recipient. Further, based on our observations of the HLA types within the U.S. study cohort, NIMA-matching correlated with the frequency of mismatched antigens within the U.S. population. Therefore, searching for a NIMA-matched UCB unit would best be facilitated by selecting a mismatched UCB unit where the mismatch is a high frequency HLA-antigen within the target population, such as HLA A*02 in Caucasians.[21] However, to truly understand the probability of finding a NIMA-match within a given population will require either complex mathematical models based on HLA haplotype frequencies or the addition of maternal HLA typing to UCB unit registries. Consultation with an HLA expert at search may allow physicians apply the surrogate approach described above to

identify a potential NIMA-matched UCB unit and request donor maternal HLA typing at time of confirmatory HLA typing of the UCB unit. The additional request for maternal HLA typing to those banks that do not routinely perform maternal HLA typing will add to their financial burden.

Taken together, the current analysis and the earlier report[16] suggest selecting units where the recipient is matched to the donor NIMA may ameliorate some of the excess mortality associated with HLA-mismatched UCB transplantations. But both reports are limited by modest numbers of NIMA-matched transplants. Although we performed a carefully controlled analysis that considered risk factors associated with higher mortality risks, there are several unknown and unmeasured factors that may have also influenced survival after UCB transplantation. Nevertheless, the marginal survival advantage associated with NIMA-matched transplants cannot be ignored. Therefore, when considering mismatched UCB transplant for hematologic malignancy, efforts to obtain donor maternal HLA typing from cord blood banks are encouraged. Matching recipients to donor non-inherited maternal antigen must be considered along with other known factors associated with lowering mortality risks.

Conflict of Interest The authors declare none**.**

Contributors

VR, SS, MJZ and ME contributed equally to study design and interpretation of data. VR and ME had primary responsibility for drafting the manuscript. MJZ did the statistical analysis. SS reviewed maternal HLA typing, donor-recipient HLA typing and assignment and contributed to interpretation of data and manuscript preparation. AR, DP, CB, LBL, EB, PB, RC, BF, GK, JK, JL, LL, AN, CN, VP, FP, JTP, VR, JJR, MMH and EG contributed to interpretation of data and approved the final report.

Acknowledgements

Supported by a Public Health Service grant (U24-CA76518) from the National Cancer Institute, the National Heart Lung and Blood Institute and the National Institute of Allergy and Infectious Diseases, Heath Resources and Services Administration (HHSH234200637015C), the Office of Naval Research, Department of Navy to the National Marrow Donor Program (N00014-10-01-0204). Mary Eapen is a Scholar in Clinical Research, the Leukemia and Lymphoma Society. Opinions, findings, and conclusions or recommendations expressed herein are those of the authors and do not reflect the views of the Office of Naval Research or the National Marrow Donor Program.

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Table 1. Examples of NIMA matching (A) and NIMA mismatching (B) in the setting of a single locus mismatched umbilical cord blood transplants.

A. NIMA matched: HLA-A *24 is not carried by UCB donor. HLA-A *24 is carried by the UCB donor's mother and the recipient. Therefore this is a NIMA-matched transplant.

	HLA-A	HLA-B	HLA-DRB1
UCB unit / donor	A*02, 32	B*18, 35	DRB1*01:01, 11:04
UCB donor mother	A***24**, 32	B*07, 35	DRB1*01:01, 13:01
Recipient	A*02, **24**	B*18, 35	DRB1*01:01, 11:04

B. NIMA mismatched: HLA-A *01 is not carried by UCB donor or the UCB donor's mother. Therefore this is a NIMA-mismatched transplant.

	HLA-A	HLA-B	HLA-DRB1
UCB unit / donor	A*02, 32	B*18, 35	DRB1*01:01, 11:04
UCB donor mother	A*24, 32	B*07, 35	DRB1*01:01, 13:01
Recipient	A*01, 02	B*18, 35	DRB1*01:01, 11:04

Table 2. Patient, disease and transplant characteristics of the study population

Variables	NIMA-mismatched transplantations	NIMA-matched transplantations
Number	116	48
Region		
Europe	37 (32%)	27 (56%)
United States	79 (68%)	21 (44%)
Age		
≤16 years	91 (78%)	30 (62%)
>16 years	25 (22%)	18 (38%)
Disease		
Acute myeloid leukemia	48 (41%)	21 (44%)
Acute lymphoblastic leukemia	47 (41%)	18 (38%)
Chronic myeloid leukemia	1 (1%)	1 (2%)
Myelodysplastic syndrome	10 (9%)	4 (8%)
Other acute leukemia	8 (7%)	3 (6%)
Non-Hodgkin lymphoma	2 (2%)	1 (2%)
Disease status		
1st complete remission/chronic phase	31 (27%)	12 (25%)
2nd complete remission/chronic phase/accelerated phase	56 (48%)	22 (46%)
Relapse, refractory anemia with excess blasts	29 (25%)	14 (29%)
Conditioning regimen		
Myeloablative		
Total body irradiation containing	58 (50%)	19 (40%)
Non-irradiation containing	43 (37%)	15 (31%)
Reduced intensity		
Total body irradiation containing	13 (11%)	10 (20%)
Non-irradiation containing	2 (1%)	4 (8%)
Infused total nucleated cell dose		
≤3 x 10^7/kg recipient body weight	25 (22%)	16 (33%)
>3 x 10^7/kg recipient body weight	91 (78%)	32 (67%)
GVHD prophylaxis		
Cyclosporine alone or with steroids	56 (48%)	23 (48%)
Cyclosporine + methotrexate	12 (10%)	5 (10%)
Cyclosporine + mycophenolate mofetil	31 (27%)	14 (29%)
Tacrolimus + methotrexate	7 (6%)	3 (6%)
Tacrolimus + mycophenolate mofetil	5 (4%)	1 (2%)
Tacrolimus alone	5 (4%)	2 (4%)
Donor-recipient HLA match		
5/6 HLA match	43 (36%)	14 (27%)
4/6 HLA match	73 (63%)	34 (71%)

FIGURE LEGEND

Figure 1A: The 5-year probabilities of transplant-related mortality are 18% (95% CI 8% – 29%) after NIMA-matched and 32% (95% CI 23% – 41%) after NIMA-mismatched transplantations.

Figure 1B: The 5-year probabilities of overall survival are 55% (95% CI 40% – 69%) after NIMA-matched and 38% (95% CI 29% – 48%) after NIMA-mismatched transplantations.

Figure 1A

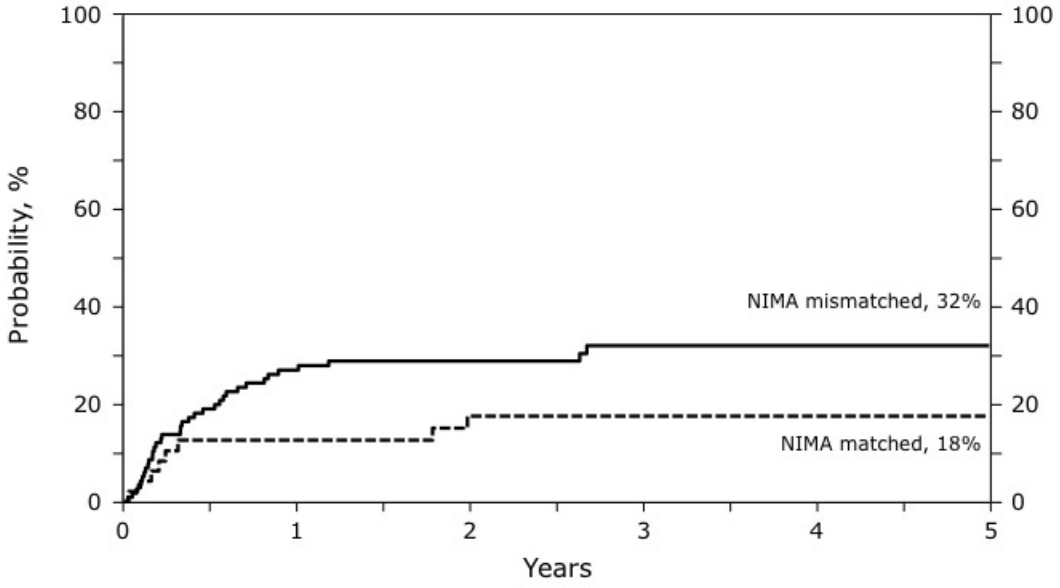

Figure 1B