A Phase 2 Trial of KIR Mismatched Unrelated Donor Transplantation Using In Vivo T-cell Depletion with ATG in AML: Children’s Oncology Group AAML05P1 Study

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Abstract

AML patients receiving killer immunoglobulin-like receptor (KIR) mismatched haploidentical HSCT have improved survival. COG AAML05P1 is a prospective phase 2 trial of unrelated donor (URD) HSCT in which KIR typing of donors was available to the treating physician at donor selection, aiming to determine feasibility (defined as the ability to obtain donor samples from unrelated donors and perform and return KIR data before transplant) of prospective selection of

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Authorship Contributions

SMD, RI and WL designed the study, analyzed data, wrote and approved the manuscript. TA, YW and RG developed datasets, performed statistical analyses and edited and approved the paper. SS, EAK, SM, PJO, LJB and SS analyzed data, edited and approved the manuscript.

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Conflict of Interest Statement

SMD has served as a consultant for Novartis and has received research support from Alexion and Prolacta; WL is currently a part-time employee of Miltenyi Biotec. RI was a full-time employee of Merck and Co., Inc. and AstraZeneca during study conduct, and is currently a full-time employee of Jazz Pharmaceuticals.
KIR mismatched donors and effect on outcomes. The study accrued 90 evaluable patients. Patients ≤30 years old with high risk AML at presentation or relapsed AML were eligible. After enrollment as many as 5 potential URD samples were KIR typed (including gene expression) in a central laboratory and results reported to the treating physician, who made the final donor selection.

Cases were categorized as KIR matched or mismatched using different published strategies. Overall survival, disease-free survival (DFS), and relapse did not differ significantly by KIR mismatch. Acute GVHD was significantly lower in recipients of KIR mismatched stem cells (35% vs 60%, p= 0.027). We examined DFS according to time to NK-receptor recovery after HSCT. NKp44 recovery was significantly associated with KIR mismatch and with decreased DFS and increased relapse risk in multivariate Cox analysis (p= 0.006 and 0.009, respectively). We show that prospective selection of URD according to KIR type was feasible, acute GVHD was reduced, but survival did not differ using any model of KIR mismatch. The study enrolled mostly matched transplants, however, so ligand-ligand mismatch was rare and therefore sample size was insufficient to determine potential benefit according to this model. Cord blood recipients demonstrated a trend towards improved DFS with KIR mismatch, but the study was not powered to detect a difference in this small subset of patients. Our data suggest that recovery of NK receptor expression might influence DFS after HSCT.

Introduction.

KIR genes participate in regulation of NK cell function and mismatch between transplant donor and recipient improves clinical outcomes in some transplant settings. Lower than expected rates of relapse, graft failure and graft-versus-host disease (GVHD) occur in KIR-incompatible, T cell-depleted, HLA-mismatched related donor hematopoietic stem cell transplant (HSCT) for AML. Similarly, Hsu et al. reported superior overall survival and disease free survival for recipients of KIR incompatible versus KIR compatible SCT for AML and MDS with HLA-identical sibling donors. In contrast, a retrospective analysis of KIR-incompatible but HLA-A, B and DRB1 matched unrelated donor SCT at the University of Minnesota did not demonstrate a reduction in relapse or improvement in survival. These results contrasted those of another retrospective unrelated donor study by Giebel et al., which reported improvement in overall survival (OS), disease free survival (DFS), transplant-related mortality (TRM) and relapse among AML patients who received KIR incompatible unrelated donor transplants. The authors concluded that the difference in results might be attributed to T cell-depletion from lymphocyte immune globulin, which was not used in the patients reported by the Minnesota group. Lymphocyte immune globulin causes T cell-depletion in the donor graft, perhaps allowing donor NK cells to expand more effectively and kill leukemia targets with less interference from donor T cells that are capable of causing a non-specific graft-versus host response. In fact, a study by Lowe et al. demonstrated that T-cell alloreactivity dominates NK cell alloreactivity in minimally T cell-depleted HLA-non-identical SCT.

Another potential limitation to prior retrospective analyses is that KIR typing was inferred from prior typing of the expected KIR ligands, namely, patient and donor HLA-C and B (ligand-ligand, L-L, method). In some transplant settings, directly typing the KIR on donor cells may provide more accurate information about the potential for graft-versus-host reaction and graft-versus-leukemia effect.
cells and its ligand on recipient cells (receptor-ligand, R-L, method) is a more accurate measure of incompatibility\(^{10}\). This difference in methodology could also contribute to explain the discrepancy in results noted in previously reported studies because misclassification based on retrospective ligand-ligand typing would result in bias toward no beneficial effect of KIR incompatibility. Furthermore, donor KIR haplotype may be a confounder, as some studies have shown that donor B haplotypes are associated with better transplant outcome when compared to A haplotypes\(^{11,12}\).

There is considerable interest in understanding potential benefits of NK cell KIR incompatibility in unrelated donor HSCT for patients without a suitably HLA-matched relative, potentially improving disease control and reducing GVHD in many more high risk AML patients. The feasibility of prospectively performing KIR typing in addition to HLA-typing in an unrelated donor setting has not previously been demonstrated.

In this prospective co-operative group trial we tested the feasibility of obtaining KIR typing in addition to HLA typing for selection of unrelated donors, in collaboration with the National Marrow Donor Program. Moreover, we tested the use of in vivo T-cell depletion with anti-lymphocyte globulin to optimize the effects of KIR mismatch in the unrelated donor setting. Multiple strategies were used to define KIR mismatch, and NK cell recovery and receptor acquisition post-transplant was measured.

**Methods**

**Transplant Recipient Eligibility**

Children and young adults aged more than one month and less than 30 years old scheduled for unrelated donor HSCT for high risk myeloid malignancies were eligible to enroll in the study. High risk myeloid malignancies included patients with primary refractory AML, defined as ≥ 5% bone marrow blasts after two induction cycles of chemotherapy, or patients with AML or MDS with −5/5q-or monosomy 7 without inv(16)/t(16;16) or t(8:21) cytogenetics or NPM or CEBP\(\alpha\) mutations, or patients with relapsed AML (≥ 5% bone marrow blasts) who meet the customary WHO criteria for AML, or patients with AML and high FLT3 internal tandem duplication allelic ratio (high FLT3-ITD AR), defined as greater than 0.4, or all cases of therapy-related AML, patients without the above high risk criteria who have evidence of residual AML (≥0.1%) at the end of Induction, or if a minimal residual disease test is not performed, then with > 15% bone marrow blasts by morphology after one induction cycle of chemotherapy. Patients with Fanconi anemia were not eligible to enroll.

**Transplant Recipient Demographics**

Demographics of the transplant recipients are shown in Table 1. The majority of children received a bone marrow graft from an unrelated donor, and most were older than one year of age. The median (range) duration of follow-up for patients alive at last contact is 2.38 yrs (0.99 – 5.13 yrs).
**Donor Identification**

The transplant recipient was enrolled on study when a search for an unrelated donor was initiated. High-resolution HLA-typing on the patient was submitted to the NMDP for a preliminary search and results of this search were reported to the transplant physician, according to usual practice. The transplant physician selected several potential donors from the preliminary search for confirmatory typing/testing, according to his or her own discretion. Blood samples from potential donors were drawn for confirmatory HLA typing and infectious disease testing by the NMDP, together with additional samples for KIR typing, which were sent to a central laboratory at St Jude Children’s Research Hospital. KIR typing was performed on up to 5 potential donors per recipient, and data were reported to the treating physician. The treating physician selected the donor used for transplant according to their usual practice. In cases where an umbilical cord blood graft (UCB) was used, KIR compatibility was determined after transplant using the patient’s pre-transplant typing and KIR typing from blood samples taken after donor engraftment, to avoid compromising the number of UCB stem cells available for transplant.

**Transplant Strategy**

A uniform transplant conditioning regimen of busulfan (0.8–1.0mg/kg, for a total of 16 doses given intravenously every 6 hours over 4 days), cyclophosphamide (50mg/kg/dose given intravenously, a total of 4 doses over 4 days) and equine anti-thymocyte globulin (Atgam) (30 mg/kg/dose for 3 doses over 3 days) was used. Stem cells were infused without ex vivo T-cell depletion. A calcineurin inhibitor and four doses of methotrexate were given as GVHD prophylaxis.

**Statistical Methods**

Data from AAML05P1 were current as of December 31, 2015. The Kaplan-Meier method was used to estimate OS (defined as time from study entry to death) and EFS (time from study entry until failure to achieve CR during consolidation, relapse, or death)\(^{13}\). Relapse risk (RR) was calculated by cumulative incidence (CI) methods defined as time from date of transplant to relapse or death where deaths without a relapse were considered competing events. Patients who have ≥5% leukemic cells, with or without extra-medullary disease (EMD), after a CR marrow has been documented, or evidence of EMD following a complete remission, were considered to have relapsed after stem cell transplant. Incidence of acute GVHD (AGVHD) was calculated by the CI method where death and relapse were considered competing events. Incidence of reconstitution was also calculated by CI method where relapse and death were considered as competing events. The pattern of NK-cell receptor expression after HCT is considered to reconstitute to the donor-specific level when the difference in quantity for each receptor is within 50% of the average of the sample obtained from the donor pre-HCT and from the recipient post-HCT (ie if (donor-recipient) \( \div \) \( \frac{1}{2} \) (donor+recipient) is <0.5\(^{14,15}\). The significance of predictor variables was tested with the log-rank statistic\(^{16}\) for OS, EFS and with Gray’s statistic\(^{17}\) for RR, acute GVHD incidence, and incidence of reconstitution. All estimates are reported with two times the Greenwood standard errors. Multivariate Cox regression was used to estimate the hazard ratio (HR) adjusting for covariates. Children lost to follow-up were censored at their date of last known
contact. The chi-squared test was used to test the significance of observed differences in proportions, and Fisher’s exact test was used when data were sparse. Differences in medians were compared by the Mann-Whitney or Wilcoxon signed-rank tests as appropriate. A p-value <0.05 was considered statistically significant.

This study was designed to have 80% power to detect a KIR-compatibility associated HR of approximately 3.5 in overall survival with two-sided 0.05 type 1 error in a multivariate Cox regression analysis for overall survival. Monitoring for excessive non-relapse mortality for patients receiving KIR mismatched transplants relative to those receiving KIR matched transplants utilized monitoring based on the Lan-DeMets criterion with $\alpha$-spending function $\alpha_t$ (truncated at 3 standard deviations) and 2.5% type I error. Formal monitoring analyses were performed every 12 months.

KIR Typing and Definition

KIR typing was performed using PCR-SSP and flow cytometry. An NK-alloreactive donor was defined by using the receptor-ligand mismatch model (i.e., the recipients’ cells lack the ligand for the inhibitory KIRs expressed on the donor’s NK cells)\(^\text{10}\). We used the specific residues of HLA-C (Asn80/Lys80) and HLA B (Arg83) to determine the presence of ligand of inhibitory KIR, as described in reference \(^\text{18}\). Additionally, cases were classified as matched or mismatched according to a ligand-ligand or missing ligand strategy, or according to centromeric or telomeric haplotypes \(^\text{5,18–19}\).

Samples Available for NK-cell Reconstitution and Receptor Acquisition Studies

Subjects were asked to submit blood samples 1, 3, 6 and 12 months after transplant for evaluation of NK-cell reconstitution and receptor acquisition. Flow cytometry was used to evaluate the absolute number of CD3– CD56+ NK cells per mL of blood and the expression of the following receptors: KIRs, NKG2A, NCRs, NKG2D, DNAM-1, 2B4, and NTBA. Fifty-one samples were analyzed for time-point at one month, 40 at three months, 32 at six months and 26 at one year after transplant.

Results

Donor Selection and Feasibility

A primary goal of this study was to determine the feasibility of selecting KIR mismatched donors in an unrelated donor setting. Feasibility was defined as the ability to obtain donor samples from unrelated donors and perform and return KIR data before transplant. The study was performed in collaboration with the National Marrow Donor Program (NMDP), who supervised donor center IRB approval and facilitated the collection and shipping of samples from potential donors to the central KIR typing laboratory. Transplanting physicians had the options of requesting as many as 5 potential donors for KIR typing. The median number of donor samples submitted for each recipient was two (range 1–7). A total of 158 patients were enrolled. Sixty-eight potential recipients failed to proceed to HSCT per study guidelines for a variety of reasons, including death prior to SCT (n=16), an appropriate HLA matched donor was not identified in preliminary search (n=10), patient failed to meet the organ function requirements for HCT (n=6), physician determines it is in the patients best
interest (n=27), withdrawal of consent for any further data submission (n=1), enrollment onto another COG study with therapeutic intent - BMT CTN 0501 (n=1), patient confirmed lost to follow-up (n=1) and no donor samples were ordered for HLA- and KIR typing (n=6). KIR typing data were made available to the treating physician who selected the donor used for transplant using their own best clinical judgment. Eighty-four of ninety evaluable patients (93%) had adequate KIR typing for classification as matched or mismatched.

**Transplant Outcomes According to Donor KIR Typing**

Three different strategies to characterize KIR mismatch have been reported, together with two strategies to characterize the presence or absence of favorable KIR haplotypes in transplant donors. We tested each of the reported strategies, and outcomes are reported in Table 2.

Overall, our data show no difference in overall survival, DFS, or relapse using any of the strategies to characterize KIR mismatch or favorable donor haplotypes (Figure 1).

The incidence of acute GVHD was significantly lower in those with KIR mismatch assessed by KIR typing (35+/−13% vs 60+/−18%, p=0.027), but not for the other KIR mismatch classification methods (e.g., ligand-ligand mismatch, missing ligand, haplotypes) (Figure 2).

Point estimates for all outcomes in the cord blood transplant recipients were non-significantly improved in those receiving a graft with KIR mismatch using any of the different models of KIR mismatch, but the study was not powered to detect a difference in this small subset of patients.

**NK-cell Reconstitution and Receptor Acquisition.**

We then examined the factors that affected the tempo of NK cell reconstitution and receptor acquisition. When compared to recipients with KIR ligand-matched donor (Table 3), those with mismatched donor had earlier expression of activating receptor NKG2D (HR=2.29, P=0.05), activating receptor NKp46 (HR=2.75, P=0.02) and activating receptor 2B4 (HR=2.85, P=0.03), but later acquisition of single inhibitory receptor KIR2DL1+ NK cells (HR=0, P<0.001) and KIR2DL2/3+ NK cells (HR=0, P<0.001).

Donors with telomeric KIR-B/x haplotypes were associated with earlier expression of activating receptor DNAM (HR=2.75, P=0.01) but slower acquisition of inhibitory NKp44+ NK cells after transplantation (HR=0, P<0.001).

After stratification for KIR receptor-ligand mismatch, higher Log_{10}CD34 count in the grafts was associated with faster acquisition of NKp44 (HR=8.53, P=0.02), but slower expression of inhibitory receptor NKG2A (HR=0.33, P=0.003) and NKp46 (HR=0.30, P=0.004).

**Association between NK-cell Receptor Acquisition and Patient Outcome**

We hypothesized that NK-cell receptor acquisition after transplantation would correlate with patient outcome. We found that NKp44 recovery was significantly associated with decreased DFS (HR 4.39; 95% CI 1.83–10.51; p=0.001) and increased relapse risk (HR 4.09; 95% CI 1.35–12.43; p= 0.013) in univariate analyses. After adjustment for log_{10}CD34 and log_{10}
CD3 cell dose, NKp44 recovery remained a statistically significant risk factor for poor DFS (HR: 3.959; 95% CI: 1.48 – 10.60; p=0.006) and relapse (HR: 4.473; 95%CI: 1.46 –13.66; p=0.009).

Discussion

We report the outcomes of a prospective, co-operative group clinical trial testing the feasibility and efficacy of selection of a KIR mismatched unrelated donor for HSCT to treat high-risk myeloid malignancies in children and young adults. The study was performed in close collaboration with the National Marrow Donor Program, and showed that in general it was possible to submit samples for KIR testing at the time of donor selection, and that data were available for more than 90% of the patients in time for the treating physician to use in donor selection if they chose to. The study did not dictate final donor selection, which was done using the treating physician’s own best judgment. We did also note that sixty-eight patients were enrolled but did not proceed to transplant per the study, illustrating the challenge of performing transplant studies with early enrollment at the start of donor search, when clinical status of the patient may change in the time between enrollment and planned transplant.

KIR mismatch has been shown to improve outcomes in aggressively T-cell depleted haploidentical transplants, and in at least one prior retrospective study of unrelated donor HSCT 5,6,18–19. In contrast to those studies, our data showed no impact of KIR mismatch on survival or relapse. It is possible that NK cells as well as T-cells were removed by in vivo T-cell depletion using ATG, as performed in our study, reducing any impact of KIR mismatch. We also recognize that the study enrolled mostly matched transplants, however, so ligand-ligand mismatch was rare and therefore insufficient size to determine potential benefit according to this model. However, our data did show a reduction in acute GVHD in recipients of a KIR mismatch as defined by KIR typing, in line with prior observation 5. Other KIR mismatch classifications (ligand-ligand mismatch, missing ligand, haplotypes) did not detect a difference in acute GVHD. Many different strategies have been used to measure and define KIR mismatch, including inferring KIR genotype from HLA typing, direct measurement of KIR genotype and expression in donor and recipient, and grouping genotypes according to favorable or unfavorable haplotypes 10,18–19. We used multiple strategies to define KIR mismatch and tested each against outcomes without finding significant differences, suggesting our negative results in survival and relapse outcomes are not a consequence of an inappropriate definition of KIR mismatch. We used in vivo T-cell depletion in our study to reduce T-cell interference with NK cell expansion and activity. Our data may differ from data reported in ex vivo T-cell deleted grafts because the degree of T-cell depletion in our study was insufficient to see benefit.

We examined the tempo of NK cell reconstitution and receptor acquisition during recovery in transplant. Our data show an important influence of KIR mismatch on NK cell reconstitution and receptor acquisition, with those with a KIR mismatched donor having slower expression of inhibitory receptors KIR2DL1 and KIR2DL2/3, but faster expression of activating receptors NKG2D, 2B4 and NKp46. In general, it would be expected that these
would be beneficial changes and similar changes may contribute to the improved outcomes seen with KIR mismatch in aggressively T-cell depleted haploidentical grafts.

NK cells are controlled by various activating and inhibitory receptors in addition to KIRs. In our study, we found for the first time that NKp44 recovery was significantly associated with decreased DFS and increased relapse risk. Previous biological studies have shown that proliferating cell nuclear Ag (PCNA), which is overexpressed in cancer cells, inhibits lysis and IFN-γ secretion by NK cells through an ITIM on the NKp44 cytoplasmic domain. The presence of NKp44 in the NK immunological synapse enables accumulation of PCNA in cell membrane recruited from the nucleus and cytoplasm of cancer cell. Thus, early acquisition of NKp44 after transplantation may inhibit NK cells and promote AML recurrence.

Our study did not support routine KIR typing to select donors for in vivo T-depleted unrelated donor bone marrow grafts. Our biological studies did show important differences in NK cell reconstitution, comparing KIR mismatched with KIR matched grafts. Further exploration and exploitation of these biological findings, perhaps in a different transplant strategy designed to maximize favorable NK cell activity may be valuable.

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Key Points:

1. KIR mismatching did not improve disease free survival in children transplanted with unrelated donor stem cells for AML using in vivo T-cell depletion with ATG.

2. KIR mismatching did alter the kinetics of NK cell receptor acquisition on donor cells after stem cell transplantation in children.
Figure 1.
Kaplan-Meier estimates of disease-free survival is similar in all groups, except recipients of KIR mismatched umbilical cord blood (UCB) grafts in whom survival was improved, although not statistically significant, \( p = 0.159 \). KIR mismatch was defined by KIR typing. UCB = umbilical cord blood. BM = bone marrow.
Figure 2.
Cumulative incidence of acute graft versus host disease was reduced in recipients of KIR mismatched grafts. KIR mismatch was defined by KIR typing. UCB = umbilical cord blood. BM = bone marrow.
Table 1.
Patient demographics are shown in recipients of KIR matched and mismatched grafts, with mismatch defined using the missing ligand model.

| KIR Matching | Match (N=30) | Mismatch (N=54) | Missing (N=6) | KIR match vs. mismatch |
|--------------|-------------|-----------------|---------------|------------------------|
|              | N %         | N %             | N %           | p                      |
| Gender       |             |                 |               |                        |
| Male         | 17 57%      | 26 48%          | 3 50%         | 0.454                  |
| Female       | 13 43%      | 28 52%          | 3 50%         |                        |
| Age          |             |                 |               |                        |
| Median (range)| 9.04 (0.39–23.35) | 6.14 (0.37–18.13) | 5.9 (1.11–15.36) | 0.046 |
| Age 0–<1     | 4 13%       | 14 26%          | 1 17%         | 0.178                  |
| Age 1–<16    | 20 67%      | 37 69%          | 5 83%         | 0.862                  |
| Age 16+      | 6 20%       | 3 6%            | 0 0%          | 0.063                  |
| Race         |             |                 |               |                        |
| White        | 25 89%      | 42 81%          | 5 83%         | 0.526                  |
| Black        | 2 7%        | 4 8%            | 0 0%          | 1.000                  |
| American Indian | 1 4%        | 0 0%            | 0 0%          | 0.350                  |
| Asian        | 0 0%        | 2 4%            | 1 17%         | 0.539                  |
| Other        | 0 0%        | 4 8%            | 0 0%          | 0.292                  |
| Unknown      | 2           | 2               | 0             |                        |
| Ethnicity    |             |                 |               |                        |
| Non-Hispanic | 25 83%      | 42 82%          | 5 83%         | 0.910                  |
| Hispanic     | 5 17%       | 9 18%           | 1 17%         |                        |
| Transplant donor type |        |                 |               |                        |
| Unrelated donor | 27 90%    | 54 100%         | 6 100%        | 0.043                  |
| Other        | 3 10%       | 0 0%            | 0 0%          |                        |
| Stem cell source |        |                 |               |                        |
| Bone marrow  | 22 73%      | 40 74%          | 4 67%         | 0.941                  |
| Umbilical cord blood | 8 27%    | 12 22%          | 2 33%         | 0.647                  |
Table 2.

Table 2 shows disease-free survival at 3 years ± 2 standard error according to KIR match or mismatch determined using three different models to define mismatch (receptor ligand match; ligand ligand mismatch and missing ligand). Models of favorable and unfavorable haplotypes are also presented.

| Model                        | Marrow Transplants | UCB Transplants | All Patients | Match vs mismatch p-value |
|------------------------------|--------------------|-----------------|--------------|---------------------------|
|                              | KIR Match          | KIR Mismatch    | KIR Match    | KIR Mismatch             | All Patients | KIR Mismatch             | Match vs mismatch p-value |
| Receptor ligand match        | 40 ± 22% (N=20)    | 43 ± 16% (N=37) | 40 ± 44% (N=5) | 89 ± 21% (N=9)          | 40 ± 20% (N=25) | 50 ± 15% (N=48)          | 0.687                     |
| Ligand/ligand mismatch       | 41 ± 13% (N=61)    | 0% (N=4)        | 42 ± 27% (N=14) | 100% (N=5)             | 40 ± 11% (N=77) | 56 ± 33% (N=9)            | 0.360                     |
| Missing ligand               | 43 ± 22% (N=21)    | 31 ± 15% (N=45) | 29 ± 34% (N=7) | 77 ± 23% (N=13)         | 39 ± 18% (N=28) | 45 ± 13% (N=60)           | 0.981                     |

| Haplotype                    | A/B+B/B            | A/A             | A/B+B/B      | A/A             | A/B+B/B          | A/A            | A/A+B/B vs AA p-value    |
|------------------------------|--------------------|-----------------|--------------|-----------------|-----------------|-----------------|-------------------------|
| KIR haplotype (centromeric)  | 51 ± 19% (N=27)    | 33 ± 17% (N=30) | 38 ± 34% (N=8) | 88 ± 23% (N=8) | 48 ± 17% (N=35) | 43 ± 16% (N=40) | 0.529                   |
| KIR haplotype (telomeric)    | 41 ± 23% (N=19)    | 42 ± 16% (N=38) | 56 ± 33% (N=9) | 71 ± 34% (N=7) | 46 ± 19% (N=28) | 45 ± 15% (N=47) | 0.600                   |

*Centromeric A haplotypes are defined as those that contain KIR2DL3 and telomeric A haplotypes as those with KIR3DL1. Centromeric B haplotypes are defined as those that contain KIR2DL2 and telomeric B haplotypes as those with KIR3DS1. Two cases received PBSC and are not included in the bone marrow and cord blood analyses (n=82) but do appear in the “all patients” analysis (n=84).
Table 3.
Table 3 shows multivariate models of time to NK cell receptor acquisition, adjusted for log $10$ CD34 cell count in the infused graft. The hazard ratio in this analysis indicates the risk of failure of normalization of NK cell count or NK cell receptor expression as listed in the table, based on the covariate KIR mismatch and adjusted for CD34 count in the graft. Data were adjusted for the number of stem cells infused (CD34 count) because our data showed that CD34 count significantly influenced the tempo of immune reconstitution. Infused CD3 count was also tested in this model but did not influence outcomes so was not retained in the model. Time dependence in this analysis refers to time to normalization of NK cell count or NK receptor expression after transplant, using levels measured at timepoints of 1, 3, 6 and 12 months after transplant.

| Receptor     | Impact of Ligand match vs. mismatch on receptor acquisition | HR   | 95% CI       | P-value |
|--------------|------------------------------------------------------------|------|--------------|---------|
| KIR2DL1 *    |                                                             | 0    | <0.001       |         |
| KIR2DL2/3 *  |                                                             | 0    | <0.001       |         |
| NKG2A *      |                                                             | 1.177| 0.11 – 13.04 | 0.894   |
| NKG2D        |                                                             | 2.285| 1.00 – 5.23  | 0.050   |
| NKp46        |                                                             | 2.754| 1.17 – 6.49  | 0.021   |
| NKp44        |                                                             | 1.866| 0.41 – 8.5   | 0.420   |
| 2B4          |                                                             | 2.845| 1.11 – 7.31  | 0.030   |
| DNAM         |                                                             | 2.082| 0.79 – 5.46  | 0.136   |

* positive for single inhibitory receptor and negative for all other MHC inhibitory receptors.