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Donor KIR Gene Content and KIR-Ligand Matching and Outcomes of Pediatric Patients with JMML Following Unrelated Donor Transplant

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Corresponding Author:	Hemalatha Geetharani Rangarajan, MD Nationwide Children's Hospital Hematology Oncology & Blood and Marrow Transplant Columbus, OH UNITED STATES
First Author:	Hemalatha Geetharani Rangarajan, MD
Order of Authors:	Hemalatha Geetharani Rangarajan, MD
	Marcelo S. F. Pereira, PhD
	Ruta Brazauskas, PhD
	Andrew St. Martin,, MS
	Ashleigh Kussman, MD
	Ezgi Elmas, BSA
	Michael R Verneris, MD
	Shahinaz M Gadalla, MD PhD
	Steven G E Marsh, BSc PhD
	Sophie Paczesny, MD PhD
	Stephen R Spellman, MBS
	Stephanie J Lee, MD MPH
	Dean Anthony Lee, MD PhD
Abstract:	Background: Natural killer (NK) cell determinants predict relapse-free survival after allogeneic hematopoietic cell transplant (HCT) for acute myeloid leukemia, and prior studies show a beneficial graft versus leukemia effect in juvenile myelomonocytic leukemia (JMML) patients. However, it is unknown if NK cell determinants predict protection against relapse for JMML patients undergoing HCT. Therefore, we investigated NK cell-related donor and recipient immunogenetics as determinants of HCT outcomes in patients with JMML. Methods: Patients with JMML (0 to < 19 years) who received a first allogeneic HCT from an unrelated donor between 2000 to 2017 and had available donor samples from the Center for International Blood and Marrow Transplant Research Repository were included. Donor KIR typing was performed on pre-HCT samples. The primary endpoint was disease-free survival (DFS); secondary endpoints included relapse, grade II-IV acute graft versus host disease (GVHD), chronic GVHD, GVHD-free/relapse-free survival (GRFS), transplant related mortality and overall survival (OS). Donor killer immunoglobulin receptor (KIR) models tested included KIR genotype (AA vs Bx), B content (0-1 vs ≥ 2), centromeric and telomeric region score (AA vs AB vs BB), B content score (best, better, neutral), composite score (2 vs 3 vs 4), activating KIR content and presence of KIR2DS4. Ligand-ligand (L-L), KIR-L mismatch effects on outcomes were analyzed in HLA-mismatched donors (≤ 7/8, n=74) only. Univariate analysis was performed for primary and secondary outcomes of interest with a p-value < 0.05 considered significant . Results: 165 patients (113 males) with a median follow-up of 85 (6-216) months met study criteria. Of these, 111 received an unrelated donor HCT and 54 cord blood HCT. Almost all (161, 98%) received a myeloablative conditioning regimen. After exclusion of recipients of reduced intensity/non-myeloablative regimens and ex-vivo T cell

	depleted grafts (n=8), by there were 42 AA and 115 Bx donors respectively. Three-year DFS, OS, relapse and GRFS for the entire cohort was 58% [95% Confidence interval (CI) 50-66], 67% (95% CI 59-74, 26% (95% CI 19-33) and 27% (95% CI 19-35) respectively. Cumulative incidence of grade II-IV aGVHD at 100 days and cGVHD at one-year were 36% (95% CI 27-44%), and 23% (95% CI 17-30%) respectively. There were no differences between AA and Bx donors for any recipient survival outcomes. Risk of grade II-IV aGVHD was lower in patients with donors with B content score of ≥ 2 (HR 0.46; 95% CI: 0.26-0.83, p=0.01), an activating KIR content score of > 3 (HR: 0.52; 95% CI: 0.29-0.95; p=0.032), centromeric A/B (HR 0.57; 95% CI: 033-0.98, p=0.041) and telomeric A/B score (HR: 0.58; 95% CI: 0.34-1.00, p=0.048). Conclusion: To our knowledge, this is the first study analyzing the association of NK cell determinants and outcomes in JMML HCT recipients. Our study identifies potential benefits of donor KIR-B genotypes in reducing aGVHD. These findings warrant further study of the role of NK cells in enhancing graft versus leukemia effect via recognition of JMML blasts.

Highlights

1. We studied the impact of NK alloreactivity in 165 JMML unrelated donor allogeneic hematopoietic cell transplant (HCT) recipients.
2. The 3-year disease-free survival, overall survival and relapse probability was 58%, 67%, 27% respectively with no difference between recipients of donors with KIR AA (n=42) vs Bx (n=115) genotypes.
3. The risk of grade II-IV acute graft-versus-host disease was lower in patients who received grafts from centromeric or telomeric A/B donors, donors with a B content score of ≥ 2, and an activating KIR content of > 3.
4. Our current study does not support the role of KIR typing of unrelated donors for HCT of patients with JMML.

Donor KIR Gene Content and KIR-Ligand Matching and Outcomes of Pediatric Patients with JMML Following Unrelated Donor Transplant

*Hemalatha G Rangarajan, MD[1], *Marcelo S.F. Pereira, PhD[2], Ruta Brazauskas, PhD[3,4], Andrew St. Martin, MS[4], Ashleigh Kussman, MD[1,2], Ezgi Elmas, BSA[2,5], Michael R. Verneris, MD[6], Shahinaz M Gadalla, MD, PhD[7], Steven G E Marsh, BSc, PhD[8,9], Sophie Paczesny, MD, PhD[10], Stephen R. Spellman, MBS[11], Stephanie J. Lee, MD, MPH[11,12], Dean A. Lee, MD, PhD[1,2]

* co-first authors

[1]Division of Pediatric Hematology, Oncology, Blood and Marrow Transplant, Nationwide Children's Hospital, Columbus, OH; [2]Center for Childhood Cancer and Blood Diseases, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH; [3]Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI; [4]Center for International Blood and Marrow Transplant Research (CIBMTR), Department of Medicine, Medical College of Wisconsin, Milwaukee, WI; [5]Molecular, Cellular, and Developmental Biology Graduate Program, Ohio State University, Columbus OH; [6]University of Colorado, Anschutz Medical Campus, Colorado Children's Hospital, Aurora, CO, [7]Clinical Genetics Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; [8]Anthony Nolan Research Institute, Royal Free Hospital, London, UK; [9] UCL Cancer Institute, University College London, London UK, [10] Department of Microbiology and Immunology and Department of Pediatrics, Medical University of South Carolina, Charleston, South Carolina. [11]Center for International Blood and Marrow Transplant Research (CIBMTR), National Marrow Donor Program/Be the Match, Minneapolis, MN, [12] Fred Hutchinson Cancer Research Center, Seattle, WA.

Corresponding Author

Hemalatha G Rangarajan

Nationwide Children's Hospital, 700 Children's Drive, Columbus, Ohio

Email: Hemalatha.rangarajan@nationwidechildrens.org

Telephone: 614 355 1689,

Fax number: 614 722 3699

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Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative neoplasm of early childhood with an incidence of 1.2 per million children per year and accounts for 2-3% of all childhood malignancies[1]. It is characterized by excessive proliferation of cells of the myelomonocytic lineage secondary to activating somatic or germline mutations in the RAS/RAF/MAPK signaling pathway. Mutations in PTPN-11, N-RAS, K-RAS, RRAS, STEBP1, CBL, or NF1 have been identified in 90% of JMML patients[2]. Some of these occur in patients with underlying genetic syndromes such as Noonan syndrome (PTPN-11) and Neurofibromatosis type 1 (NF1)[3-5]. While most patients with CBL and a few with N-RAS mutations can have spontaneous resolution of disease[6,7], survival in untreated patients can be as short as 10-12 months[1]. Allogeneic hematopoietic cell transplantation (HCT) remains the sole curative therapy and is associated with a 5-year disease-free survival (DFS) of ~50%[8-11]. Although the outcomes post-HCT have improved over time[12], relapse remains a major cause of treatment failure, with rates as high as 30-50%[8,9,12]. Myeloablative conditioning (MAC) regimens utilizing busulfan (Bu) and melphalan (Mel) with either cyclophosphamide (Cy), or fludarabine (Flu) are preferred with recent studies demonstrating increased relapse with the use of a less intense regimen (BuFlu)[12,13]. Total body irradiation (TBI)-based MAC regimens on the other hand historically have been associated with increased transplant related mortality (TRM) and decreased overall survival (OS) without a decrease in relapse rates, and are generally not preferred in this younger population[12,14,15]. Therefore, it is unlikely that further intensification of the conditioning regimens without a concomitant increase in toxicity can be achieved in this malignancy.

On the other hand, the potential benefit of the graft versus leukemic (GVL) effect has been reported in JMML patients who developed GVHD post HCT. In a European Bone Marrow Transplant - Center for International Blood and Marrow Transplant Research (EBMT-CIBMTR) study of 110 umbilical cord blood (UCB) HCT recipients, decreased relapse was seen in patients with grade II-IV acute GVHD [8]. Recent smaller studies have also revealed that molecular responders[16] and recipients of intensive conditioning regimens[13] who developed chronic GVHD (cGVHD) had decreased chances of relapse and improved OS. In a Japanese cohort of 129 JMML HCT recipients, patients who developed cGVHD in comparison to unaffected patients had improved 5-year OS (84% vs 63%), event free survival (82% s 52%), and decreased relapse (15% vs 36%)[12]. In patients with imminent relapse, early withdrawal of immunosuppressive therapy (IST) and a second allogeneic HCT have been shown to prevent overt relapse [17-19]. The role of donor lymphocyte infusions (DLI) in JMML HCT recipients is debatable with some reports demonstrating its efficacy[20-23] and others showing no benefit [18,19] . Based on these observations, the use of adoptive immunotherapy other than DLI, such as natural killer (NK) cells to enhance GVL, remains an unexplored but potentially attractive strategy for disease control and prevention of relapse in this rare but aggressive malignancy.

Although the role of NK cells and their alloreactivity as determined by Killer-cell Immunoglobulin-like Receptors (KIR) has been extensively investigated in myeloid leukemias [24-27], there are no reports of the impact of these factors on relapse and survival in JMML. We previously demonstrated that the mature monocytic population of JMML cells express similar profiles of NK cell ligands as healthy-donor monocytes and are relatively resistant to NK cell cytotoxicity[28]. On the other hand, putative JMML stem cells (defined as Lin-CD34+CD38-) express ligands for NKG2D, NKp30, and NKp44 at levels equal or greater than AML stem

cells[28]. Further, JMML colony-forming units were significantly reduced following incubation with NK cells in comparison to cord blood mononuclear cells co-cultured with NK cells[28]. Based on these observations we hypothesized that NK cell-dependent mechanisms are a major component of the GVL protection from relapse of JMML after HCT, and that specific determinants of greater donor NK cell function (e.g. KIR Bx donors or KIR ligand mismatch) are associated with reduced relapse. We therefore investigated NK cell-related donor and recipient immunogenetics as determinants for outcomes in a large contemporary cohort of children undergoing first allogeneic transplant for JMML utilizing the CIBMTR registry. We hypothesized that determinants of greater NK cell function will be associated with improved DFS, OS, and relapse- and GVHD-free survival (GRFS), and decreased relapse.

Methods: Patient inclusion and exclusion criteria: We included all pediatric patients (0 to < 19 years) with a diagnosis of JMML who received their first allogeneic HCT from a matched or mismatched unrelated donor between 2000 to 2017 and had available donor samples from the CIBMTR Research Repository. Patients were censored at their second allogeneic HCT if applicable. Patient whose disease transformed to AML prior to HCT were allowed, but none were identified in this cohort. All graft sources: peripheral blood (PB), bone marrow (BM) or UCB and conditioning regimens: myeloablative conditioning (MAC), reduced intensity conditioning (RIC) and non-myeloablative (NMA) regimens were eligible. Classification of the intensity of conditioning regimens as MAC, RIC, or NMA was done as previously reported.[29] The Institutional Review Board of the National Marrow Donor Program approved this study. Disease status at the time of HCT was determined as per standard CIBMTR definitions for MDS (for HCT prior to 2015)[30] or JMML (for HCT ≥ 2015) response criteria[31].

Donor KIR typing and KIR ligand assessment of both donor and recipients: Donor KIR typing was performed on pre-HCT samples and results were correlated with clinical data extracted from the CIBMTR database. Available pre-HCT donor samples included frozen peripheral blood (n=104), amplified DNA (n=40), dried whole blood on filter papers (n=14), and B-lymphoblastoid cell lines (B-LCL) (n=7). Genomic DNA was extracted from frozen donor peripheral blood mononuclear cells or extracted from filter paper using DNeasy Blood and Tissue Kit - Qiagen® -following the manufacturer's protocol. KIR typing was then performed using Miltenyi Biotec KIR typing kits. Briefly, DNA was diluted into a resuspension buffer. Approximately 75 ng/ul of DNA was used in each well of 96 well PCR plates containing lyophilized enzyme mix, including Taq DNA polymerase and positive controls. The presence or absence of KIR genes was analyzed by PCR using sequence specific primers (SSP) which enable the detection of all alleles of the 15-known human KIR genes with two pseudogenes. *KIR* genes were typed as described elsewhere. [26] *KIR* genes of the centromeric and telomeric segments of *A* and *B* haplotypes[32-34] were analyzed as described elsewhere.[24] Donor *KIR* genotypes are indicated as *A/A* when they did not contain *B* haplotypes; the centromeric segment is termed "*Cen-A/A*" and the telomeric "*Tel-A/A."* Donor *KIR* genotypes are indicated as *B/x* when they contained at least 1 *B* haplotype; the centromeric segments are termed "*Cen-B/x*" and the telomeric "*Tel-B/x*." The KIR B-content score of the donors were calculated according to the number of centromeric and telomeric gene motifs containing B haplotype defining genes, with possible values being ranging from 0 to 4. Classification of the donor KIR B status as best, better, or, neutral was determined using online calculator at http://www.ebi.ac.uk/ipd/kir. [35,36] Based on presence, or absence of Human Leukocyte Antigen (HLA) ligands, donors and

recipients were grouped as C1, C2, or Bw4 as previously described[37-40] . This was determined by high resolution HLA typing of donors and recipients already available in the CIBMTR database.

Endpoints: The primary endpoint was DFS comparing AA and Bx donor KIR genotypes. Secondary endpoints included relapse, acute GVHD (aGVHD), chronic GVHD (cGVHD), TRM, GRFS, and overall survival (OS). OS was defined as the probability of survival regardless of disease status at any time point with death from any cause considered an event. Surviving patients were censored at last follow-up. DFS was defined as the probability of being alive and free of disease at any time point with death and relapse considered as events Patients who were alive and disease free were censored at last follow up. Relapse was defined as the probability of relapse post-HCT with death in remission being considered a competing event. TRM was defined as death due to any transplantation-related cause other than disease relapse. Acute and chronic GVHD was diagnosed and graded at each transplant center according to standard criteria[41-43]. GRFS was defined as the absence of grade III-IV acute GVHD (aGVHD), systemic therapy-requiring cGVHD, relapse, or death, all of which were considered as events for this outcome We identified 8 patients who received either NMA/RIC regimens or *ex-vivo* T cell depleted grafts (Table 1). While the former group is at increased risk of relapse[44], the latter group is likely to have much different NK cell reconstitution and alloreactivity in the setting of minimal to no post-transplant immunosuppression. Given these differences, and the small number of patients in each category, these patients were excluded from the analysis of primary and secondary endpoints.

Statistical Methods: Patient and transplant-related variables included age (< 2 years vs ≥ 2 years), sex, race, performance score (< 90 vs >90), disease status, graft type (bone marrow, peripheral blood, and cord blood), HLA matching (8/8 vs others), conditioning intensity

(myeloablative vs others), use of serotherapy, GVHD prophylaxis (calcineurin inhibitor (CNI)+ methotrexate (Mtx) ± others vs others) and year of HCT (2000-2007 vs 2008-2017). We compared the primary and secondary outcomes using various KIR models that have previously been associated with survival of patients with other myeloid malignancies [24,45-47]**,** including variables of interest: KIR genotype (AA vs Bx) as well as donor KIR-B content (0-1 vs ≥ 2), centromeric and telomeric region score (*A/A* vs *A/B* vs *B/B*), donor KIR-B content score (best, better, neutral, KIR composite score (2 vs 3 vs 4), activating KIR content and presence of activating KIR2DS4. Based on HLA typing of recipient, donor, and KIR typing of donor, the following models were also tested: Ligand-Ligand (L-L) Mismatch[27], KIR receptor-ligand (KIR-L) mismatch[48], and missing ligand (present vs absent)[49]**(Figure 1).**

Probabilities for OS, DFS, and GRFS were calculated using the Kaplan-Meier estimator with variance estimated by Greenwood's formula. For analysis of GVHD, patients were censored at the time of 2nd HCT. Cumulative incidence rates for relapse, acute grade II-IV and chronic GVHD were calculated with death as a competing risk. Relapse was considered a competing risk for TRM. Log-rank test and Gray's test were used to compare respective survival curves and cumulative incidence curves between AA and Bx donors. Cox proportional hazards models were used to perform univariate analyses for primary and secondary outcomes of interest. The proportional hazards assumption was checked for all covariates. Apart from patient and transplant-related characteristics, the various KIR models were included in the risk factor analysis for primary and secondary endpoints. L-L and KIR-L mismatch effects were studied for all outcomes in a subgroup of HLA-mismatched donors (high resolution match <8/8, n=74) only. A significance level of 0.05 was used throughout. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

Results:

Patient demographics: The study population included 165 (113 males) children with JMML who underwent their first allogeneic HCT between 2000-2017 with available samples for donor KIR typing **(Table 1)**. The median age of the cohort was 2 (range < 1 -8) years with a male: female ratio of 2:1. ~~Half of the recipients (55%) had a HCT co-morbidity index of ≤1~~ Majority of the recipients were White (77%) with a performance score ≥90 (81%). More transplants (61%) were performed in recent years (2008-2017). The median follow-up of all patients was 85 (range 6-216) months.

Disease characteristics: The median fetal hemoglobin (HbF), platelet count, peripheral blood blasts at diagnosis were 19%, 65 x 10^9/L, and 3% respectively. The CIBMTR database lacked information on genetic syndromes (e.g. Noonan's, NF1) and molecular mutations (e.g. PTNPN1, CBL, etc.) for most recipients. Of the 55 patients with available cytogenetic data, 10 patients had monosomy 7, trisomy 8 or both ~~Only 14 (8%) patients had splenectomy pre-HCT and 15 (9%) had progressive disease prior to HCT~~**(Table 1).**

Transplant characteristics (Table 2): The graft source was unrelated BM in 58%, PB in 9% and UCB in 33% of recipients. Amongst these, there were 78 HLA-mismatched (≤ 7/8) donor HCTs, which included 30 BM and 48 UCB recipients. Almost all patients (161, 98%) received a MAC regimen followed by a CNI + Mtx based GVHD prophylaxis (91, 49%). The most common regimens (not shown) were BuCyMel (48%), followed by TBI/CY (15%) and Bu/Melphalan (15%). Half of the patients (91, 55%) received in-vivo T cell depletion with ATG. The median time from diagnosis to HCT was 4 (range <1 -32) months.

Distribution of donor by KIR variables (Table 3): After excluding patients who received RIC/NMA or ex-vivo T cell depleted graft recipients (n=8), based on donor KIR B content, 42 patients received grafts from AA and 115 from Bx donors respectively. The AA and Bx recipients were similar with regards to both baseline and disease characteristics (**Table 1-3**). Table 3 shows the distribution of donors by various models of KIR gene content as described earlier. Eighty nine percent of Bx donors had a B content score of 1-2 with the remaining 11% having a score of 3-4. Similarly, only a few Bx donors had centromere (12%) and telomere scores (7%) with the highest B content (i.e. *B/B*). KIR2DS4 was present in 48% of all donors, L-L mismatch, KIR-Ligand mismatch, and missing ligand in the GVH direction was observed in 10%, 63%, and 63% of donor-recipient (D-R) pairs, respectively.

Association of KIR gene content with transplant outcomes: The primary goal of the study was to determine the association of KIR content (AA vs B/x) with transplant outcomes. The 3-year DFS, OS and GRFS for the entire cohort was 58% (95% confidence interval [CI]: 50-66), 67% (95% CI: 59-74) and 27% (95% CI: 19-35) respectively **(Table 4).** The cumulative incidence of relapse at 3 years post HCT was 26% (95% CI: 19-33), grade II- IV aGVHD at day 100 was 36% (27-44%) and cGVHD at 1 year was 23% (95% CI: 17-30%). In a univariate analysis, there were no differences between AA and BX cohorts with regards to both the primary and secondary outcomes of interest **(Table 4, Figure 2, Figure 3)**. The risk of grade II-IV aGVHD was lower in patients with donors with B content score of ≥ 2 (HR 0.46; 95% CI: 0.26-0.83, p=0.01), an activating KIR content score of > 3 (HR: 0.52; 95% CI: 0.29-0.95; p=0.032), *Cen-A/B* (HR 0.57; 95% CI: 033-0.98, p=0.041) and *Tel-A/B* donors (HR: 0.58; 95% CI: 0.34-1.00, p = 0.048) (**Table 5).** Considering the various KIR models, in the subgroup of HLA mismatched donors, there was no impact of L-L mismatch or KIR-L mismatch on primary or

secondary outcomes **(Table 6).** Although there was trend towards an improved OS (i.e., lower mortality) in L-L mismatched donors in the non-GVHD direction this was not statistically significant (HR: 0.46; 95% CI: 0.21-1.02; p=0.05) (Table 6). Relapse was the most common cause of death in the entire study cohort as well as in the subgroups of L-L mismatched and L- L matched donor-recipient pairs **(Table S1).** The type of graft also did not impact any of the above outcomes.

The impact of patient-related factors such as age, sex, race, performance score, disease status, and transplant-related factors like HLA matching, conditioning intensity, use of in vivo T cell depletion, GVHD prophylaxis, and year of transplant (2000-2007 vs 2008-2017) was also studied (not shown). TRM was increased in patients younger than 2 years (HR 2.91; 95% CI: 1.17-7.20 p = 0.021) and female recipients (HR 2.19 95% CI: 1.01- 4.74, p = 0.046), while the risk of cGVHD was increased in non-Caucasians (HR 2.22; 95% CI: 1.08-4.56, p=0.03). ~~Amongst patients who died (n=62), relapse was the most common (40%, n=25) primary cause of death.~~

Discussion:

While several studies have investigated the impact of donor NK cell-mediated alloreactivity in myeloid and lymphoid malignancies post allogeneic HCT [26,45-47,50], there have been none in JMML HCT recipients. Preclinical studies have demonstrated the sensitivity of JMML stem cells to NK mediated lysis[28]. These findings along with the reports of decreased relapse in JMML patients who developed GVHD post allogeneic HCT suggest the beneficial effect of GVL in this rare myeloproliferative neoplasm[8,12,13,16]. We therefore hypothesized that NK mediated alloreactivity enhancing the GVL effect may be protective against relapse in JMML HCT recipients. Hence, we studied the impact of donor NK alloreactivity as determined

by KIR gene content and various other published KIR models [45-47,51] on transplant outcomes in a large contemporary cohort of JMML patients who underwent an unrelated donor HCT. However, we found that most of the KIR variables were not associated with HCT outcomes and also observed a reduced risk of grade II-IV aGVHD with donors with B content score ≥ 2, an activating KIR content score of > 3, and *Cen-A/B or Tel-A/B* scores. While we are unable to completely explain the above findings, a few factors could have contributed to these observations. Nearly half of the study cohort received ATG which could have not only depleted donor T cells but also NK cells thereby reducing any impact of a NK mediated GVL effect. Since a greater degree of mismatch is tolerated in UCB HCT we attempted to ascertain if L-L mismatch cohort was heavily biased toward UCB resulting in the stem cell source overshadowing the L-L effect. However, our analysis revealed no impact of graft source on primary or secondary outcomes in the sub-cohort of HLA mismatched donors (n=74). Additionally, more than half of the study cohort received matched transplants, wherein NK-cell mediated alloreactivity via the KIR-L and L-L mismatch may be less impactful than in the mismatched setting[26,50,52,53]. Although we identified 24 recipients of haploidentical HCT in the CIBMTR database these were not included both because of small numbers in this category and lack of available donor samples for KIR typing. Matched-related donors were excluded because they would have lacked KIR-Ligand mismatch, though they may be considered as a confirmatory cohort to assess the KIR-B content effect. Finally, we were also limited by small patient numbers in various categories leading to low statistical power in our subset analysis.

Early evidence of NK cell mediated GVL favorably impacting HCT outcomes was first reported by Ruggeri et al. In their report the authors observed that in patients with acute myeloid leukemia (AML) undergoing haploidentical HCT, increased OS and decreased relapse was only

seen when there was a L-L mismatch based on HLA differences between donor and recipients [27,54]. Similar outcomes have also been observed in HLA mismatched transplants when there is a mismatch of KIR genes between donor-recipient pairs[55,56] . Although the protection offered by NK cells has been mostly observed clinically in myeloid malignancies[24,46] a few studies have also shown similar benefits in patients with ALL and non-Hodgkin's lymphoma[45,51,57]. However negative studies similar to ours have reported a lack of benefit of NK mediated alloreactivity in both myeloid and lymphoid malignancies.[47,58,59] In a recent CIBMTR study of 714 pediatric patients with acute leukemia, who underwent a T replete unrelated donor HCT, the authors did not find any impact of NK alloreactivity as determined by various KIR models on transplant outcomes.[47] Similar to the unexpected findings in our study, in an analysis of 119 adults with AML who underwent a myeloablative T cell depleted HLA matched unrelated HCT, the presence of donor encoded centromeric KIR B content conferred an increased risk of infectious mortality translating into decreased OS.[60]

Increased TRM was seen in children < 2 years and female HCT recipients. Two prior registry studies have reported a trend towards increased TRM in female JMML HCT recipients[8,9] while younger children have been shown to be more vulnerable to transplant related toxicities secondary to organ immaturity. [61,62] We observed an increased risk of cGVHD in non-whites (n=27) compared to others (n=120). We postulate that this could be partly attributed to greater genomic diversity beyond what is captured in HLA-matching status. Older age[8,9,63,64], higher fetal Hb, presence of somatic PTPN-11 mutation[63,65], monosomy 7 or other cytogenetics[8,63,65], decreased platelet count[64,66] have been associated with increased relapse risk.[1,67] Recently reported prognostic factors include AML gene expression signature[63], aberrant DNA methylation [68-70] and somatic mutations (SETBP1, ASL1, SH2B3, JAK3) [13,66,71,72]. We could not analyze the

impact of some of these prognostic variables including molecular mutations, cytogenetic abnormalities, underlying genetic syndromes or pre HCT chemotherapy on HCT outcomes as these data were unavailable or missing in this retrospective database.

The transplant outcomes of our cohort mirror the reports by recent large registry studies[8,9,12]. In an earlier EBMT study which included 100 children who either received a HLA-identical relative or matched/one antigen disparate unrelated donor transplant between 1993-2002, the 5 year DFS was 52% (95% CI 42-62) and relapse rate was 35% (95% CI 27-46)[9,15]. ~~This cohort included only 7 UCB HCT recipients.~~ Similarly, in the combined CIBMTR-EBMT registry-based study of UCB recipients (1995-2010), the 5 year OS, DFS and cumulative incidence of relapse were was 52± 5 %, 44%%± 5% and 33%± 5% respectively [8]. In a more recent study from Japan (2000-2011) which included 129 children who received HCT from all stem cell sources (including 30 UCB recipients) the 5-year OS, EFS and RR were 64% (55 to 72%), 46% (37 to 55%) and 34 % (26 to 43%)[12]. In their multivariate analysis, use of UCB was associated with worse EFS (HR 1.96 95% CI: 1.10-3.51, p=0.05). We did not observe a difference between AA and BX donors with respect to these outcomes. Although, nearly 1/3 of our cohort received UCB HCT, we did not observe any impact of graft type on primary or secondary outcomes. As improvements in supportive care have likely contributed to improved transplant outcomes for JMML in recent years, further improvement in DFS may be achieved only with use of targeted agents or adoptive immunotherapies without compromising toxicity in this population. The use of epigenetic modifiers namely 5-azacytidine[15,73], and MEK inhibitors to reduce pre HCT disease burden are currently being explored [2,15]. Although our study does not support the use of KIR for selecting unrelated donors for children undergoing T replete transplants, the findings should be confirmed or corroborated in a larger or more uniform cohort.

To our knowledge, this is the first study analyzing NK determinants in pediatric JMML HCT recipients. Acute GVHD was the only outcome associated with NK cell immunogenetics, but not in the expected directions. These unexpected findings may be due to our limited sample size, heterogeneity in or bias introduced by graft sources, heterogeneity in treatment regimens, or extensive use of *in-vivo* T cell depletion. Although our study identifies a potential benefit of decreased risk of grade II-IV aGVHD in JMML patients following HCT from donors with a B content score ≥ 2, an activating KIR content score of > 3, and *Cen-A/B or Tel-A/B* scores, these observations require further investigation in larger cohorts

Contributions: H.G.R, designed study, analyzed, reviewed the data, and wrote the manuscript. M.S.F.P performed experiments, analyzed data, reviewed data and manuscript. A.K, E.E performed experiments. R.B, A.S.M: performed statistical analysis and reviewed the manuscript. M.R.V, S.M.G, S.G.E.M, S.P provided scientific input, and edited the manuscript. S.R.S and S.L assisted in study design, facilitated data acquisition, provided scientific input, reviewed the data and edited the manuscript. DAL designed study, analyzed, reviewed the data and edited the manuscript.

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Legend for Figures:

Figure 1: KIR models predicting NK alloreactivity according to HLA typing of donor, recipient, and donor KIR typing. **A)** Ligand- Ligand Mismatch Model: Requires HLA typing of Donor and

Recipient. Predicts alloreactivity in the Graft versus host direction when recipient lacks expression of inhibitory KIR ligand (e.g. in this case C1), that is present the donor**. B)** KIR Receptor- Ligand Mismatch Model: requires donor KIR typing and recipient HLA typing. Donor has at least one inhibitory KIR receptor (e.g. KIR2DL2 in this case) whose ligand is missing in the recipient (e.g. C1 in this case) **C)** Missing Ligand Model requires HLA typing of recipient only. Recipient is missing at least one of the inhibitory KIR ligands e.g. missing C1

Figure 2: Relapse in JMML HCT recipients by donor KIR B haplotype: AA (n=42) vs Bx (n=115) donors.

Figure 3: Disease-free survival in JMML HCT recipients by donor KIR B haplotype: AA (n=42) vs Bx (n=115) donors

Table 1: Baseline characteristics of patients receiving their first allogeneic HCT for JMML, 2000 – 2017, grouped by KIR genotype

Characteristic	AA N=43	Bx N=122	Full Cohort N=165
Patient-related			
Age - no. (%)			
Median (range)	2 (<1-7)	2 (<1-8)	2 (<1-8)
< 5	39 (91)	113 (93)	152 (92)
≥ 5	4 (9)	9 (7)	13 (8)
Sex - no. (%)			
Male	27 (63)	86 (70)	113 (68)
Female	16 (37)	36 (30)	52 (32)
Race - no. (%)			
White	34 (79)	93 (76)	127 (77)
Black or African American	4 (9)	9 (7)	13 (8)
Asian	3 (7)	8 (7)	11 (7)
More than one race	2 (5)	4 (3)	6 (4)
Missing	0	8(7)	8(5)
KPS - no. (%)			
90 – 100	31 (72)	102 (84)	133 (81)
≤ 90	9 (21)	18 (15)	27 (16)
Missing	3(7)	2(2)	5(3)
HCT-CI - no. (%)			
0 -1	30 (69)	62 (51)	92 (55)
≥ 2	0	9 (7)	9(6)
Missing	13(30)	51 (42)	64 (40)
Year of transplant - no. (%)			
2000 – 2007	14 (33)	50 (41)	64 (39)
2008 - 2017	29 (67)	72 (59)	101 (61)
Follow-up (months) - median (min-max)	74 (31-170)	85 (6-216)	85 (6-216)
Disease Characteristics			
Cytogenetic abnormalities			
Monosomy 7 (%)	4 (9)	3 (2)	7 (4)
Trisomy 8 (%)	1 (2)	0 (0)	1 (<1)
Monosomy 7 and Trisomy 8	0	1 (1)	1 (<1)
No Monosomy 7 or Trisomy 8	11 (26)	35 (29)	46 (28)

Not reported	27 (63)	83 (68)	110 (67)
Splenectomy pre HCT			
No	10 (23)	28 (23)	38 (23)
Yes	5 (12)	9 (7)	14 (8)
Not reported	28 (66)	85 (70)	113 (68)
Lab values at diagnosis			
WBC count ($\times 10^9$/L)			
n / N	20/43	43/122	63/165
Median (Range)	29 (5-193)	27 (7-127)	29 (5-193)
Platelet count ($\times 10^9$/L)			
n / N	19/43	41/122	60/165
Median (Range)	63 (19-599)	67 (0-455)	65 (0-599)
Monocytes ($\times 10^9$/L)			
n / N	17/43	41/122	58/165
Median (Range)	18 (4-47)	20 (1-45)	19 (1-47)
Blasts in blood (%)			
n / N	15/43	40/122	55/165
Median (Range)	1 (0-13)	3 (0-36)	3(0-36)
Fetal hemoglobin (%)			
n / N	12/43	24/122	36/165
Median (Range)	23 (2-66)	14 (0-65)	19(0-66)
Blasts in marrow (%)			
n / N	12/43	24/122	36/165
Median (range)	9 (3-26)	8 (0-71)	9 (0-71)
Disease status pre-HCT - no. (%)			
Complete response	9 (21)	14 (11)	23 (14)
Partial response	8 (19)	18 (15)	26 (16)
Minimal response	0	3 (2)	3 (2)
Stable disease	9 (21)	33 (27)	42 (25)
Progressive disease	6 (14)	9 (7)	15 (9)
Missing	11 (26)	45(37)	56(35)

KPS- Karnofsky performance status, HCT-CI- hematopoietic cell transplantation-specific comorbidity index

Table 2: Transplant-associated variables of patients receiving their first allogeneic HCT for JMML, 2000 – 2017, grouped by Donor KIR genotype

Characteristic	AA N=43	Bx N=122	Total N=165
Stem cell source			
BM	24 (56)	72 (59)	96 (58)
PBSC	6 (14)	9 (7)	15 (9)
UCB	13 (30)	41 (34)	54 (33)
Unrelated donor high-resolution HLA typing			
8/8	24 (80)	57 (70)	81 (73)
≤7/8	6(20)	24 (30)	30 (27)
Cord blood high-resolution HLA typing			
8/8	0	2 (5)	2 (4)
≤7/8	12 (100)	36 (95)	48 (96)
Missing	1	3	4
Conditioning intensity - no. (%)			
MAC	43 (100%)	118 (97)	161 (98)
RIC/NMA	0	4 (3)	4 (2)
Conditioning regimen - no. (%)			
TBI based*	6(14)	28 (23)	34 (21)
Bu based**	37 (86)	90 (74)	127 (77)
Others (Flu or Mel based)	0	4 (4)	4 (2)
In-Vivo T cell depletion - no. (%)			
Yes (ATG)	21 (49)	70 (57)	91 (55)
No	21 (49)	44 (36)	65 (39)
Missing	1 (2)	8 (7)	9 (5)
GVHD prophylaxis - no. (%)			
CNI alone	0	8 (6)	8 (5)
CNI + MTX ± other(s) (except MMF)	22 (42)	59 (42)	91 (49)
CNI + MMF ± other(s) (except tacrolimus)	17 (39)	35 (29)	52 (31)
CNI + other(s) (except MMF, MTX)	3 (7)	17(14)	20(13)
Others including Ex-vivo T-cell depletion	1 (2)	3 (3)	4 (3)
Time from diagnosis to HCT (months) – median (range)	4 (1-32)	5 (<1-30)	4 (<1-32)

BM- bone marrow, PBSC- peripheral blood stem cells, UCB- umbilical cord, HLA: Human leucocyte Antigen, MAC- myeloablative conditioning. RIC/NMA- reduced-intensity conditioning/non-myeloablative, TBI- total body irradiation, Bu: Busulfan, Flu: Fludarabine, Mel: Melphalan, ATG- anti-thymocyte globulin, CNI: Calcineurin inhibitor (Tacrolimus or Cyclosporine), MTX- methotrexate, MMF- mycophenolate mofetil,*TBI based regimens included TBI/Cyclophosphamide (Cy), TBI/CY/Flu, TBI,Cy/Thiotepa (TT),** Bu based regimens included BuCyMel, BuCY, BuMel, BuFlu.

Table 3: Donor characteristics for patients receiving their first allogeneic HCT for JMML, 2000 – 2017, grouped by KIR genotype

Characteristic	AA N=42	Bx N=115	Total N=157
Donor B Content of KIR - no. (%)			
0	42 (100)	0	42 (27)
1	0	54 (47)	54 (34)
2	0	48 (42)	48 (31)
3	0	11 (10)	11 (7)
4	0	2 (2)	2 (1)
Centromeric regions score - no. (%)			
A/A	42 (100)	27 (23)	69 (44)
A/B	0	74 (64)	74 (47)
B/B	0	14 (12)	14 (9)
Telomeric regions score - no. (%)			
A/A	42 (100)	34 (30)	76 (48)
A/B	0	73 (63)	73 (46)
B/B	0	8 (7)	8 (5)
Donor KIR B content ranking score - no. (%)			
Best	0	15 (13)	15 (10)
Better	0	47 (41)	47 (30)
Neutral	42 (100)	53 (46)	95 (61)
KIR Composite score no. (%)			
2	0	34 (30)	34 (22)
3	42 (100)	54 (47)	96 (61)
4	0	27 (23)	27 (17)
Activating KIR content - no. (%)			
0-1	41 (98)	0	41 (26)
2-3	1 (2)	40 (35)	41 (26)
4-6	0	75 (65)	75 (48)
Presence of activating KIR2DS4 - no. (%)			
Absent	20 (47)	65 (53)	85 (52)
Present	23 (53) (	57 (47)	80 (48)
Ligand-Ligand Mismatch - no. (%)			
GVH	6 (14)	8 (7)	14 (9)
GVH, HVG	1 (2)	1 (1)	2 (1)
HVG	2 (5)	17 (15)	19 (12)
Matched	33 (79)	89 (77)	122 (78)
KIR-Ligand Mismatch - no. (%)			
GVH	33 (79)	66 (57)	99 (63)
Matched	9 (21)	49 (43)	58 (37)
Missing Ligand - no. (%)			

Presence	10 (24)	48 (42)	58 (37)
Absence	32 (76)	67 (58)	99 (63)

GVH- graft-versus-host direction, HVG- host-versus-graft direction

Table 4. Univariate outcomes in JMML HCT recipients by donor KIR B haplotype

	AA (N = 42)		Bx (N = 115)			Total (n=157)
Outcomes	**N Eval**	**Prob (95% CI)**	**N Eval**	**Prob (95% CI)**	**p-value**	
Grade II-IV acute GVHD	29		87		0.09	
100-day		48 (31-66)%		31 (22-41)%		36 (27-44)%
Chronic GVHD	41		111		0.79	
1-year		23 (11-37)%		23 (16-32)%		23 (17-30)%
3-year		26 (14-41)%		27 (19-36)%		27 (20-34)%
Relapse	42		113		0.10	
1-year		17 (7-29)%		25 (17-33)%		23 (16-30)%
3-year		17 (7-29)%		29 (21-38)%		26 (19-33)%
5-year		17 (7-29)%		30 (22-39)%		27 (20-34)%
TRM	42		113		0.64	
1-year		19 (9-32)%		13 (8-20)%		15 (10-21)%
3-year		19 (9-32)%		15 (9-22)%		16 (11-22)%
5-year		19 (9-32)%		16 (10-24)%		17 (11-23)%
DFS	42		113		0.31	
1-year		64 (49-78)%		62 (53-71)%		63 (55-70)%
3-year		64 (49-78)%		55 (46-65)%		58 (50-66)%
5-year		64 (49-78)%		53 (44-63)%		56 (48-64)%
OS	42		113		0.81	
1-year		71 (57-84)%		75 (67-83)%		74 (67-81)%
3-year		67 (52-80)%		67 (58-75)%		67 (59-74)%
5-year		67 (52-80)%		63 (54-72)%		64 (56-71)%
GRFS	29		87		0.67	
1-year		31 (16-49)%		29 (20-39)%		29 (21-38)%
3-year		28 (13-45)%		26 (18-36)%		27 (19-35)%
5-year		28 (13-45)%		26 (18-36)%		27 (19-35)%

GVHD: Graft versus host disease, TRM: Transplant related mortality, DFS, Disease Free survival, OS: overall survival, GRFS: GVHD free relapse free survival.

Table 5: Analysis of NK- KIR related determinants and primary and secondary outcomes.

KIR Model	Variable	OS HR (95% CI)	Relapse HR (95% CI)	RFS HR (95% CI)	TRM HR (95% CI)	GRFS HR (95% CI)	aGVHD II-IV HR (95% CI)	cGVHD HR (95% CI)
Donor KIR haplotype	AA Bx	1.00 1.08 (0.59-1.98)	1.00 1.90 (0.84-4.29)	1.00 1.35 (0.76-2.39)	1.00 0.87 (0.38-1.99)	1.00 0.88 (0.58-1.34)	1.00 0.62 (0.37-1.06)	1.00 1.11 (0.54-2.28)
Donor KIR B content	0-1 ≥ 2	1.00 1.23 (0.72-2.08)	1.00 1.05 (0.56-1.97)	1.00 1.21 (0.75-1.95)	1.00 1.48 (0.69-3.14)	1.00 0.73(0.50-1.08)	1.00 **0.46 (0.26-0.83)***	1.00 0.84 (0.44-1.62)
Donor KIR Centromeric Region Score	AA AB BB	1.00 0.92 (0.53-1.59) 0.92 (0.35-2.45)	1.00 1.32 (0.70-2.49) 0.60 (0.14-2.58)	1.00 1.25 (0.76-2.06) 0.83 (0.31-2.17)	1.00 1.15 (0.51-2.58) 1.13 (0.30-4.25)	1.00 0.86 (0.58-1.26) 0.58 (0.26-1.28)	1.00 **0.57 (0.33-098)**** 0.85 (0.33-2.17)	1.00 0.95 (0.50-1.84) 0.77 (0.23-2.61)
Donor KIR Telomeric Region Score	AA AB BB	1.00 1.40 (0.80-2.43) 1.65 (0.57-4.79)	1.00 1.43 (0.74-2.73) 2.47 (0.82-7.38)	1.00 1.27 (0.77-2.10) 2.10 (0.87-5.07)	1.00 1.06 (0.48-2.37) 1.00 (0.36-7.22)	1.00 0.92 (0.63-1.35) 1.02 (0.44-2.36)	1.00 **0.58 (0.34-1.00)**#** 0.70 (0.22-2.29)	1.00 0.92 (0.48-1.77) 1.52 (0.45-5.15)
Donor KIR B content ranking score	Best Better Neutral	1.00 0.95 (0.37-2.45) 0.90 (0.37-2.17)	1.00 1.45(0.41-5.09) 1.36 (0.41-4.50)	1.00 1.33 (0.53-3.33) 1.16 (0.49-2.77)	1.00 1.21 (0.31-4.69) 0.96 (0.27-3.41)	1.00 1.33 (0.61-2.89) 1.68(0.81-3.50)	1.00 0.56 (0.19-1.61) 1.29 (0.51-3.26)	1.00 1.51 (0.43-5.29) 1.38(0.41-4.59)
KIR Composite Score	2 3 4	1.00 1.59 (0.74-3.43) 1.79 (0.73-4.38)	1.00 0.86 (0.39-1.86) 1.33 (0.54-3.27)	1.00 1.10 (0.59-2.06) 1.19 (0.55-2.57)	1.00 1.65 (0.56-4.85) 0.89 (0.20-3.96)	1.00 1.00 (0.62-1.62) 1.20 (0.66-2.16)	1.00 0.79 (0.43-1.48) 1.00 (0.46-2.16)	1.00 0.79 (00.36-1.72) 1.10 (0.43-2.86)
Activating KIR content	0-1 2-3 4-6	1.00 0.62 (0.27-1.43) 1.28 (0.68-2.40)	1.00 1.47 (0.56-3.86) 2.02 (0.87-4.70)	1.00 1.03 (0.50-2.10) 1.44 (0.79-2.63)	1.00 0.64 (0.21-1.96) 0.94 (0.39-2.26)	1.00 0.83 (0.50-1.40) 0.82 (0.52-1.28)	1.00 0.73 (0.38- 1.41) **0.52 (0.29-0.95)**##	1.00 1.48 (0.65-3.38) 0.86 (0.39-1.90)
Presence of KIR DS4	Absent Present	1.00 0.66 (0.38-1.14)	1.00 0.60 (0.32-1.12)	1.00 0.74 (0.46-1.21)	1.00 1.05 (0.49-2.26)	1.00 0.69 (0.47-1.01)	1.00 0.66 (0.39-1.10)	1.00 1.70 (0.90-3.23)

Overall Survival, RFS: Relapse Free Survival, GRFS: Graft versus host disease, Relapse Free Survival, aGVHD: acute Graft versus host disease, cGVHD: Chronic graft versus host disease**, *p=0.01, **p=0.0410, #p=0.0479, ##p=0.0327 .**

Table 6: Analysis of Ligand- Ligand (L-L) and KIR-Ligand (KIR-L) mismatch in mismatched unrelated donors (n=74) and primary and secondary outcomes.

KIR model	Variable	OS HR (95% CI)	Relapse HR (95% CI)	RFS HR (95% CI)	TRM HR (95% CI)	GRFS HR (95% CI)	Grade II-IV aGVHD HR (95% CI)	cGVHD HR (95% CI)
L-L Mismatch	GVH direction vs Others	**1.00** **0.46 (0.21-1.02)**	1.00 0.65 (0.24-1.77)	1.00 0.66 (0.31-1.40)	1.00 0.52 (0.18-1.48)	1.00 0.85 (0.44-1.65)	1.00 0.66 (0.31-1.40)	1.00 0.67 (0.32-1.43)
KIR-L Mismatch	GVH direction vs Others	1.00 0.81 (0.37-1.76)	1.00 1.44 (0.61-3.42)	1.00 1.18 (0.61-2.30)	1.00 0.82 (0.29-2.32)	1.00 1.07 (0.62-1.83)	1.00 1.18 (0.61-2.30)	1.00 1.15 (0.59-2.24)

GVH: Graft versus Host, OS: Overall Survival, RFS: Relapse Free Survival, GRFS: Graft versus host disease, Relapse Free Survival, aGVHD: acute Graft versus host disease, cGVHD: Chronic graft versus host disease.

Figure 1: KIR models predicting NK alloreactivity according to HLA typing of donor, recipient, and donor KIR typing.

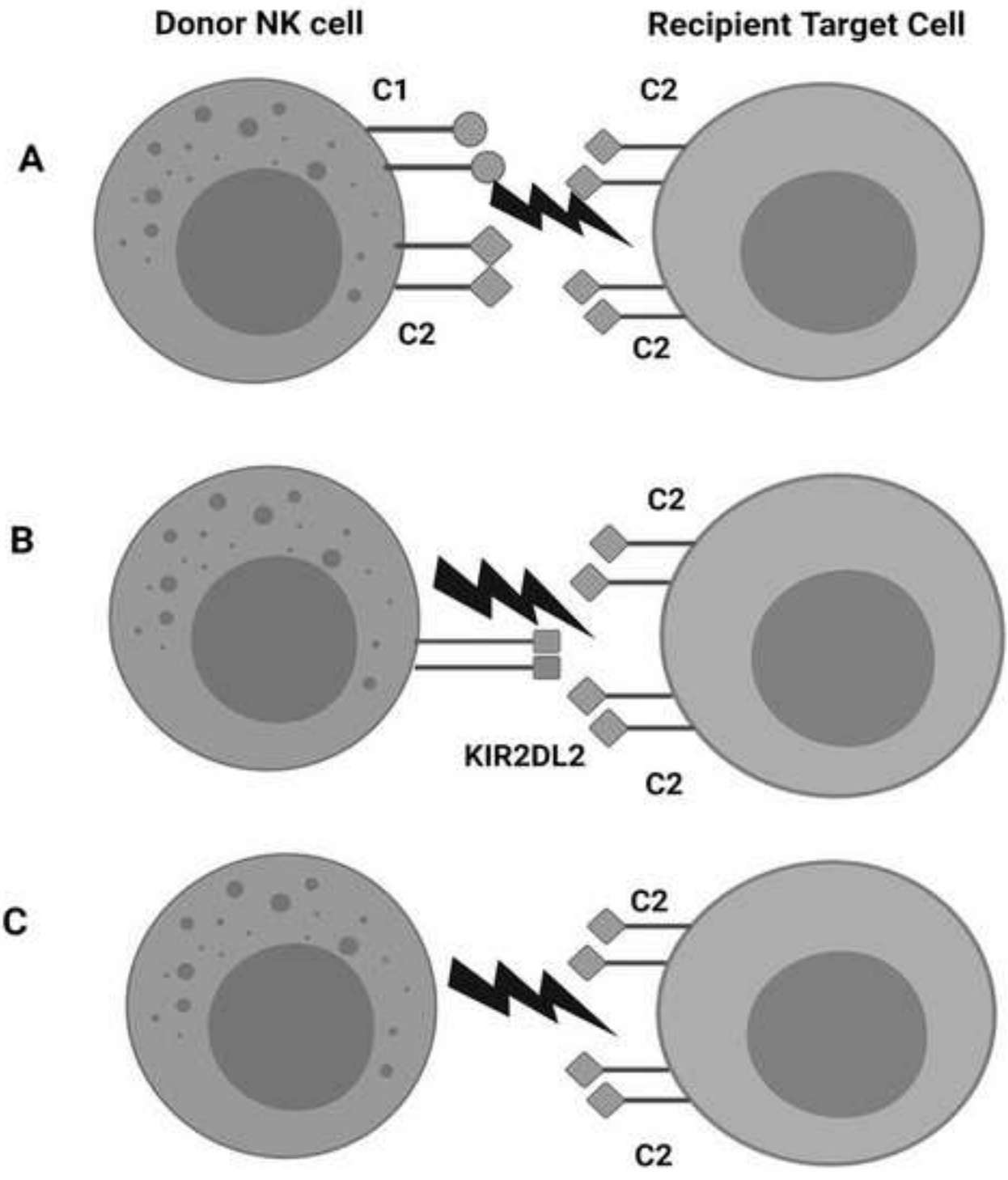

Figure 2: Relapse in JMML HCT recipients by donor KIR B haplotype: AA (n=42) vs Bx (n=115) donors.

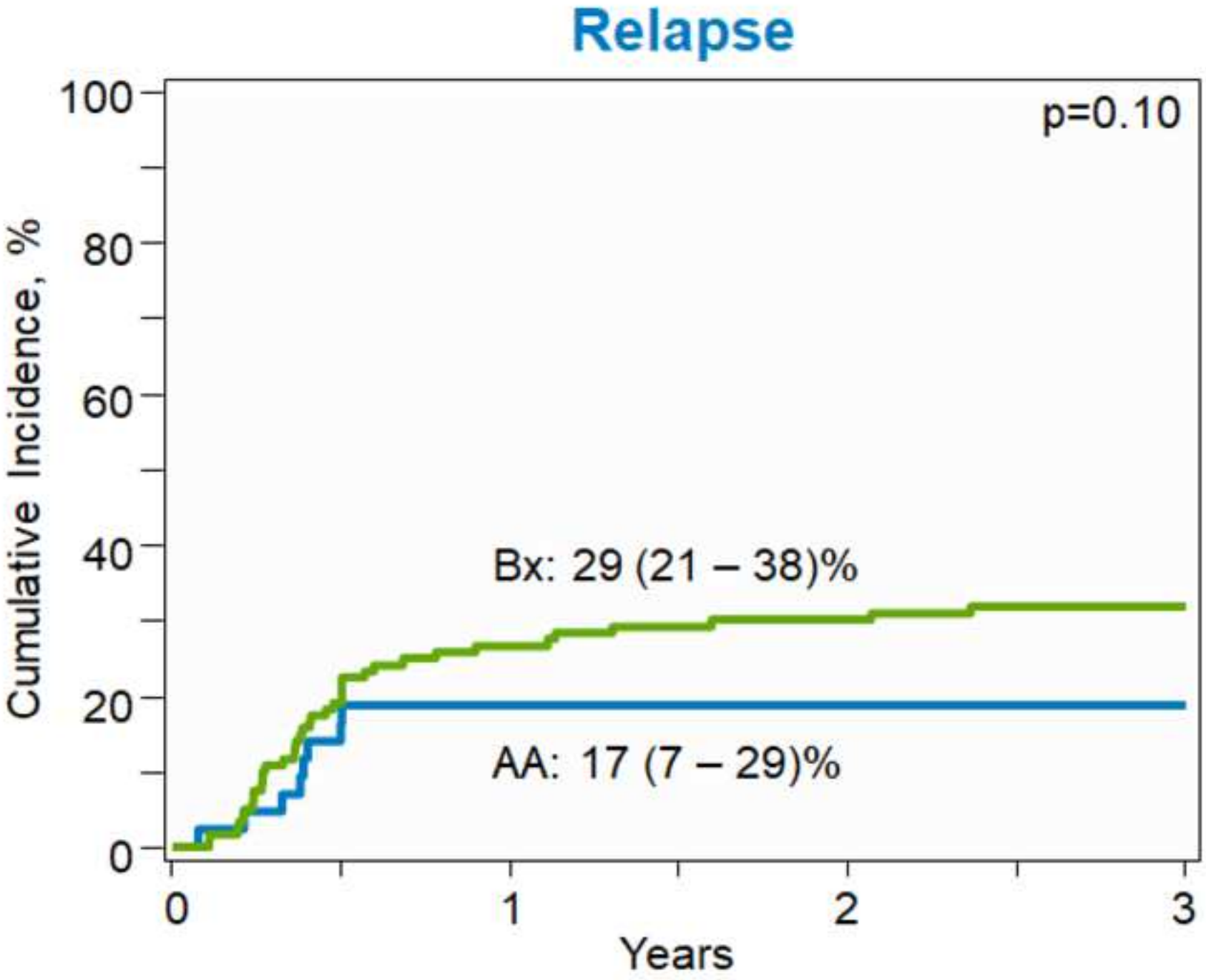

Figure 3: Disease-free survival in JMML HCT recipients by donor KIR B haplotype: AA (n=42) vs Bx (n=115) donors

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