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## Natural Killer Cell Therapy in Children with Relapsed Leukemia

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### Abstract

**Background**—Novel therapies are needed for children with relapsed or refractory leukemia. We therefore tested the safety and feasibility of haploidentical natural killer cell therapy in this patient population.

**Procedure**—Twenty-nine children who had relapsed or refractory leukemia were treated with chemotherapy followed by the infusion of haploidentical NK cells. Cohort 1 included 14 children who had not undergone prior allogeneic hematopoietic cell transplantation (HCT), whereas Cohort 2 included 15 children with leukemia that had relapsed after HCT.

**Results**—Twenty-six (90%) NK donors were KIR mismatched (14 with one KIR and 12 with 2 KIRs). The peak NK chimerism levels were >10% donor in 87% of the evaluable recipients. In Cohort 1, 10 had responsive disease and 12 proceeded to HCT thereafter. Currently, 5 (36%) are alive without leukemia. In Cohort 2, 10 had responsive disease after NK therapy and successfully proceeded to second HCT. At present, 4 (27%) are alive and leukemia-free. The NK cell infusions and the IL-2 injections were well-tolerated.

**Conclusions**—NK cell therapy is safe, feasible, and should be further investigated in patients with chemotherapy-resistant leukemia.

### Keywords

NK cell; childhood leukemia; relapse; cell therapy

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### AUTHORSHIP CONTRIBUTIONS

W. L., H.I., and J.R. designed the study, analyzed and interpreted data, and wrote the paper. G.L. performed statistical analyses. W.L., H.I., K.G., C.H., B.M.T., M.D., D.S., T.G., and C-H.P provided study material and patient information, and contributed to the interpretation of data. W.L., H.I., and J.R drafted the paper. All authors contributed to the revisions of the draft and approval of the final manuscript. The authors declare no conflicts of interest.

## INTRODUCTION

Children with newly diagnosed acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) experience long-term disease free survival rates greater than 80% and 60%, respectively, when treated on contemporary clinical trials.[1–9] However, the outcome of patients with refractory or relapsed disease remains poor, with survival rates of less than 40%.[10–14] Except for the small group of patients with late recurrences of B-lineage ALL, children with relapsed ALL or AML are unlikely to be cured with chemotherapy alone, and hematopoietic cell transplantation (HCT) is the treatment of choice.[15, 16]

The development of novel methods of graft preparation has led to high success rates of HCT in children regardless of matched donor availability.[17–19] In addition, modern supportive care measures have reduced the risk of death related to regimen toxicity, infection, and graft-versus-host disease (GVHD) to less than 15%. Nevertheless, HCT is not always successful, as leukemia relapse remains common. In this regard, the most powerful predictor of relapse after HCT is the level of minimal residual disease (MRD) at the time of transplantation.[20–22] In this high risk, often heavily pretreated pediatric oncology population, additional attempts at MRD reduction with chemotherapy combinations prior to HCT or dose escalation of conditioning chemotherapy during HCT are unlikely to improve outcome and may contribute to overall morbidity and mortality. Therefore, new therapeutic strategies that have non-cross resistant mechanisms of action and no overlapping toxicities are desirable.[23] One such approach is immunotherapy with the use of allogeneic natural killer (NK) cells.

NK cells can target and kill leukemia cells without prior exposure to those cells.[24] In the setting of allogeneic HCT, several studies have demonstrated the powerful effect of NK cells against leukemia.[25–30] Recently, we demonstrated that infusions of haploidentical NK cells in patients with AML were well tolerated and void of GVHD.[31] In the present study, we infused haploidentical NK cells from parental donors to 29 patients with relapsed or refractory childhood leukemia. The safety, feasibility and outcome strongly support further investigations into the use of NK cells in frontline leukemia treatments.

## METHODS

### Patients

Two cohorts of patients were treated with haploidentical NK cells. The first cohort included patients who had refractory leukemia and had not received prior HCT. The second cohort had leukemia that had relapsed after prior allogeneic HCT. Availability of matched donors was not an exclusion criterion; these patients were eligible to proceed to HCT with a matched donor if they had stable or responsive disease after the haploidentical NK cell therapy.

### Conditioning and NK cell purification

Patients were enrolled on the St. Jude NKAML or NKHEM protocols (ClinicalTrials.gov NCT00697671 and NCT00187096) and received clofarabine 40 mg/m<sup>2</sup>, etoposide 100 mg/m<sup>2</sup>, and cyclophosphamide 400 mg/m<sup>2</sup> on days-6 through -2 before NK cell infusion.

[32] On alternate days, 1 million units/m<sup>2</sup> of IL-2 was administered subcutaneously for 6 doses starting on day -1 to activate and expand circulating donor NK cells. The donors underwent apheresis on day-1 and the product was purified for CD3<sup>-</sup> CD56<sup>+</sup> cells in our Human Applications Laboratory by a 2-step procedure using magnetic activated cell sorting. [33] First, the CliniMACS system (Miltenyi Biotec, Cambridge, MA) was used to deplete T cells from the mononuclear cell product obtained by leukapheresis by using anti-CD3 antibody-coated beads. Second, the CD3-depleted product was enriched for CD56<sup>+</sup> cells by incubation with anti-CD56 antibody-coated beads. The entire cell-purification process lasted less than 12 hours and the final products were infused immediately without in vitro exposure to IL-2 or other cytokines.

### Response criteria

MRD was studied by flow cytometry as previously described.[34, 35] Briefly, bone marrow mononuclear cells were labeled with various combinations of monoclonal antibodies conjugated to fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, and allophycocyanin directed against surface antigens, or isotype-matched nonreactive monoclonal antibodies which served as a control for non-specific binding to Fc receptors. After 10 minutes incubation at 20°C in the dark, the 4-color stained cell preparations were washed in PBSA twice, fixed in 0.5% paraformaldehyde, and analyzed with a dual laser-FACSCalibur flow cytometer with Cell Quest Pro software (Becton Dickinson, San Jose, CA). Results were reported as percentage of nucleated cells with the leukemia-associated immunophenotype.

For this study, patients were considered to have attained complete remission (CR) if bone marrow blasts were less than 5% with evidence of trilineage hematopoietic recovery. Complete remission with incomplete blood count recovery (CRi) was defined as less than 5% bone marrow blasts without trilineage hematopoietic recovery. Patients achieved a partial response (PR) if the bone marrow contained 5% to 25% blasts and a decrease of at least 50% in blast percentage. All other patients were considered to have no response (NR).

### Statistics

Summary statistics were provided for baseline variables at the time of NK cell therapy and for cell composition before and after NK cell purification. Survival rates were estimated by the method of Kaplan-Meier. All reported *P* values are two sided and are considered significant if <.05. Statistical analyses were performed with SAS software (version 9.2, Cary, NC).

## RESULTS

The 29 patients who received haploidentical NK cell infusions included 14 children with relapsed or refractory leukemia (no prior HCT), and 15 children with leukemia that had relapsed after HCT (Table I). Among the 14 children with relapsed or refractory leukemia who had not undergone prior HCT (8 with ALL and 6 with AML), 1 was in morphologic remission, but with detectable MRD, whereas 4 had 5% to 25% blasts, and 9 had more than 25% blasts in the marrow at the time of NK cell therapy (Table II). Following NK cell

therapy, 7 patients achieved CR or CRi, 3 attained PR, and 4 had NR; 12 patients proceeded to subsequent HCT. Currently, 5 patients (36%) are alive with no evidence of disease, 6 died of progressive disease, and 1 died from HCT-related toxicity (Fig. 1).

Among the 15 patients (9 with ALL and 6 with AML) whose leukemia had relapsed after prior allogeneic HCT, all had detectable leukemia at the time of NK cell therapy, including 10 patients with greater than 25% blasts (Tables I and II). Despite having undergone prior HCT and having high leukemic burdens at the time of NK cell therapy, 10 of these 15 had responsive disease (7 CR/CRi and 3 PR) and successfully proceeded to second HCT. At last follow-up, 4 (27%) are alive and leukemia-free, 3 died of progressive leukemia after the second HCT and 3 died of HCT-related toxicity (Fig 1).

All NK cell donors underwent apheresis without complications. Patients received a median of  $18.6 \times 10^6/\text{kg}$  NK cells (range, 3.5 to  $103 \times 10^6/\text{kg}$ ), with undetectable or minimal contamination of products by B or T cells (Table III). Among the 29 donors, 26 (90%) were KIR mismatched (14 with one KIR and 12 with 2 KIRs; Table IV). The NK cell infusions and the IL-2 injections were well-tolerated with no attributable side effects. Specially, none of the patients developed GVHD, cytokine storm, or effusion syndrome. Among the 29 patients, 23 had sufficient numbers of NK cells to perform chimerism studies. The donor chimerism levels peaked between Day 7 and Day 14, reaching 100% donor in 16 recipients, 12–98% in 4 recipients, 1% in one recipient, and 0% in two recipients.

## DISCUSSION

The outcome of children with primary refractory or relapsed ALL or AML remains poor, with survival rates approximately 30%. [10, 11] Although allogeneic HCT is potentially curative for patients who have relapsed, short and long-term treatment-related morbidity remain significant problems. In addition, the risk of subsequent relapse is high for patients who undergo HCT with high disease burden. [22] Clearly, alternative therapies, such as NK cell therapy, are needed for patients with chemotherapy resistant disease.

In the setting of allogeneic HCT, the beneficial effects of donor NK cells have been well documented. [25, 26, 30] We and others have also demonstrated the safety and feasibility of NK cell infusions in the absence of HCT. [31, 36–38] The key determinant of NK cell activity is the KIR gene family. As KIRs are highly polymorphic in gene alleles and cell surface expression, KIR typing is essential for donor selection. [24, 39–41] Better outcomes were observed in pediatric leukemia if the donor NK cells expressed a certain KIR that the cognate ligand was absent in the recipient. [42] In the present study, 20 of the 29 patients achieved at least a PR, including 11 of 17 patients with ALL and 9 of 12 with AML. We attribute the favorable responses in part to the preferential use of KIR mismatched donors. KIR typing did not delay the NK cell therapy as the results were available before donor infectious evaluations were completed in all recipients.

Although this report includes the largest number of NK recipients published to date, our study is still limited by relatively small number of patients. In addition, the contribution of the NK cell infusions cannot be determined, since all patients received intensive

chemotherapy. Randomized studies are needed to address this issue. Moreover, future studies are needed to determine the optimal timing of NK cell therapy, the best preparative regimen, and the most effective methods to enhance NK cell activity. In this regard, we have established methods to generate large numbers of activated NK cells for clinical use.[43, 44] Addition of  $RxR\gamma$  agonists or lineage-specific antibodies such as those against CD19 or CD33 may further augment the donor NK cell activity.[45, 46] Enhancement of NK activity by using anti-KIR antibodies to block inhibitory KIRs is another attractive approach; this strategy has been proven to be safe in patients with AML and was shown to enhance NK-cell-mediated cytotoxicity against CD20+ lymphomas.[47, 48] A more potent reagent to overcome inhibitory signals and enhance NK activity may be bispecific killer cell engagers, antibodies that are designed to bind both NK cell and target antigens. Recently, a novel bispecific antigen that activates NK cells through CD16 and binds AML cells via CD33 was shown to enhance the antileukemic effects of NK cells.[49] An alternative approach is to deplete host regulatory T cells, which may inhibit the proliferation of donor NK cells. The results of a recent clinical trial demonstrated that depletion of host regulatory T cells by an IL-2 diphtheria toxin fusion protein may improve the efficacy of NK cell therapy for AML. [50]

The present study demonstrates that haploidentical NK cell therapy can be readily administered even in patients with rapidly progressive leukemia, is well-tolerated, and may be useful in reduction of disease burden prior to HCT. Among the 29 patients with relapsed or refractory leukemia who received clofarabine, etoposide, cyclophosphamide, and NK cells, 22 proceeded to HCT and 9 are alive. Although the small patient numbers and potential differences in patient characteristics preclude formal comparisons, only 2 of 15 patients previously treated at our institution with the same chemotherapy, but without NK cells, are alive.[32] The results from this study, together with those described above, should encourage future investigations of NK therapy for patients with refractory leukemias, as well as for those with newly diagnosed high-risk disease.

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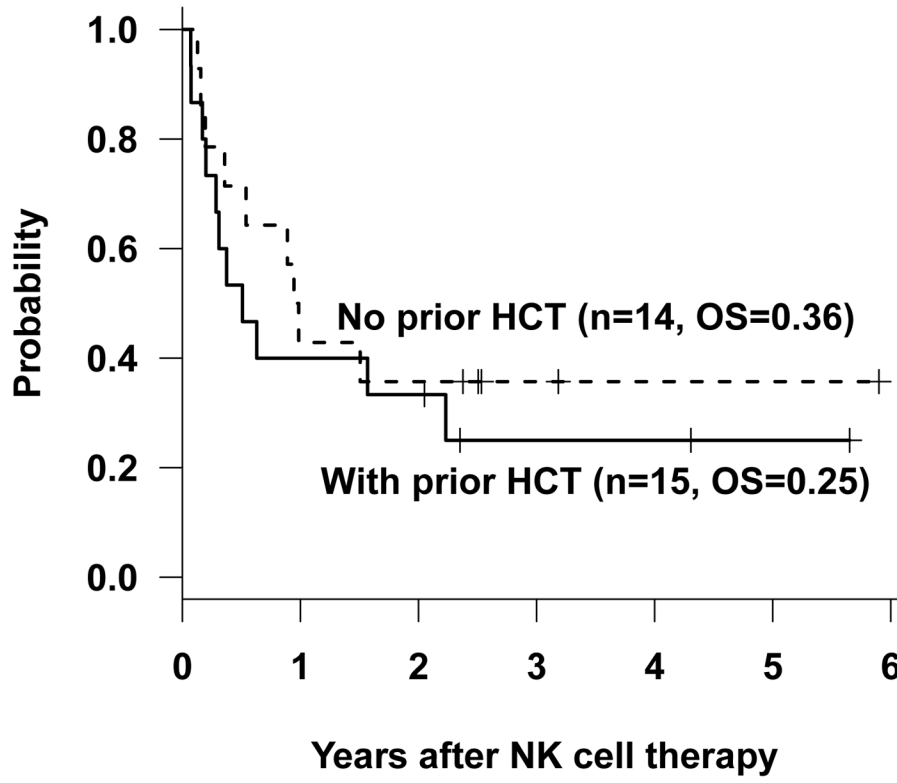


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**Figure 1.**  
Survival probability after NK cell therapy and HCT.

**Table I**

## Patient summary

<b>Characteristic</b>	<b>No prior HCT N</b>	<b>Prior HCT N</b>
Number of patients	14	15
Age (years)		
<10	8	10
>10	7	5
Sex		
Male	9	9
Female	5	6
Race		
White	9	15
Non-white	5	0
Disease		
ALL	8	9
AML	6	6
Leukemia blast % in BM at the time of NK therapy		
<0.01%	0	0
0.01% – 5%	1	1
5% – 25%	5	4
>25%	8	10
Haploidentical NK donor		
Parent	13	15
Other adult relatives	1	0
Current report HCT donor		
Matched unrelated	5	6
Haploidentical	7	4

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**Table II**

Patient details

Patient	Prior HCT	Diagnosis	Relapse	Peak Chimerism (%)	Pre NK Blasts (%)	Post NK Blasts (%)	Response	HCT after NK	Status
1	No	ALL	1	0	40	18	PR	yes	Dead
2	No	ALL	1	1	2	Neg	CR	yes	Alive
3	No	AML	1	12	80	91	NR	yes	Dead
4	No	ALL	1	17	30	Neg	CR	yes	Alive
5	No	ALL	1	82	97	Neg	CR	yes	Dead
6	No	ALL	1	100	15	Neg*	CRi	no	Dead
7	No	AML	1	100	80	14	PR	no	Alive
8	No	ALL	1	100	37	6	PR	yes	Dead
9	No	ALL	1	100	92	100	NR	yes	Dead
10	No	ALL	1	100	20	96	NR	yes	Dead
11	No	AML	1	100	40	31	NR	yes	Dead
12	No	AML	1	100	56	2	CR	yes	Dead
13	No	AML	1	100	12	0.3	CR	yes	Alive
14	No	AML	1	QNS	15	Neg*	CRi	yes	Alive
15	Yes	ALL	2	0	100	100	NR	yes	Dead
16	Yes	ALL	2	24	30	Neg	CR	yes	Dead
17	Yes	AML	2	49	10	Neg*	CRi	yes	Dead
18	Yes	ALL	2	98	90	90	NR	no	Dead
19	Yes	ALL	2	100	14	Neg	CR	no	Dead
20	Yes	ALL	2	100	100	100	NR	no	Dead
21	Yes	ALL	2	100	95	95	NR	yes	Dead
22	Yes	ALL	2	100	97	Neg*	CRi	yes	Alive
23	Yes	ALL	2	100	14	3	PR	yes	Alive
24	Yes	AML	2	100	90	36	PR	yes	Alive
25	Yes	AML	1	100	93	Neg*	CRi	yes	Alive
26	Yes	AML	1	100	13	4	PR	yes	Dead
27	Yes	AML	1	QNS	11	Neg*	CRi	no	Dead

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Patient	Prior HCT	Diagnosis	Relapse	Peak Chimerism (%)	Pre NK Blasts (%)	Post NK Blasts (%)	Response	HCT after NK	Status
28	Yes	AML	2	QNS	52	NE	NE	no	Dead
29	Yes	ALL	2	QNS	90	Neg*	CRi	yes	Dead

Neg, negative; Neg\*, no detectable blasts, but hypocellular marrow; PR, partial response; CR, complete remission; CRi, complete remission with incomplete count recovery; QNS, quantity not sufficient; NE, not evaluable.

**Table III**

Cell populations before and after NK cell purification

<b>Cells</b>	<b>Before cell processing Median (range)</b>	<b>After cell processing Median (range)</b>
TNC/kg ( $10^6$ )	401 (46 – 2044)	18.8 (3.8 – 104)
CD56 <sup>+</sup> NK cell %	10.1 (4.47 – 24.5)	98.4 (84.7 – 99.6)
CD56 <sup>+</sup> cells/kg ( $10^6$ )	49.9 (6.75 – 248)	18.6 (3.5 – 103)
CD3 <sup>+</sup> 56 <sup>-</sup> T cell %	56.1 (15.5 – 69.2)	0 (0 – 0.14)
CD3 <sup>+</sup> 56 <sup>-</sup> cells/kg ( $10^6$ )	202 (26.3 – 990)	0 (0 – 0.01)
CD19 <sup>+</sup> B cell %	10.2 (3.13 – 21.4)	0.31 (0 – 1.69)
CD19 <sup>+</sup> cells/kg ( $10^6$ )	40.1 (4.85 – 239)	0.04 (0 – 0.71)

TNC; total nucleated cells.

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**Table IV**

KIR mismatch between NK cell donor and recipient

<b>Characteristic</b>	<b>No prior HCT N</b>	<b>Prior HCT N</b>
Number of patients	14	15
Mismatched KIR(s)		
2DL1	4	5
3DL1	3	2
2DL1, 3DL1	3	3
2DL2, 2DL3	3	3
2DL3, 3DL1	0	0
None	1	2

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