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RESEARCH

Ex vivo-expanded and activated haploidentical natural killer cells infusion before autologous stem cell transplantation in high-risk neuroblastoma: a phase I/II pilot study

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Abstract

Given that natural killer (NK; CD3 − CD56 +) cells-mediated antibody-dependent cell cytotoxicity (ADCC) plays an important role in targeting neuroblastoma (NB) cells, adoptive cell therapy (ACT) utilizing expanded and activated haploidentical NK cells has emerged as a promising immunotherapeutic approach in pediatric patients with high-risk NB. In this pilot study, five pediatric patients with high-risk NB were enrolled. After harvesting hematopoietic progenitor cells (HPCs), patients received an intravenous infusion of high-activity iodine-131 (^{131}I)-meta-iodobenzylguanidine (^{131}I-MIBG). Seven days after the ^{131}I-MIBG infusion and before the delivery of a single infusion of haploidentical purified NK cells, patients were administered a preparative regimen to establish a lymphodepleted host environment conducive to improved donor NK cell survival. Four days after the NK cell infusion, patients underwent the conditioning regimen, then received autologous hematopoietic stem cell transplantation (AHSCT). All patients achieved successful neutrophil and platelet engraftment. No adverse reactions were noted during or after the infusion of NK cells. Our study shows that incorporating NK cell infusion before AHSCT as a component of the conditioning regimen for consolidative therapy in pediatric patients with high-risk NB can be safe and well tolerated. IRCT Registration Number: IRCT20140818018842N32.

Keywords Neuroblastoma · Natural killer cell therapy · Autologous hematopoietic stem cell transplantation · Adoptive cell therapy

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Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor in childhood that arises from the neural crest during embryogenesis [1]. It is quite heterogeneous at clinical presentation, with approximately 50% of the patients classified as having high-risk diseases via the International Neuroblastoma Risk Group (INRG) staging system [2]. For high-risk NB, major international cooperative groups (German Pediatric Oncology and Hematology Society (GPOH), Children's Oncology Group (COG), and the International Society of Pediatric Oncology (SIOP)) use intensive multimodal approaches, including induction with multiagent chemotherapy and surgical resection, consolidation with radiation therapy, myeloablative chemotherapy followed by autologous hematopoietic stem cell transplantation (AHSCT), treatment of minimal residual disease (MRD) with retinoids, and immunotherapy using a tumor-specific anti-disialoganglioside (GD2) antibody, granulocyte–macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-2 (IL-2) [3].

Additionally, patients with diagnostic iodine-123 (^{123}I)-meta-iodobenzylguanidine (^{123}I-MIBG) avid tumors may receive iodine-131 (^{131}I)-MIBG targeted radiation therapy before AHSCT as consolidation treatment [4]. However, the outcome is particularly dismal, with long-term progression-free survival (PFS) of up to 50–60%, highlighting the need for novel approaches [5].

The incorporation of anti-GD2 antibody as MRD treatment following AHSCT for high-risk NB has emerged as the standard of care, resulting in a remarkable improvement in event-free survival (EFS) by 20% [6]. The effectiveness of anti-GD2 monoclonal antibodies is influenced by natural killer (NK; CD3-CD56 +) cell-mediated antibody-dependent cell cytotoxicity (ADCC), which is facilitated by GM-CSF and IL-2.

NK cells, as immune effector cells, are a heterogeneous group of lymphocytes belonging to the innate immune system, with different maturation statuses and functional specificities [7]. They are presumed to be key effectors in the immunosurveillance of cancers and viral infections by integrating multiple signals of activating and inhibitory killer cell immunoglobulin-like receptors (KIR; also known as CD158), which bind to specific ligands expressed on tumor and virus-infected cells [2].

NK cells can target and lyse tumor cells lacking human leukocyte antigen (HLA)-class I molecules, which represent ligands of killer inhibitory receptors. Together with the presence of different ligands for activating receptors such as NKG2D, NK cell-mediated ADCC contributes to the killing of NB cells [8, 9]. NKG2D is a multi-subunit activation receptor. Unlike normal cells, where NKG2D ligands are expressed transiently, many primary tumors persistently express NKG2D ligands. Nevertheless, the prolonged interaction of NKG2D with tumor cell-bound ligands impairs NKG2D function both in vitro and in vivo [10].

IL-2 may not be the optimal cytokine for activating NK cells because of its potential to enhance the proliferation of regulatory T cells (Tregs), which could diminish NK cell antitumor efficacy [11]. On the other hand, NB is considered a poorly immunogenic tumor (cold tumor) due to low/absent HLA-class I expression, downregulation of molecules that activate T and NK cells, secretion of immunomodulatory cytokines, release of exosomes containing immune suppressive molecules, and secretion of immunomodulatory cytokines (e.g., IL-10 and transforming growth factor-β1 (TGF-β1)) [12–14]. Nevertheless, the presence of tumor-infiltrating lymphocytes (TILs), comprising T cells and NK cells (constituting up to approximately 30% of TILs), has been demonstrated in NB in several studies [15, 16].

Given the factors mentioned earlier, it may be essential to explore alternative approaches to enhance NK cell cytotoxicity against neuroblasts to address the high relapse rate following this treatment. Innovative transplant approaches involving the selection of the most appropriate hematopoietic stem cell (HSC) donor within an allogeneic context are under investigation. This selection may involve alloreactive NK cell subsets that lack inhibitory KIRs specific to the HLA-I alleles of the recipient, as observed in procedures such as HLA-haploidentical hematopoietic stem cell transplantation (HSCT) [17].

Consequently, adoptive cell therapy (ACT) utilizing expanded and activated haploidentical NK cells has emerged as a promising immunotherapeutic approach for pediatric patients with high-risk NB. The objective of this study was to evaluate the safety, feasibility, and possible efficacy of haploidentical NK cell ACT to eradicate MRD throughout the consolidation treatment phase before AHSCT.

Patients and methods

Study design, definitions, and endpoints

This prospective phase I/II study was conducted at the Research Institute for Oncology, Hematology, and Cell Therapy (RIOHCT), affiliated with Tehran University of Medical Sciences (TUMS), Tehran, Iran after receiving approval from the institutional review board. Between August 2022 and September 2023, five pediatric patients (age < 21 years) with newly diagnosed high-risk NB, who were candidates for their first AHSCT as consolidation treatment, were included in this study. High-risk NB was defined using the INRG staging system, MYCN status, patient age, and other

biological features in a schema identical to that used by the COG [4, 18, 19].

All patients had adequate organ function and were categorized as complete response/remission (CR) or very good partial response (VGPR) according to the International Neuroblastoma Response Criteria (INRC, A Consensus Statement from the National Cancer Institute Clinical Trials Planning Meeting) after completion of the induction phase chemotherapy evaluated by bone marrow morphology (bilateral routine staining of bone marrow), computed tomography (CT), magnetic resonance imaging (MRI), and/or MIBG scans. Patients were excluded from ^{131}I-MIBG therapy if the MIBG uptake at diagnosis was negative.

All donors and the parents or legal guardians of the patients provided written informed consent before proceeding with the trial, authorizing the use of their data for research purposes in accordance with the Declaration of Helsinki. No individual data were reported, and all authors were committed to protecting the privacy of the patients and donors recruited in this study.

The main goal of this study was to assess the safety and feasibility of the adoptive transfer of haploidentical donor NK cells to patients with high-risk NB. The secondary objective was to investigate preliminary efficacy signals, particularly PFS, after the administration of alloreactive haploidentical NK cells in conjunction with AHSCT.

Autologous hematopoietic progenitor cell collection and processing

All patients received peripheral blood as the source of autologous hematopoietic progenitor cells (HPCs). To mobilize HPCs, granulocyte colony-stimulating factor (G-CSF) was administered at a daily dose of 10 µg/kg body weight for four days. Peripheral blood leukapheresis was performed when the absolute peripheral CD34 + count was at least 20/µL to collect a minimum of 2×10^6 CD34 + cells/kg. Adequate products were obtained from all five patients. The unpurged HPC product was cryopreserved according to standard institutional operating procedures.

^{131}I-MIBG therapy

After harvesting HPCs, patients received an intravenous infusion of high-activity (8.8–14.2 mCi/kg) ^{131}I-MIBG in a radiation-protected isolation room until radiation emissions met institutional regulations. Concurrent implementation of thyroid protection with potassium iodide and potassium perchlorate, and a Foley catheter for bladder protection were also considered.

The required organ function parameters at the time of ^{131}I-MIBG administration included an absolute neutrophil count (ANC) of $\geq 0.5 \times 10^9$/L, a platelet count of $\geq 20 \times 10^9$/L, total bilirubin levels below twice the upper limit of normal, alanine aminotransferase (ALT) levels less than five times the upper limit of normal, a glomerular filtration rate (GFR) or creatinine clearance exceeding 60 mL/min/1.73 m^2, and the absence of clinically significant cardiac dysfunction or infection.

Haploidentical NK cell collection, processing, and infusion

Eligible donors were HLA-haploidentical parents who met the standard criteria for cell donation, according to the guidelines of the Foundation for the Accreditation of Cellular Therapy (FACT)/National Marrow Donor Program (NMDP). On day -20, approximately 50 ml of whole blood was collected via intravenous blood sampling. Peripheral blood mononuclear cells (PBMCs) were isolated, and NK cells were expanded under good manufacturing practice (GMP) conditions. CD3 + T cell-depleted PBMCs were expanded at a seeding density of 2×10^5 cells/mL in ex-vivo 10 media with 5% albumin, 2×10^6 irradiated autologous PBMCs (25 Gy), 10 ng/mL anti-CD3 monoclonal antibody (OKT3; *Orthoclone*, USA), and 500 IU/mL of MACS GMP recombinant human IL-2 (Miltenyi Biotec, USA). OKT3 was added once at the start of expansion to stimulate the T cell population in the irradiated feeder cells. NK cells received fresh media with 500 IU/mL of IL-2 every 2–3 days to maintain cellular concentration at $1–2 \times 10^6$ until harvesting on day -10.

Following expansion in culture, the cells were harvested, and NK cell purity was assessed by flow cytometry (CD3-/CD56 +). To assess the functional status of NK cells, intracellular flow cytometry (interferon (IFN)-γ), CD107a degranulation (Biolegend-USA), and lactate dehydrogenase (LDH) release assays (Sigma-Germany) against K562 (IBRC-Iran) were used as targets. The cells were washed using the Sepax System (Biosafe, Eysins, Switzerland) and suspended in 100 ml Plasma-Lyte supplemented with 0.5% human serum albumin. All NK cell products met the release criteria, including negative Gram staining, endotoxin assay < 5 EU/kg patient weight, absence of mycoplasma contamination, and visual inspection showing no contamination with a cell viability of ≥ 80%. Regarding product quality, the median purity was 85.6 ± 3.6% (CD3-CD56 +) with minimal CD3 + T cell contamination of < 5%.

To establish a lymphodepleted host environment conducive to improved donor NK cell survival, patients received cyclophosphamide (60 mg/kg on days -13 and -12) 7 days after receiving a high-activity ^{131}I-MIBG infusion and before the delivery of haploidentical purified NK cells. A single infusion of haploidentical purified NK cells (1×10^6/kg CD56 + cells of patient body weight), expanded over 10 days of collection, was provided on day -10 without

cryopreservation and via intravenous drip over 1 h employing a Y infusion set with a filterless chamber. The premedication included chlorpheniramine and diphenhydramine.

Autologous hematopoietic stem cell transplantation and conditioning

The conditioning regimen prior to the reinfusion of autologous HPCs, which consisted of intravenous busulfan (Bu, administered at a weight- and age-adjusted dose of 0.8–1.2 mg/kg every 6 h from days -6 to -3), followed by melphalan (Mel, 70 mg/m^2/day for patients weighing > 10 kg or 2.3 mg/kg/day for patients weighing ≤ 10 kg, given intravenously on days -2 and -1), was initiated 4 days after the NK cell infusion. Autologous HPC infusion with a median dose of 4.7×10^6 CD34 + cells/kg (recipient body weight) was given the day after completing chemotherapy (day 0). Subsequently, all patients were administered weight-adjusted G-CSF (filgrastim, 5 μg/kg daily), starting on day + 5 post-AHSCT and continued until neutrophil recovery.

According to our institutional transplant protocol, patients were administered levetiracetam as seizure prophylaxis during Bu conditioning. Veno-occlusive disease (VOD) prophylaxis was provided through oral ursodeoxycholic acid (UDCA). Infection prophylaxis included antiviral (aciclovir), antifungal (fluconazole), and trimethoprim-sulfamethoxazole (TMP-SMX) for coverage of Pneumocystis jiroveci.

Following recovery from the transplant, the patients underwent radiotherapy targeting the primary tumor site. Subsequently, they received maintenance therapy with retinoids (cis-retinoic acid) at a dose of 160 mg/m^2/day for 14 consecutive days every 4 weeks for 9–12 months. This treatment regimen aims to enhance patient outcomes and reduce the risk of cancer recurrence. Retinoids have shown promise in reducing the risk of cancer recurrence in high-risk NB patients after high-dose chemotherapy and stem cell transplantation. Figure 1 summarizes the overall treatment schema used in this study.

Toxicity monitoring and response evaluation

Neutrophil recovery was defined as the 1st day of 3 consecutive days with an ANC ≥ 0.5×10^9/L, and platelet recovery was defined as the 1st day of 7 consecutive days with a platelet count ≥ 20×10^9/L without transfusion. Hematological and non-hematological adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. NK cell infusion-related immediate adverse reactions were defined as adverse reactions that developed from the initiation of NK cell infusion to 6 h after the completion of infusion.

Responses were determined by MIBG scintigraphy and CT imaging scans every 6 months for up to 2 years, and defined using response criteria developed by the INRC group.

Results

Patient characteristics

Five pediatric patients with high-risk NB and a strong affinity for ^{131}I-MIBG were enrolled in the study. Upon entering the trial, four of the five participants were identified as being in CR at the time of their post-induction chemotherapy evaluation. Notably, none of the patients received radiation therapy during the initial induction chemotherapy course. One individual exhibited amplification of the MYCN oncogene, whereas two patients were diagnosed with NB at age < 18 months. The baseline characteristics of the patients

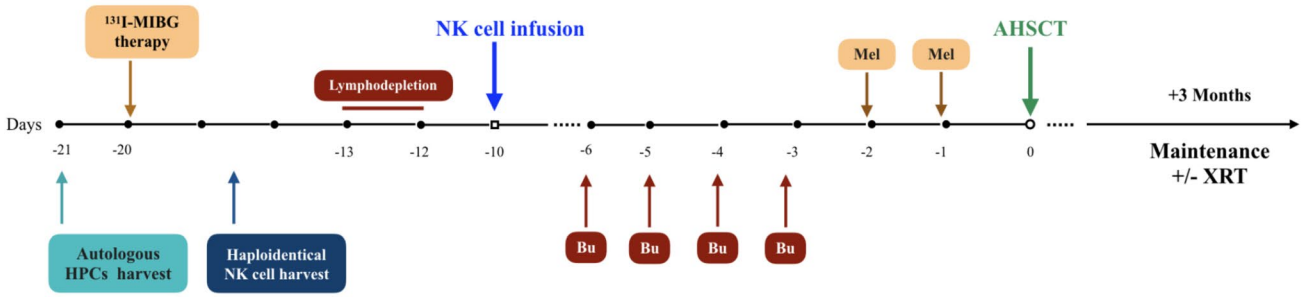

Fig. 1 Time diagram of treatment schema

and treatment details are summarized in Tables 1 and 2, respectively.

Hematologic and non-hematologic toxicity

All patients successfully achieved neutrophil and platelet engraftment before day 30 after HPCs infusion. The AHSCT conditioning regimen led to febrile neutropenia that was effectively resolved with broad-spectrum antibiotics and antifungal agents, with no deaths linked to the treatment protocol. After the reinfusion of HPCs, one patient experienced engraftment syndrome characterized by skin rash, fever, and diarrhea, which responded positively to a low dose of steroids. Importantly, no adverse reactions were noted during or after infusion of NK cells and MIBG treatment. No patient developed acute graft-versus-host disease (GvHD).

Response and survival

Six months after AHSCT, all patients demonstrated a CR based on MIBG scintigraphy and CT imaging, according to the INRC. All patients were prescribed cis-retinoic acid starting on day + 90 post-transplant, combined with radiotherapy. However, due to the limited availability of the anti-GD2 antibody, only one patient received it, subsequently developing pancytopenia after completing the second cycle of anti-GD2 antibody therapy. Bone marrow aspiration confirmed a diagnosis of acute myeloid leukemia (AML) with normal cytogenetic analysis. This AML development was likely secondary to the cumulative genotoxic effects of prior therapies, including ^{131}I-MIBG and alkylating agents, both recognized contributors to therapy-related myeloid neoplasms (t-MN). Despite the prompt initiation of induction chemotherapy, the patient exhibited refractory disease and ultimately died. The only patient who experienced disease progression at the primary adrenal site and in the bone marrow 12 months post-transplant (UPN NB-01) did not respond to salvage chemotherapy and subsequently died. The three other patients are alive and progression-free at their last follow-up (Table 3).

Discussion

Given that NK cell-mediated ADCC plays an important role in targeting NB cells, several studies are currently exploring the feasibility and safety of combining adoptively transferred ex vivo-expanded autologous or allogeneic NK cells with anti-GD2 antibody therapy to enhance treatment responses [19–21]. However, despite these efforts, no significant clinical advantages have been observed with ACT for the treatment of this aggressive tumor [22].

This study demonstrated the potential of incorporating ex vivo-expanded and activated haploidentical NK cells into

Table 1 Patients' baseline characteristics

UPN	Sex	Age at Dx (years)	Age at AHSCT (years)	MYCN status	Stage at Dx	Relapse	Disease status prior to AHSCT	Time from Dx to AHSCT (months)
NB-01	F	3	4	NA	IV	No	CR	13
NB-02	M	< 1	2	NA	IV	No	CR	18
NB-03	F	5	5	A	IV	No	CR	9
NB-04	M	4	5	NA	IV	No	CR	10
NB-05	M	< 1	2	NA	IV	No	VGPR	14

A, amplified; AHSCT, Autologous hematopoietic stem cell transplantation; CR, complete response; Dx, diagnosis; F, female; M, male; NA, non-amplified; UPN, unique patient number; VGPR, very good partial response

Table 2 Treatment details of patients

UPN	Total dose of ^{131}I-MIBG	Dose of ^{131}I-MIBG (mCi/kg)	Conditioning regimen for AHSCT	CD34 + cell dose ($\times 10^6$/kg)	NK cells donor relation	NK cell dose ($\times 10^6$/kg)	Post-AHSCT maintenance
NB-01	210	11	Bu + Mel	5	Father	1	Retinoids
NB-02	97	8.8	Bu + Mel	5.42	Father	1	Retinoids
NB-03	180	10.2	Bu + Mel	3.2	Father	1	Retinoids + Anti GD2
NB-04	200	14.2	Bu + Mel	4.6	Father	1	Retinoids
NB-05	168	12	Bu + Mel	5.29	Father	1	Retinoids

AHSCT, Autologous hematopoietic stem cell transplantation; Bu, busulfan; 131I-MIBG, 131I-meta-iodobenzylguanidine; Mel, melphalan; NK, natural killer; UPN, unique patient number

Table 3 Treatment outcomes of patients

UPN	Neutrophil engraftment (days)	Platelet engraftment (days)	Grade 3 to 4 adverse events					Final outcome
			Mucositis (grade)	Febrile neutropenia	Sepsis	VOD	ES	
NB-01	9	11	+ (II)	+	–	–	–	Death/Relapse
NB-02	10	13	+ (II)	+	–	–	–	Alive/CR
NB-03	10	14	+ (II)	+	–	–	–	Death/AML
NB-04	10	10	+ (III)	+	–	–	+	Alive/CR
NB-05	9	11	+ (III)	+	–	–	–	Alive/CR

AML: acute myeloid leukemia; CR, complete response; ES, engraftment syndrome; UPN, unique patient number; VOD, veno-occlusive disease

the consolidation treatment phase before AHSCT. By targeting the MRD in patients with high-risk NB, this innovative approach may significantly enhance the efficacy of pretransplant conditioning regimens. Indeed, this study is the first to integrate the adoptive transfer of NK cells before transplantation. These findings open new possibilities for improving outcomes in patients with high-risk NB and highlight the promising role of NK cells in the field of cancer treatment.

Importantly, as infusion of donor NK cells into a leukopenic setting has extensively been shown to support in vivo NK cell expansion [23, 24], to establish a lymphodepleted host environment consistent with improved donor NK cell survival, patients received a high-dose cyclophosphamide-based chemotherapy regimen known for its anti-NB activity, immediately before NK cell infusion.

Talleur et al. reported the results of a phase II trial in which the adoptive transfer of parental haploidentical NK cells was integrated into consolidation therapy comprising a Bu/Mel conditioning regimen, AHSCT, and experimental immunotherapy involving hu14.18K322A (a humanized anti-GD2 monoclonal antibody), GM-CSF, and IL-2 for high-risk NB patients. Their study concluded that toxicities were comparable between patients who received NK cells and those who did not [19]. Similarly, no adverse reactions were observed during or after NK cell infusion in our patients.

Another study by Federico et al. was conducted in patients with recurrent or refractory NB, and no complications were observed during NK cell administration. The overall objective response rate was 61.5% (8 of 13 patients) with a CR/VGPR rate of 38.5% (5 of 13 patients) [25]. In contrast to our study, unmanipulated haploidentical NK cells have also been used following AHSCT with subcutaneous IL-2 and daily intravenous GM-CSF in newly diagnosed NB, demonstrating increased NK cell cytotoxicity throughout the treatment period compared to the diagnosis [26].

A significant contributing factor to the recurrence and metastasis of NB tumors is the presence of cancer stem cells (CSCs) within the tumor. These cells are typically resistant to chemotherapy. However, the presence of NK cell-activating receptor ligands on CSCs makes them potential targets for NK cell-based immunotherapy. Therefore, focusing on CSCs may offer a novel and important avenue for the future development of NK cell-based immunotherapies [27–30].

In this context, a promising therapeutic approach that has gained attention for stimulating NK cell-mediated antitumor responses involves plasmacytoid dendritic cells (pDCs). These specialized dendritic cells play a role in both innate and adaptive immune responses and are recognized for their production of substantial amounts of type I IFNs, cytokines, and chemokines, all of which are closely linked to enhancing NK cell cytolytic activity, exhibiting the potential of pDCs to enhance immune responses against tumors [31, 32]. Cordeau et al. illustrated that pDC-mediated NK cell activation, combined with anti-GD2 treatment, heightened the cytotoxicity of NK cells against NB cells. This interaction results in increased surface expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), increased expression of Fas ligand (FAS-L) and CD69 (a classical marker of T cell activation) in CD56dim cytotoxic cells, and the promotion of robust IFN-γ production [11]. These findings highlight the therapeutic potential of activated pDCs in high-risk NB patients.

The hypoxic tumor microenvironment is another obstacle for invading immune cells, resulting in immune suppression. The presence of tumor-associated macrophages (TAMs) can inhibit T cell responses, cause T cell apoptosis via Fas–Fas ligand interactions, activate myeloid-derived suppressor cells (MDSCs), and stimulate Tregs, thereby suppressing the active immune response [33, 34]. Furthermore, NB cells express high levels of gangliosides, including GD2, which contribute significantly to an immunosuppressive microenvironment [35].

A recent study revealed that metastatic NB exhibits greater infiltration of TAMs than locoregional tumors [36]. Given the complexity of NB tumor microenvironment, the development of innovative immunotherapeutic approaches

appears to be a fundamental aspect of future directions in NB treatment [37].

Natural Killer T cells (NKTs; CD3 + CD56 +) are innate-like lymphocytes that exhibit features of both NK and T cells. NKTs are divided into type I, known as invariant NKTs (iNKTs), and type II NKTs. The presence of iNKTs within a tumor or in the bloodstream has been linked to enhanced survival and decreased progression of various cancers including NB. Adoptive transfer of iNKTs could potentially play a therapeutic and complementary role in NB by targeting TAMs and boosting or restoring the cytotoxicity of NK and T cells [38].

The involvement of NK cells in GvHD development remains a topic of debate. Various studies have explored the adoptive transfer of haploidentical NK cells following lymphodepleting preparative regimens in a non-HSCT setting and have noted graft-versus-tumor (GVT) effects, transient expansion of NK cells, and no evidence of GvHD occurrence [39–41]. To mitigate the risks of GvHD and B-cell lymphoproliferative disease, we used highly purified NK cells with minimal T- or B-cell contamination.

Although the number of infused NK cells plays a crucial role in their persistence after infusion, the optimal dose or timing of NK infusion has not been definitively established [42]. Studies have shown that administration of a large number of ex vivo-expanded and highly activated NK cells is safe, feasible, and effective in maintaining the effector arm of the host immune response [43]. It is important to highlight that in the present study, NK cells were used to target the remaining NB cells before AHSCT, with the long-term persistence of NK cells not being the main focus of this study.

Conclusion

Our study showed that incorporating NK cell infusion before AHSCT as a component of the conditioning regimen for consolidative therapy in pediatric patients with high-risk NB can be safe and well tolerated. However, in this pilot study, the efficacy of allogeneic NK cells in eliminating residual disease could not be definitively determined.

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Author contributions T.R., Mo.A. (Mohammad Ahmadvand), and A.Ki. conceptualized the study. T.R., A.Ka., M.R.R., and A.Ki. organized the study. Mo.A. conducted NK cell collection and processing. B.C. and M.R.S.N. performed flow cytometry assessments and molecular tests. T.R., Mj.A. (Mojtaba Azari), and Mr.A. (Morteza Azari) wrote the manuscript. T.R., A.Ki., R.A., and G.J. reviewed and revised the whole manuscript. All authors agreed on all aspects of the work and approved the final version of the manuscript.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Committee on Medical Ethics of the Research Institute for Oncology, Hematology, and Cell Therapy (RIOHCT), affiliated with Tehran University of Medical Sciences (TUMS), Tehran, Iran (IR.TUMS.HORCSCT.REC.1400.003).

Consent to participate Written informed consent was obtained from all the donors and the parents or legal guardians of the patients.

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