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Clinical Trial

Evaluating the safety and feasibility of allogeneic NK cell infusion in high-risk lymphoma patients post-autologous stem cell transplantation

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Abstract
Lymphoma, a cancer with poor prognosis is a growing global health challenge that encompasses two primary types, Hodgkin (HL) and non-Hodgkin lymphoma (NHL), each further divided into various subtypes with distinct biological behaviors. Conventional therapeutic strategies include chemotherapy, radiation, surgery, and autologous hematopoietic stem cell transplantation (auto-HSCT). Natural killer (NK) cells exhibit intrinsic cytotoxicity against tumor cells without the need for prior immunization or activation. In this prospective clinical trial, we evaluated the feasibility of allogeneic NK cell therapy in patients with high-risk lymphoma who had a poor prognosis. Each patient received 1×107 NK cells/kg infusion without interleukin-2 (IL-2) supplementation. Therapy was tolerated without graft-versus-host-disease, cytokine release syndrome, or neurotoxicity. During the follow-up period, 7 had complete responses (CR) (87.5%) and one case exhibited stable disease (SD) (12.5%). In summary, our investigations support the development of allogeneic NK cellular therapies for advanced lymphoma to overcome chemoresistance. Therapeutic efficacy may be further improved by disrupting the immunosuppressive environment and infusion of exogenous IL-15. This approach presents a promising and pragmatic strategy for managing high-risk lymphoma post-HSCT. Future research should focus on optimizing NK cell dosages and infusion frequency to maximize treatment effectiveness.

Keywords Natural killer cells · Lymphoma · Clinical trial · Phase I · Immunotherapy · Cellular immunotherapy

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1 Introduction

Lymphoma, a cancer originating in the lymphatic system, represents a significant and growing global health challenge [1]. Lymphoma encompasses two primary types, Hodgkin and non-Hodgkin lymphoma (NHL), each further divided into various subtypes with distinct biological behaviors [2]. Traditional therapeutic approaches include chemotherapy, radiation, surgery, and autologous hematopoietic stem cell transplantation (auto-HSCT). Advancements in the genetic analysis of lymphoma have enhanced the prognosis of these patients through the introduction of monoclonal antibodies, including rituximab, and immune checkpoint inhibitors. However, the prognosis of patients with relapsed or refractory forms of this disease remains poor, particularly in NHL, where high relapse rates persist despite aggressive treatment. So, the common therapeutic approaches are often limited by substantial relapse rates and non-relapse mortality due to treatment-associated toxicity and immune complications [3–5].

In recent years, immunotherapy has emerged as a promising avenue for treating lymphoma by leveraging the patient's immune system to target malignant cells. Among these therapies, natural killer (NK) cell-based therapies have gained particular attention due to their unique capacity to recognize and destroy cancer cells without needing antigen-specific priming [6]. NK cells exhibit cytotoxic mechanisms akin to T cells, yet demonstrate a more favorable safety profile and are more suitable for producing allogeneic, ready-to-administer products [7]. Unlike T cell-based therapies, such as chimeric antigen receptor T (CAR-T) cells, NK cell therapies carry a reduced risk of graft-versus-host disease (GVHD) due to their innate mechanism of action, which limits alloreactivity while maintaining effective antitumor activity [8].

NK cells, as innate lymphoid immune entities, play a crucial role in antitumor immunity by inhibiting tumor proliferation and metastasis [9]. These cells demonstrate significant cytotoxicity towards tumor cells in the absence of prior sensitization or immunization, and they elicit the production of various cytokines that activate the adaptive immune response [10]. Previous studies have indicated that the adoptive transfer of allogeneic NK cells is safe and well-tolerated and can exert antitumor effects in lymphoma patients without inducing severe toxicities [9, 11, 12]. Furthermore, ex vivo expansion techniques have enabled the generation of large quantities of highly activated NK cells with enhanced cytotoxic capabilities, allowing for scalable treatment options such as "off-the-shelf" therapy for relapsed or refractory NHL [13].

This study evaluates the safety and preliminary efficacy of allogeneic NK cells following auto-HSCT in patients with high-risk NHL. This phase I trial aims to determine the safety and feasibility of this strategy in improving outcomes for patients with limited treatment options.

2 Materials and methods

2.1 Study design and patients

This phase I clinical trial was a non-randomized, interventional, single-group, and non-blinded study. We evaluated the safety and preliminary efficacy of allogeneic NK cell injection in patients with high-risk lymphoma who received auto-HSCT. All patients failed the third-line therapeutic setting, including lenalidomide-based, tazemetostat, zanubrutinib/Obinutuzumab. The trial coded IRCT20140818018842 N26 on 28/11/2022 was added to the Iranian clinical trial data set (IRCT). This study was conducted at the Tehran University of Medical Sciences'Shariati Hospital's Cell Therapy and Hematopoietic Stem Cell Transplantation Research Center.

Patient enrollment was performed chronologically according to the timeline from the start of the study, with no patient selection preference. After providing informed consent from participants, patients were selected based on the following criteria: histologically confirmed malignant lymphoma, relapsed lymphoma after auto-HSCT, failure of at least 2 prior lines of therapy, age 18 years or older, adequate organ function at the time of infusion, and adequate performance score: Karnofsky performance status (KPS) > 70 or Eastern Cooperative Oncology Group (ECOG) = 0–2, leukocyte count ≥ 3000/mm^3 and ≤ 12,000/mm^3, neutrophil count ≥ 1500/mm^3, platelet count of ≥ 50,000/μL, hemoglobin concentration ≥ 10 g/dL, serum creatinine (Cr) ≤ 1.5 mg/dL, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 100 IU/L, blood urea nitrogen (BUN) level ≤ 25 mg/dL, and normal cardiac function on echocardiography. The exclusion criteria were as follows: active or treatment-refractory infections, positive

history of allergy or sensitivity to organic proteins, advanced physical or mental disability, candidate for receiving chemotherapy, radiotherapy, immunotherapy, or transplantation during NK cell transplantation, positive pregnancy test for woman of childbearing age, central nervous system lymphoma or lymphomatous meningitis, positive record for hepatitis B or C virus (HBV or HCV), human immunodeficiency virus (HIV), human T-lymphotropic virus-1 (HTLV-1), or syphilis infection, patients with a history of autoimmune disease, and enrollment in other clinical trials.

2.2 Endpoints and evaluation criteria

The primary endpoint of this prospective trial was to assess the safety and tolerability of adoptive transfer of allogeneic NK cells in patients with high-risk lymphoma. For this purpose, the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 5.0) checklist and Bearman scale were used. The secondary endpoints included complete remission, complete response (CR), partial response (PR), and stable disease (SD). Patient responses were evaluated using computed tomography (CT) or positron emission tomography (PET) scans, with radiologic assessments conducted locally in accordance with the Lugano classification for NHL.

2.3 Donor screening and eligibility criteria

Unrelated healthy donors were randomly selected for the study upon obtaining informed consent. All donors were transplanted with hematopoietic progenitor cells before NK cell therapy. For this purpose, the cells were collected via apheresis using a COBE Spectra device. Donors were mobilized with G-CSF for 5 days before collection. Collected cells were cryopreserved in 10% DMSO using a controlled-rate freezer and stored in liquid nitrogen tanks at – 196 °C. Finally, the cells were thawed rapidly in a 37 °C water bath and infused intravenously within 30 min of thawing. Patients received pre-medication with acetaminophen and diphenhydramine.

For NK cell isolation, all donors underwent comprehensive screening, including tests for HIV, HBV, HCV, HTLV, syphilis, and blood cultures. Following successful screening, 20 mL of whole blood were collected intravenously using heparinized syringes and Falcon tubes. The collected samples were promptly transported to a cold storage facility to ensure optimal preservation.

2.4 NK cell isolation and preparation procedures

Peripheral blood mononuclear cells (PBMCs) were isolated from unrelated healthy donors using Ficoll Paque Premium (GE Healthcare, USA) through density gradient centrifugation at 300 g for 20 min. After separating the final mixture into three layers, the middle layer was carefully collected using a pipette, moved to a different tube, and washed twice with PBS that had been preheated to 25 °C. The cell pellets were re-suspended in 1 mL of RPMI1640 (Gibco, USA). Cell viability was then visually evaluated and counted using 4% trypan blue dye (Sigma-Germany).

Highly purified NK cells were obtained according to the manufacturer's instructions (Miltenyi Biotec, Germany), and non-NK cells were depleted using a magnetically activated cell sorting (MACS) system. NK cell purity was evaluated by flow cytometry using anti-human CD3 and CD56 mAbs. For NK cell expansion, isolated cells were seeded at 5×10^5 cells/mL and cultured with an irradiated (100 Gy) k562-genetically modified cell line (5×10^6 cells/mL) in a T-25 culture flask. The culture medium was RPMI1640 (Gibco-USA) containing 1000 IU/mL IL-2 (IL-2, Miltenyi Biotec-USA), 10 ng/mL IL-15, and 5% of human serum (Sigma-Aldrich). On day 5, expanded NK cells were transferred to a larger flask in the RPMI medium containing 1000 IU/mL IL-2, 10 ng/mL IL-15, and 5% of human serum. A fresh culture medium containing 1000 IU/mL IL-2, 10 ng/mL IL-15, and 5% of human serum was added to the flask every 2 to 3 days for 21 days. The procedures for NK cell expansion were conducted in accordance with Good Manufacturing Practice (GMP) principles.

2.5 Characterization and functional assessment of NK cell products

The purity of peripheral blood-derived NK cells (PB-NK) was measured by quantitatively evaluating the proportions of $CD3^-$ and $CD56^+$ markers using flow cytometry (FACSCalibur Becton Dickinson, USA). Briefly, a total of 1×10^5 cells were collected, washed with phosphate buffer saline (PBS), and stained with fluorochrome-labeled antibodies (CD56-APC and CD3-FITC, Biolegend, USA) for 20 min at room temperature and in the dark. Subsequently, the cells were washed, and the percentage of labeled cells was analyzed using a flow cytometry device and FlowJo software. To assess the in vitro cytotoxic effect of human-activated NK cells (effector cells), the K562 cell line was selected as the target cell line.

The effector (E) and target (T) cells were co-cultured in ratios of 1:1, 5:1, and 10:1, followed by measuring the presence of lactate dehydrogenase (LDH) in the cell culture medium after 24 h. The frequency of LDH is directly associated with the necrosis and cytotoxicity of NK cells. For cytokine analysis, the supernatants were collected from activated NK cells that were co-cultured with the K562 cell line for 24 h and were analyzed for the IFN-γ and TNF-α content using ELISA kits (Sigma Aldrich, USA) according to the manufacturer's instructions.

In addition, the viability of cells was determined by the trypan blue exclusion method. Regarding quality control of the final product, we checked bacterial and fungal contamination by 48-h culture of isolated cells. Mycoplasma contamination was evaluated by PCR-based MycoAlert™ Mycoplasma Detection Kit (Lonza), and endotoxin investigation was measured using the kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay (Charles River). The assay has a detection range of 0.005–50 EU/mL. Samples with < 0.25 EU/mL were considered acceptable for clinical use.

2.6 NK cell administration and conditioning regimen

Following hospitalization, the conditioning regimen commenced on day −7 relative to HSCT. This regimen included the administration of Bendamustine at a dosage of 200 mg/m^2 per day from days −7 to −6, Cytarabine at 200 mg/m^2 twice daily from days −5 to −2, and Etoposide at 200 mg/m^2 per day from days −5 to −2. On day −1, Melphalan was administered at a dose of 140 mg/m^2. On day 0, HSCT was infused and on day + 5 fresh PB-NK cells (1×10^7 cells/kg) were injected intravenously for one hour with 200 mL of physiological serum containing 2% human albumin solution (Albumedix, United Kingdom). To prevent infections and manage potential complications, all patients received prophylactic antibiotics: ciprofloxacin at 500 mg twice daily, fluconazole at 300 mg daily, and acyclovir at 600 mg daily until the NK cell infusion.

2.7 Clinical assessment

The primary objective of the prospective trial was to evaluate the safety and tolerability of this therapy and in vitro expansion of allogeneic NK cells. The secondary endpoint was the investigation of the CR, PR, and SD during the 6-month follow-up through evaluation of PET/CT results for lymphoma.

2.8 Laboratory findings and hematological assessments

The amount of total white blood cells (WBC) and platelets were evaluated for patients before and after HSCT and after NK cell administration. Alkaline phosphatase (ALP) levels with a normal range of 44 to 147 international units per liter (IU/L), aspartate aminotransferase (AST) with a normal range of 12 to 38 U/L, alanine aminotransferase (ALT) with a normal range of 53 to 120 µmol/L, total bilirubin, and creatinine were assessed daily, and the results before and after HSCT, and after NK cell therapy were reported for each patient.

2.9 Study design and statistical analysis

Descriptive statistics were used to summarize cell frequencies. Paired t-tests were used for statistical analysis (GraphPad Prism, version 6; ElCamino, CA). Statistical significance was defined as a $P < 0.05$. Based on average values, representative histograms or pictures were selected.

3 Results

3.1 Patient demographics and disease characteristics

A total of eight patients with high-risk lymphoma were enrolled in this study based on the specified eligibility criteria. The characteristics of these patients are detailed in Table 1. Two patients (25%) had NHL, five patients (62.5%) had classic HL, and one (12.5%) had nodular lymphocyte predominant Hodgkin (NLPHL), classic Hodgkin lymphoma (nodular Sclerosis (NS), mixed cellularity (MC)), and diffuse large B-cell lymphoma (DLBL). The average age of the patients who were enrolled in the study was 36 years (range, 25–51 years), five patients were male (63%), and three patients were female (37%). In addition, all participants underwent lymphodepletion chemotherapy and auto-HSCT before the administration of PB-NK cells. All patients were evaluated for safety, and exploratory endpoints.

Table 1 Patient characterization

Patients	Age	Sex	Diagnose	Diagnose to HSCT interval (year)	Adverse effect	Grade of side effects	Remission status at transplant	Best overall response	F/U duration (m)	Final outcome
P-1	25	Female	Classic HL	5	Headache and Cough	1	CR	CR	22	Alive
P-2	33	Male	Classic HL	2	None	–	PR	CR	12	Alive
P-3	34	Male	NLPHL	22	Fever and Headache	1	CR	CR	12	Alive
P-4	34	Female	Classic HL	9	respiratory distress and tachypnea	1	PR	SD	12	Alive
P-5	51	Female	DLBCL	9	Diarrhea	1	CR	CR	11	Alive
P-6	42	Male	Classic HL	3	fever	1	PR	CR	11	Alive
P-7	37	Male	DLBCL	3	None	–	CR	CR	9	Alive
P-8	31	Male	Classic HL	-	Fever and Headache	1	CR	CR	6	Alive

SD: Stable disease; CR: Complete response; PR: Partial response; F/U: Follow up

3.2 NK cell expansion and functional characterization

Expansion of PB-NK cells to the desired dosage was successfully achieved in all 8 participants, with an average NK cell purity of 86.9% (ranging from 83.9% to 89.9%) (Fig. 1a). Consistent with our preclinical findings, the NK cells exhibited high functionality, evidenced by the production of IFN-γ and TNF-α upon stimulation with the K562 cell line (Fig. 1b), also demonstrating in vitro cytotoxic activity against the K562 cell line (Fig. 1c).

The final acceptance criteria for all NK cell products included a negative culture of fungal and microbial cultures, negative Gram stain result, endotoxin levels below 5 EU per kilogram of patient weight, absence of mycoplasma contamination, a visual inspection confirming no contamination, and a cell viability of at least 80%. All desirable data was reached during quality control tests.

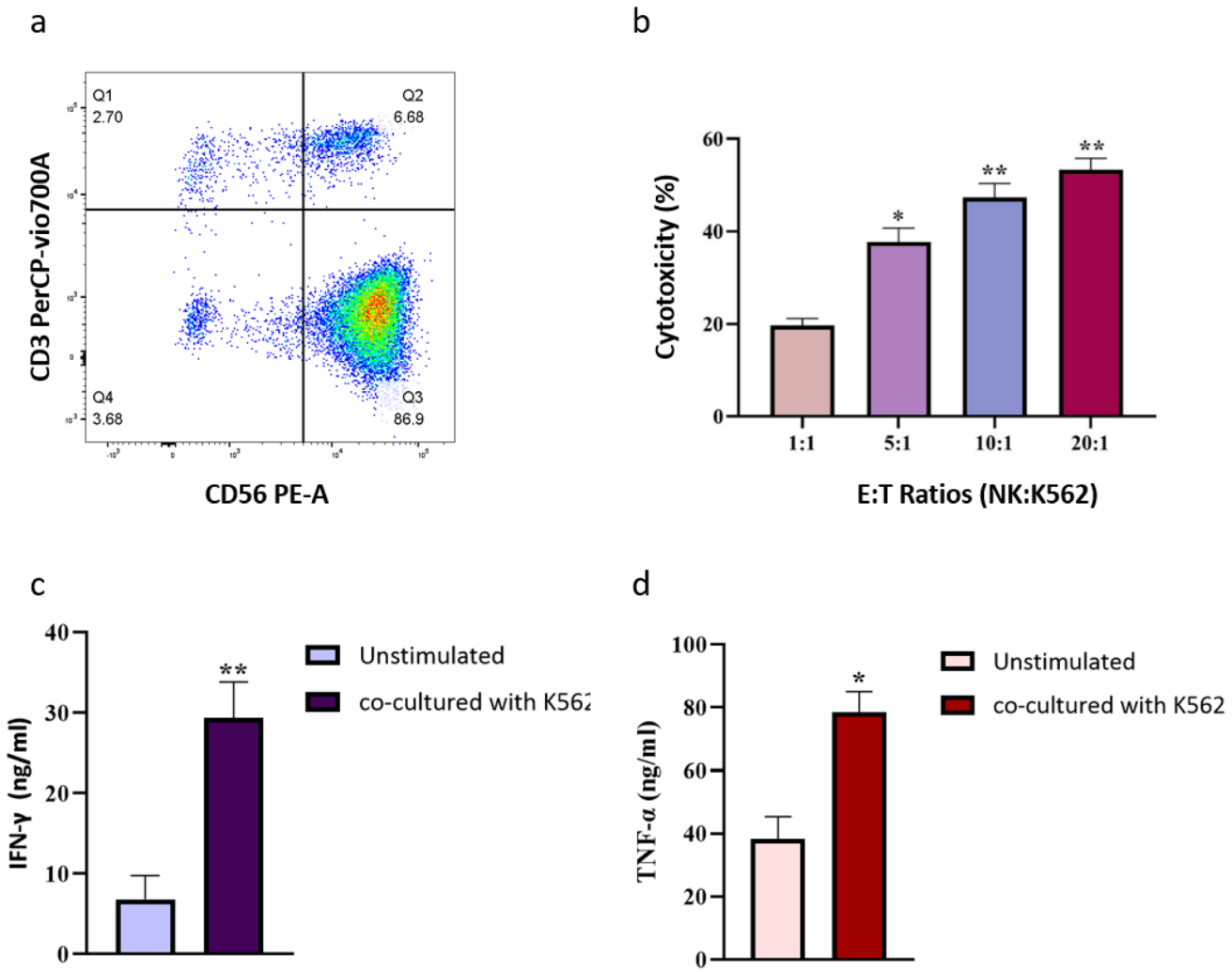

Fig. 1 Expansion and functional characterization of NK Cells. **a** The frequency of $CD56^{+}CD3^{-}$ NK cells derived from peripheral blood mononuclear cells (PBMCs) exceeded 80% following magnetic-activated cell sorting (MACS). **b** Co-culturing of activated NK cells (E) and K562 cells (T) at different E: T ratios using LDH assay, indicated the most cytotoxic effect in 10:1 E: T. ratio **c**, **d** On day 21 post-activation, cytokine release was assessed, revealing mean concentrations of approximately 29.33 pg/mL for IFN-γ and 78.5 pg/mL TNF-α in NK cells co-cultured with K562 cell line, both significantly elevated compared to the control NK groups. All data are presented as means ± standard deviation (M ± SD, n = 3). **P ≤ 0.01, and ***P ≤ 0.001

3.3 Safety evaluation of allogeneic NK cell infusion

The CTCAE criteria were used to record the adverse events. The CTCAE parameters, which are predicated on clinical manifestations and physiological indicators, were documented to include myocardial infarction, alterations in respiratory function, pyrexia, dermatological eruptions, and manifestations indicative of anaphylactic shock, all of which were assessed during the duration of cell infusion until 48 h. The patient's physiological signs, laboratory evaluations, and trajectory of physical recovery were meticulously observed, documented within their medical records, and systematically classified utilizing the CTCAE checklist (Table 1). Additionally, the Bearman scale was used for reaching more details about safety (Table 2). In this guideline, GI, pulmonary, cardiac, bladder, renal, hepatic, CNS, stomatitis toxicities were checked 1, 3, and 6 months after NK cell therapy. Safety was the main goal of this experiment. Based on CTCAE guideline, three patients experienced fever and headache. Based on Beraman scale, one patient experienced respiratory distress and tachypnea, and one patient experienced diarrhea. However, GVHD, neurotoxicity, and treatment-related mortality were not observed. The adverse effects noted were transient and effectively managed with supportive care, including antipyretic therapy and intravenous hydration. The findings indicate that PB-NK cell administration was both safe and practicable, with 4 out of 8 patients experiencing temporary (< 1 day) and manageable adverse events directly attributed to the cell infusion.

3.4 Clinical response to PB-NK cell infusion

All 8 patients received PB-NK cell infusions and had their responses assessed (Table 1). During the 3 months, all patients (100%) presented complete responses (CR). At the last follow-up, one patient (12.5%) showed stable disease (SD), and seven exhibited CR (Table 1).

3.5 Hematological and biochemical laboratory findings

After the administration of the conditioning regimen, the white blood cell (WBC) and platelet counts decreased in all patients immediately (Table 3). The levels of liver enzymes, including AST, ALT, and ALP, as well as total bilirubin and creatinine, which are markers of kidney function, remained within normal limits throughout hospitalization, and our intervention had no substantial effect on these values. The absence of electrolyte imbalance indicates normal homeostasis following intervention. The biochemical test results of each individual are presented in Table 4.

4 Discussion

NK cells are critical immune effector cells that influence survival after autologous peripheral blood HSCT. It was revealed that following HSCT, the absolute NK cell count directly affects the clinical outcomes of lymphoma [14]. The effectiveness of allogeneic hematopoietic cell transplantation in patients with lymphoma indicates that donor-derived NK cells may exert an effective graft-versus-lymphoma impact that protects against recurrence [15, 16]. A broad family of inhibitory NK cell receptors, such as C-type lectins and killer cell immunoglobulin-like receptors (KIRs), that identify particular MHC-I alleles are employed for NK cell recognition of"self"to ensure immunologic tolerance to self-antigens without the requirement for prior sensitization [17]. Tumor-transformed cells without self-MHC-I are prone to NK cell-mediated

Table 2 Safety evaluation according to NCI Common Terminology Criteria for Adverse Events

CTCAE guideline				
Fever	Tachycardia	Headacke	Chill	Dyspnea
P-3, P-8, P-4	P-4	P-1	–	P-4
Bearman scale				
CNS toxicity	Cardiac toxicity	GI toxicity	Bladder/renal toxicity	Pulmonary toxicity
–	p-4	p-5, p-6	–	p-4

Table 3 Hematologic laboratory findings

Patient	Dose of CD34 + for trasplant	WBC at transplant day	WBC after NK cell infusion	Time of WBC engraftment	platelet count on HSCT transplant day	Time of platelet engraftment	Platelet count after NK cell infusion
P-1	4.2	3000	400	9	142.000	11	198.000
P-2	$6.63*10^6$	10.000	2.400	10	7.000	13	7.500
P-3	$2.8*10^6$	2.300	2.700	9	75.000	12	27.000
P-4	3.1	100	3.800	7	10.000	11	7.000
P-5	$2.6*10^6$	200	13.500	7	94.000	10	20.000
P-6	$4.13*10^6$	1.200	3.500	9	68.000	12	11.000
P-7	$2.14*10^6$	1.000	650	8	37.000	12	7.500
P-8	$4.8*10^6$	2.900	5.500	12	96.000	14	36.000

lysis since several stimulating NK cell receptors interact with their ligands expressed on tumor cells [18]. Faster and more robust NK cell recovery after HSCT is an important aspect that may influence clinical outcomes [7]. It was observed that on day 15 after autologous HSCT, individuals with NHL who had an absolute NK cell count of at least 80 cells/μL had longer overall survival (OS) and progression-free survival (PFS) than those with lower counts [19]. In addition, patients with lymphoma who received an autograft with an absolute NK cell count $\geq 0.5 \times 10^9$ cells/kg had 5-year PFS and 5-year OS rates of 71% and 87%, respectively [20].

Moreover, low-dose subcutaneous recombinant IL-2 has been used in many early trials to support NK cell recovery and cytotoxic activity as a successful strategy to eliminate residual disease and avoid recurrence in lymphoma patients after autologous HSCT [21, 22]. Ishikawa et al. also sought to co-culture autologous, patient-derived NK cells with IL-2 and irradiated human feeder cell line (HFWT). An appropriate effectiveness and safety profile was observed when they administered these cells intravenously, with or without localized injections, coupled with low-dose IFN-β to patients who had recurring malignant gliomas [23]. Several studies have examined the ex vivo transfer of activated autologous NK cells as a therapeutic option for patients with lymphoma, according to findings of earlier research [24, 25]. Although only a minor anticancer effect was observed, adoptive transfer of autologous NK cells was determined to be safe and feasible [11]. This restriction mainly resulted from autologous NK cells'inhibitory receptors matching self-MHC class I on cancer cells, which produced "self" recognition signals that inhibited NK cell stimulation and the anti-cancer effects that followed [11]. Additionally, the dosage of transferred NK cells is restricted to around 10^7 cells/kg and adoptive transfer of autologous NK cells are expensive and sometimes necessitate numerous apheresis operations [26]. To address these obstacles, scientists have employed allogeneic NK cells in the immunotherapy of lymphoma and other malignancies [27–30].

Yang et al. assessed the safety and potential therapeutic efficacy of allogeneic NK cells (MG4101) collected from unrelated, healthy donors in patients with advanced solid tumors or malignant lymphoma. According to their findings, MG4101 was administered at dosages ranging from 1×10^6 cells/kg to 3×10^7 cells/kg without causing any severe toxicity or GVHD symptoms. Nine patients (52.9%) had progressing illness and eight patients (47.1%) had SD out of the 17 evaluable cases. Additionally, MG4101 treatment decreased regulatory T cells and myeloid-derived suppressor cells, increased chemokines that attract T cells and increased NKG2D expression in $CD8^+$ T cells [31]. Moreover, the efficacy and safety of rituximab in conjunction with ex vivo-expanded allogeneic NK cells (MG4101) for relapsed or resistant B cell non-Hodgkin lymphoma treatment were assessed in a recent phase I clinical study. The trial results demonstrated the safety of the combination treatment, with an objective response rate (ORR) of 55.6%, a PR in four patients, and CR in one patient. Furthermore, two individuals exhibited low T cell exhaustion marker levels and prolonged responses [9]. Moreover, ex vivo expanded allogeneic NK cells with IL-15 and nicotinamide were employed in a study on high-risk NHL and multiple myeloma patients, and they were injected with low-dose IL-2 and elotuzumab or rituximab to improve ADCC and proliferation. Adoptive NK cell transfer has promising efficacy in advanced-stage disease cases [32].

In this study, we used Gy-irradiated—Epstein-Barr Virus- (EBV-) Immortalized LCLs and recombinant IL-2 to expand PB-NK cells [33]. Also, IFN-γ and TNF-α cytokine release measurements were used to assess cell function. Based on our obtained data, one patient (12.5%) had SD throughout the 3-month monitoring period, five (62.5%) were in remission, and two (25%) had CR. Three patients (37.5%) had CR at the six-month follow-up, four patients were in remission (50%), and one patient (12.5%) had SD. All serum creatinine, SGOT, SGPT, ALP, and total bilirubin values were within the normal range. Normal liver

Table 4 Biochemical laboratory findings

Case	AST			ALT			ALP			Total Bilirubin			Creatinine		
	Before HSCT	After HSCT	After NK infusion	Before HSCT	After HSCT	After NK infusion	Before HSCT	After HSCT	After NK infusion	Before HSCT	After HSCT	After NK infusion	Before HSCT	After HSCT	After NK infusion
P-1	21	18	12	26	19	14	180	167	177	0.9	0.9	0.7	1	0.8	0.8
P-2	19	12	10	22	18	28	218	156	173	0.9	1.1	0.7	0.9	0.7	0.7
P-3	35	18	17	39	28	20	130	143	123	0.9	0.8	0.3	1.1	0.9	0.9
P-4	16	11	7	17	10	6	160	141	128	0.9	0.7	0.6	0.9	1	1
P-5	23	8	4	23	15	6	329	163	118	0.5	0.2	0.4	1.2	1.1	0.7
P-6	132	19	14	89	41	17	173	117	94	0.8	0.6	0.3	1.2	1.2	1.17
P-7	174	12	8	147	34	14	267	213	189	0.6	0.4	0.5	2	1.6	1.7
P-8	35	32	18	60	62	29	419	150	143	0.6	1	0.6	1.1	1	0.7

function demonstrated that the conditioning regimen and cell infusion had no negative effects on normal homeostasis [34]. Thus, allogeneic NK cell transfer is a potential treatment for high-risk lymphoma due to its favorable safety profile. Our trial's sample size was small, so it was impossible to draw firm conclusions, but NK cell therapy by itself showed some promise in treating auto-HSCT high-risk lymphoma.

Similar to our study, Ahmadvand et al. conducted a phase I non-randomized clinical study to evaluate the potential effectiveness, safety, and feasibility of adoptive NK cell transfer in patients with refractory/relapsed AML. They discovered that the target cell dosage was 10×10^6 cells/kg and that dose escalation was well tolerated. Their techniques comprised immunosuppressive regimes that would not have prevented the expansion of allogeneic NK cells obtained from random donors but were necessary for the in vivo persistence of allogeneic NK cells. Additionally, based on their results, there were no grade 2–5 toxicities following infusion of PB-NK cells, and the NK cell infusion process was well tolerated. After each PB-NK cell injection, four patients experienced grade 1 transitory chills, headaches, nausea, and bone pain; these side effects did not require hospitalization [35]. Another study evaluated the safety of injecting allogeneic, ex vivo-expanded, and primed NK cells into patients with relapsed/refractory neuroblastoma following auto-HSCT. Based on the reported data, no acute or subacute adverse effects were recorded after NK cell injections (1 and 5×10^7 cells/kg for the first and second injections, respectively). In addition, after 18 months, one patient achieved CR, and the other experienced PR after 9 months. During the 26-month and 15-month follow-ups, two patients remained alive. It is noteworthy that the former demonstrates typical development and functioning. Thus, even as a neoadjuvant treatment for high-risk cases, allogeneic transfer of NK cells is a potential treatment for neuroblastoma patients due to its favorable safety profile [36].

In conclusion, we present the findings of a pilot study to treat patients with high-risk lymphoma after auto-HSCT by administering allogeneic ex vivo expanded NK cells. The results of this experiment demonstrate that expanded PB-NK cells are safe and feasible for use in "off the-shelf" immunotherapy and that allogeneic cell treatment may be safely enhanced by their intrinsic alloreactivity. This feature, together with the seemingly low HLA-matching criteria between NK cell donors and recipients, might lead to a true off-the-shelf product that could make therapy more accessible. Due to some limitations in our clinical investigation, such as limited sample size and lack of randomization and comparability, it was challenging to evaluate the effectiveness of allogeneic NK cell treatment. To assess this therapy's effectiveness, further research is needed with larger cohorts, greater NK cell dosages, and more frequent injections.

Author contributions M. A: conceptualization, data curation, and validation M. B: conceptualization, data curation, and validation. M.S.S.P: methodology, writing—review and editing. S. Tavakoli: methodology, writing—review and editing M.V: writing—review and editing, validation. A.M: writing—review and editing H.S.S: writing—review and editing.

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Data availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate The present study was approved by the Research and Ethics Committee of the Tehran University of Medical Sciences, and all experiments were performed in accordance with relevant guidelines and regulations (Approval No. IR.TUMS.MEDICINE.REC.1399.500). The participants or their legal guardian/next of kin provided written informed consent to participate in this study.

Competing interests The authors declare no competing interests.

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