NK Cells for Adoptive Immunotherapy

Subjects: Hematology

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NK (Natural Killer) cell-mediated adoptive immunotherapy has gained attention in hematology due to the progressing knowledge of NK cell receptor structure, biology and function. Today, challanges related to NK cell expansion and persistence in vivo as well as low cytotoxicity have been mostly overcome by pioneering trials that focused on harnessing NK cell functions. Recent technology advancements in gene delivery, gene editing and chimeric antigen receptor (CARs) have made it possible to generate genetically modified NK cells that enhance the anti-tumor efficacy and represent suitable 'off-the-shelf' products with fewer side effects. The recent advanced in NK cell biology along with current approaches for potentiating NK cell proliferation and activity was highlighted, redirecting NK cells using CARs and optimizing the procedure to manufacture clinical-grade NK and CAR NK cells for adoptive immunotherapy.

Keywords: NK cells ; CAR NK cells ; adoptive immunotherapy

1. Introduction

Immunobiology and immunotherapy of hematological malignancies have captured great interest in recent years. NK (Natural Killer) cells are components of the innate system that identify and kill tumor- and virus-infected cells in a major histocompatibility complex (MHC) unrestricted fashion. Unlike T cells, which recognize through an antigen-specific T-cell receptor (TCR) and express receptors encoded by rearranging genes, NK cells have activating and inhibitory receptors (killer immunoglobulin receptors, or KIRs) that ligate MHC molecules [1][2][3]. Tumor cells down-regulate or lose the MHC class I expression and become susceptible to lysis by NK cells. Several activating NK cell receptors and co-stimulatory molecules recognize tumors [4]. NK cells also exhibit antigen-dependent cellular cytotoxicity (ADCC) by detecting antibodies on tumor cells through the low-affinity Fcγ CD16 receptor [5].

NK cells have become an attractive modality in adoptive immunotherapy during the last two decades due to the growing research about NK cell biology that has elucidated the insufficient anti-tumor effect and expansion. Initially, trials examined the ex vivo activated and expanded primary peripheral blood (PB) NK cells or NK cell lines (e.g., NK-92) in autologous and allogeneic settings [1][2]. Umbilical cord blood (UCB)-derived NK cells are demonstrated to be younger, recover better after cryopreservation and have stronger proliferation potential. Manufacturing NK cell-based immunotherapies from induced pluripotent stem cells (iPSCs) has prevented long production times while maintaining “off-the-shelf” capabilities [6].

NK-cell mediated antitumor immunotherapy can be enhanced by checkpoint blockade, bi- and tri-specific killer engagers (BIKEs and TriKEs), anti-KIR monoclonal antibodies and chimeric antigen receptor (CAR)-engineered NK cells (CAR-NK cells). Cytokines play essential roles in NK cell expansion and potentiating NK cell therapy products [7][8]. Genetic modifications have further improved the specificity, strength and efficacy of NK cell-based immunotherapies. Today, the optimal time for NK cell infusions has not been determined. Non-modified or modified NK cells can be used as maintenance therapy after chemotherapy or can be combined with autologous or allogeneic stem cell transplantation.

2. Recent Advances in NK Cell Biology

NK cells have historically been considered as “naturally” cytotoxic cells with limited life span and proliferative capacity, but recent research indicates that NK cells also require priming of various factors such as IL-15, IL-2, IL-12 or IL-18 for maximum effector function [9]. Early clinical trials showed that administration of exogenous IL-2 facilitated NK cell expansion and persistence [10]. IL-15 plays a role in NK cell development and promotes NK cell survival through expression of anti-apoptotic factor Bcl-2 [11]. Miller and colleagues showed that IL-15 has superior activity to IL-2 for in vivo NK cell persistence [12].

NK cells not only function in innate immunity but also obtain immunological memory like T and B cells in adaptive immunity. Memory-like NK cells develop following infection with, for example, human cytomegalovirus (CMV) and respond to a cytokine cocktail (IL-12, IL-15 and IL-18) [13]. The memory-like response was correlated with the expression of CD94, NKG2A and CD69 and a lack of CD57 and KIR in CD56-dim NK cells [14]. When NK cells are stimulated with cytokines, immunomodulator-semaphoring 7A (SEMA 7A) is upregulated on NK cells, maintaining increased functionality [15].

3. The Role of NK Cell Therapy in Hematological Malignancies

3.1. Administration of Autologous NK Cells

The initial study administering autologous IL-2-activated NK cell-rich populations or intravenous IL-2 infusions in lymphoma patients did not produce a significant effect compared with controls [16]; see **Table 1**. Several approaches have augmented the antibody-dependent cellular cytotoxicity (ADCC) of autologous NK cell therapy: inserting anti-tumor monoclonal antibody, checkpoint receptor blockers, bi- and tri-specific killer engagers (BiKEs and TriKEs) and cytokine-induced memory NK cells.

Table 1. Clinical trials with administration of autologous and allogeneic NK cells (aGVHD: Acute Graft versus Host Disease, AML: Acute Myeloid Leukemia, cGVHD: Chronic Graft versus Host Disease, CR: Complete Response, CRS: Cytokine Release Syndrome, CML: Chronic Myeloid Leukemia, Cy: Cyclophosphamide, DLI: Donor Lymphocyte Infusion, Flu: Fludarabine, F/U: Follow-up, GVHD: Graft versus Host Disease, iPSC: Induced Pluripotent Stem Cells, MDS: Myelodysplastic Syndrome, N/A: Not Applicable, NHL: Non-Hodgkin Lymphoma, ORR: Overall Response Rate, PBMC: Peripheral Blood Mononuclear Cells, R: Rituximab, SD: Stable Disease, UCB: Umbilical Cord Blood, * Posttransplant application). The numbers in the first column represent the number of patients.

Patients	Donor/NK Cell Source	NK Cell Expansion Method	Conditioning Regimen Prior to NK Infusion	Adverse Event/Toxicity	Response	Reference
4 Follicular Lymphoma, 5 Diffuse Large B Cell Lymphoma	Autologous/PBMC	IL-2 and IL-15 stimulation	None	None	CR in 7/9, median F/U: 44 months	[17]
9 AML	Allogeneic/PBMC	IL2, IL-12, IL-15, and IL-18 stimulation, CD3 depletion, CD56-positive selection	Flu + Cy	N/A	ORR 55%, CR 45%	[18]
4 AML, 1 CML	Haploidentical/PBMC	CD3 depletion, CD56 enrichment	None *	None	2/5 patients donor chimerism	[19]
19 AML	Haploidentical/PBMC	CD3 depletion, IL-2 stimulation	Flu + Cy	Pleural effusion in 1 patient	CR in 5/19	[10]
10 AML	Haploidentical/PBMC	CD3-depletion, CD56-enrichment, IL-2 stimulation	Flu + Cy	None	CR 100%	[20]
41 hematological malignancies	Haploidentical/PBMC	CD3-depletion, IL-15, IL-21 stimulation	None *	None	Significant reduction of leukemia progression 46% vs. 74% (historical cohort)	[21]
29 lymphoma	Autologous/PBMC	Ex vivo IL-2 stimulation	None	None	No change in outcome compared to historical controls	[16]

Patients	Donor/NK Cell Source	NK Cell Expansion Method	Conditioning Regimen Prior to NK Infusion	Adverse Event/Toxicity	Response	Reference
41 AML	Haploidentical/PBMC	CD3-depletion, IL-15, IL-21 and hydrocortisone stimulation	None *	Grade 2 to 4 aGVHD 28%, cGVHD 30%,fever 73%	CR 57%, 3-year leukemia progression 75%	[22]
6 B cell NHL	Allogeneic/PBMC	CD3-depletion, IL-2 stimulation	Flu + Cy + R	None	4/6 clinical response	[23]
7 AML	"Off-the-shelf"/NK-92	IL2 stimulation	None	None	1 blast reduction, 2 SD	[24]
26 AML	Haploidentical/PBMC	CD19 and CD3 depletion, rhIL15 stimulation	Flu + Cy	CRS in 56% of patients, neurologic toxicity in 5/9 patients	CR: 40%	[25]
8 AML, 5 CML	Haploidentical/PBMC	CD3-depletion K562 Clone9.mbIL21 feeder cells	None *	aGVHD grade 1–2 54%	CR: 11/13 median F/U: 14.7 months	[26]
9 AML	"Off-the-shelf"/iPSC	IL2 stimulation	Flu + Cy	3 patients Grade 3 febrile neutropenia	4/9 CR	[27]
11 B cell NHL	"Off-the-shelf"/iPSC	IL 2 stimulation	Flu + Cy	None	8/11 had objective response, CR median F/U: 5.2 months	[28]
3 AML	"Off-the-shelf"/iPSC	IL2 stimulation	Flu + Cy	None	1/3 CR	[27]
14 B cell NHL	"Off-the-shelf"/iPSC	IL2 stimulation	Flu + Cy + R	None	10/14 patients achieved objective response, 7 CR	[28]
10 AML	Allogeneic/UCB	$CD34^+$ selection	Flu + Cy	None	4/10 disease free	[29]
12 MM	Allogeneic/UCB	CD3 depletion, K562-9.mbIL21, IL-2 stimulation	Lenalidomide/melphalan	None	10 patients achieved at least VGPR, Median F/U 21 months	[30]

Daratumumab, a monoclonal antibody against CD38, is a feasible option when NK cells have CD38 knocked out by CRISPR/Cas9 to prevent fratricide [31]. Checkpoint receptor blockage through PD-1 or PD-L1 activated an NK response in mouse models of several cancers, including lymphoma. Activated NK cells express PD-1, interact with PD-L1+ tumor cells and down-regulate NK cell-mediated immunity. Other checkpoint inhibitors against CD96, TIGIT or TIM-3 enhance anti-tumor activity in various solid tumors [32].

3.2. Administration of Allogeneic NK Cells

Although autologous NK cell therapies have useful effects, the aggressiveness of hematological malignancies, tumor escape and manufacturing failures because of the low number and compromised function of patient-derived NK cells have prompted interest in allogeneic NK cells for an "off-the-shelf" approach. Indeed, this process requires depleting T cells and/or regulatory T cells from the product to prevent graft versus host disease (GVHD) or lympho-proliferative disorders [33].

A pioneering study with successful allogeneic adoptive transfer of NK cells from a HLA-haploidentical donor in AML, demonstrated by Miller et al., confirmed the notion that KIR mismatch with tumor MHC may lead to greater cytotoxicity [10]. Complete hemtologic remission was achieved in 5 of 19 in poor-prognosis patients with AML under intensive cyclophosphamide and fludarabine conditioning regimens. In 10 pediatric patients, complete remissions (CRs) were achieved by KIR ligand-mismatched CD3-depleted and CD56-enriched NK cells (median dose, 26 × 10^6/kg) and six doses of IL-2 (1 million U/m^2) without graft versus host disease (GVHD) and remained in CR for 2 years [20]. In 57 refractory AML patients, the expansion of haploidentical NK cells was greater in 15 patients that received host regulatory T cell-depleted IL-2 diphtheria fusion protein (IL2DT) following cyclophosphamide and fludarabine than in patients who did not receive IL2DT (27% vs. 10%). The CR rate at day 28 was improved in patients with IL2DT (%53 vs. %21, p = 0.002) [34]. Bachanova et al. reported six patients with advanced B-cell non-Hodgkin lymphoma that received rituximab, cyclophosphamide and fludarabine followed by CD3-depleted NK cell-enriched cell products followed by subcutaneous IL-2 (10 × 10^6 units/6 doses). The treatment did not cause major toxicity, and four of six patients showed a clinical response at 2 months. However, the inadequate immunodepletion and host Treg population affects NK cell survival and expansion unfavorably [23].

Transfusing haploidentical, T-cell depleted, KIR-ligand mismatched NK cells after conditioning therapy with melphalan and fludarabine in advanced multiple myeloma following autologous stem cell transplantation caused no significant toxicity; further blocking of inhibitory KIR ligands with anti-human leucocyte antigen antibody enhanced killing of multiple myeloma cells [35]. Lymphodepletion with busulfan, fludarabine and ATG followed by IL-2 activated haploidentical NK cells showed increased efficacy with delivery of CD56+ cells (p = 0.022) in high-risk AML, MDS and CML without an increase of GVHD [36]. NK cells isolated from haploidentical donors and activated with CTV-1 leukemia cell line lysate in a phase I trial showed a prolonged relapse-free survival (RFS) period in high dose of infusion (337 days, 3 × 10^6) [37]. Recombinant human IL-15 also induced NK cell expansion and haploidentical transfer-induced remission in 35% of AML patients [25]. Another approach to maximize the anti-leukemia potential of NK cells is to pre-activate NK cells with IL-12, IL-18 and IL-15 to differentiate them into cytokine-induced memory-like NK cells. In a phase I trial using adoptively transferred cytokine-induced memory-like NK cells in AML, four of nine patients achieved CR [18].

The first-in-human study of NK cell products generated from CD34+ hematopoietic stem and progenitor cells (HSPC) of partially HLA-matched UCB units demonstrated that UCB-derived NK cells were well tolerated without a significant toxicity, and two of four patients with minimal residual disease (MRD) before infusion became MRD negative for 6 months [29]. From twelve multiple myeloma cases, UCB-derived NK cells were administered for MM patients undergoing high dose chemotherapy and autologous hematopoietic stem cell transplantation (auto-HCT), and 10 patients achieved at least very good partial responses [30].

3.3. NK Cell Engineering—“CAR-NK Cell Therapy”

Despite the tremendous efforts and considerable progress that has been achieved in adoptive NK-cell immunotherapy, a certain number of tumor cells with genetic or epigenetic variations can still bypass immunological surveillance [38]. To overcome the inhibition of the immune response and tumor escape, genetic modulation of NK cell-associated receptor expression is promising. A CAR is a genetically engineered protein composed of three parts: an extracellular domain derived from a single-chain fragment (scFv) targeted to a special antigen with high affinity binding, a transmembrane domain and an intracellular signaling domain. CAR technology was first applied to T cells, generating CAR-T cells, but some drawbacks have been demonstrated. Major limitations include the high risk of graft versus host disease (GVHD) in allogeneic use, high manufacturing costs and adverse events such as cytokine release syndrome (CRS) or neurotoxicity [39][40][41]. Conversely, CAR-NK cells do not cause GVHD and can be obtained from healthy third-party donors, making them suitable for “off-the-shelf” use. Adverse events have been observed less frequently; activated NK cells produce useful and safe cytokines such as IFN-γ and GM-CSF [42]. CAR-NK cells can also produce cytotoxic effects by using their native activating NK receptors, especially when tumor cells lose the antigen expression targeted by CARs, so the cytotoxic function of CAR-NK cells is not CAR-restricted [43]. On-target/off-tumor effects occur rarely because of the short life of NK cells [44].

Although less activity was observed with CD137-CD3 ζ co-stimulation, all CAR NK-92 cells retained cytotoxicity in vitro and in a Raji xenograft model in vivo [45]. UCB-derived NK cells transduced with a retroviral vector incorporating genes for CAR-CD19, IL-15 and inducible caspase-9-based suicide gene (iC9) killed CD19 positive cell lines and prolonged survival in a Raji xenograft lymphoma murine model [46]. CAR-NK cells against CD20, CD138 and CS-1 showed promising results in B-cell malignancies and multiple myeloma CD5 in T-cell malignancies in preclinical studies (**Table 2**). To our knowledge, different activation signals in CAR-NK cells have been compared in only solid tumors [47].

Table 2. Several preclinical studies of CAR-NK cells in hematological malignancies.

Target	Tumor Type	NK Cell Source	Structure of CAR Constructs	References
CD19	B-cell leukemia	NK-92 cell line	Anti CD19 scFv + CD3ζ	[48]
CD19	B-cell leukemia	Peripheral blood	Anti CD19 scFv + 41BB-CD3ζ	[49]
CD19	B-cell malignancies	NK-92	Anti-CD19 scFV + CD3ζ, CD28 + CD3ζ or CD13 + CD3ζ	[45]
CD19	B-cell malignancies	Cord blood	Anti-CD19 scFv + 4-1BB + CD3ζ + iCasp9 + IL-15	[46]
CD19	B-cell malignancies	Peripheral blood	Anti CD19 scFv + 41BB + CD28 + CD3ζ	[50]
CD20	B-cell malignancies	Peripheral blood	Anti CD19 scFv + 41BB-CD3ζ	[51]
CD20	Burkitt lymphoma	Peripheral blood	Anti CD19 scFv + 41BB-CD3ζ + IL15	[52]
CD138	Multiple myeloma	NK-92MI	Anti CD19 scFv + CD3ζ	[53]
CS-1	Multiple myeloma	NK-92	Anti CD19 scFv + CD28 + CD3ζ	[54]
CD5	T-cell malignancies	NK-92	Anti CD19 scFv + 41BB + CD28 + CD3ζ	[55]

Not many clinical trials of CAR-NK cells against hematological malignancies are listed in clinicaltrials.gov (**Table 3**). Tang et al. safely administered the first CD33-CAR-NK-92 cells against relapse refractory acute myeloid leukemia (AML) in three patients. Patients had mild fever and cytokine release, but the response was transient (NCT02944162) [56]. HLA-mismatched anti-CD19 CAR-NK cells derived from cord blood with IL-15 and iCas9 were expanded on K562-mbIL21 and 4-1BB ligand feeder cells and administered to 11 relapse refractory B-cell lymphoma patients. The treatment was tolerated without major toxic effects or cytokine release syndrome. Among the 11 patients, 8 (73%) had a response, 7 had complete remission and 1 had remission [57] (NCT03056339).

Table 3. Human trials of CAR-NK cells for hematological malignancies listed at ClinicalTrials.Gov (iPS: induced pluripotent stem).

Antigen Target	Tumor	NK Cell Source	Structure of the CAR Construct	Phase of the Study	ClinicalTrials.Gov Identifier # (Number)
CD22	B lymphoma	Unknown	Anti-CD22 + CD244	I	NCT03692767
CD19	B lymphoma	NK-92	Anti-C19 + CD244	I	NCT03690310
CD19/CD22	B lymphoma	Unknown	Anti-CD19/22 + CD244	I	NCT03824964
CD19	B lymphoma	Unknown	Unknown	I	NCT04639739
CD19	B lymphoma	Unknown	Unknown	I	NCT04887012
BCMA	Multiple myeloma	NK-92	Anti-BCMA + CD8αTM-4-1BB-CD3ζ	I/II	NCT03940833
CD7	NK/T-cell lymphoma	Unknown	Unknown	I	NCT04264078
CD19	B-lymphoid malignancies	Cord blood NK cells	Anti-CD19 + CD28-CD3ζ	I/II	NCT03056339
CD33	Acute myeloid leukemia	NK-92	Anti-CD33 + CD28-4-1BB-CD3ζ	I/II	NCT02944162
CD7	T-cell leukemia/lymphoma	NK-92	Anti-CD7 + CD28-4-1BB-CD3ζ	I/II	NCT02742727
CD19	B-cell malignancies	NK-92	Anti-CD19 + CD28-4-1BB-CD3ζ	I/II	NCT02892695
CD19	B lymphoma	iPS-derived NK cells	Anti-CD19 + CD244	I	NCT03824951
CD19	B-cell leukemia	Peripheral blood	Anti-CD19 + CD8αTM + 4-1BB + CD3ζ	I	NCT00995137

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