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CAR-NK cell therapy: latest updates from the 2024 ASH annual meeting

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Abstract
Natural killer cells, integral to the innate immune response, exhibit the inherent capacity to identify and eliminate cancer cells without prior exposure, positioning them as prime candidates for immunotherapeutic strategies. Chimeric antigen receptor-engineered natural killer (CAR-NK) cells obviate the requirement for human leukocyte antigen compatibility, simplifying personalized schedules and facilitating the manufacture of off-the-shelf products. In addition, CAR-NK cell therapy possesses lower risk of cytokine release syndrome and neurotoxicity, benefitting patients with higher security. Nevertheless, CAR-NK cell therapy is also confronted with challenges, including but not limited to short lifespan and restrictions from tumor microenvironment. Here, we summarized the latest advancements in the preclinical investigations and clinical trials of CAR-NK cell therapy from the 2024 ASH Annual Meeting.

Keywords Cellular therapy, CAR-NK, Anti-tumor therapy

To the editor

Chimeric antigen receptor (CAR)-engineered immune cells have exhibited significant therapeutic potential in hematologic malignancies. Adoptive cell therapy based on natural killer (NK) cells appears to have more manageable safety profiles and fewer graft restrictions in contrast to T-cell based treatment strategies, presenting potent competence [1, 2]. Nevertheless, challenges such as limited lifespan, constrained expansion, and host immune rejection remain. To mitigate these issues and augment the anti-tumor efficacy of NK cell therapy, extensive research and development have been undertaken. Here, we overviewed the latest clinical and preclinical updates on CAR-NK cell therapy presented at the 2024 ASH Annual Meeting.

Generation of CAR-NK cells

The clinical application of NK cells necessitates efficient expansion. To augment the in vivo expansion of CAR-NK cells, improvements have been made in two aspects. First, the feeder cell culture system is typically considered the most effective strategy, utilizing immortalized cell lines as artificial antigen-presenting cells (aAPC) that express membrane-bound (mb) stimulatory molecules such as mbIL-18, mbIL-21, OX40L and 4-1BBL to stimulate NK cells expansion. The 721.221 cell, a type of feeder cell, engineered to express co-stimulatory molecules mbIL18/21 along with 4-1BBL/OX40L, and B7H6, significantly enhanced the expansion and activation of peripheral blood (PB)-NK cells and induced-pluripotent stem cells (iPSC)-NK cells compared with traditional K562-based aAPC [3]. In addition to employing genetically engineered feeder cells, the provision of stimulatory

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factors within an autologous context represents an alternative strategy for expansion of NK cells. Scientists have developed a multifunctional fusion protein capable of binding to B cells. In co-culture experiments conducted with B cells, this fusion protein facilitated the trans-presentation of IL-15 and provided 4-1BB co-stimulation to NK cells within an autologous setting, thereby significantly enhancing the expansion of NK cells derived from PB of healthy donors, cord blood, multiple myeloma patients and acute myeloid leukemia (AML) patients [4].

In addition to enhancing the expansion of CAR-NK cells, reducing the host's cytotoxic response against the graft is also crucial for extending the lifespan of NK cells. Downregulating human leukocyte antigen (HLA)-ABC expression could reduce the T-cell-mediated destruction on infused CAR-NK cells in vivo, while upregulating HLA E/G, and CD47 in CAR-NK cells could inhibit the cytotoxicity of host NK cells, thereby diminishing the destruction of CAR-NK cells. Traditionally, this process required at least two steps to generate CAR-NK cells. In order to simplify manipulation, researchers have engineered a novel shRNA that can specifically downregulate HLA-ABC expression while preserving HLA-E expression, providing a one-step method to create allogeneic CAR-NK cells. By transducing NK cells with a gene construct that combines a CAR, shRNA, and PD-L1 or single chain HLA-E (SCE) expression, they have successfully generated CAR-NK cells that evade host immune rejection and exhibit enhanced anti-tumor responses [5].

Preclinical studies

In the absence of cytokine support, NK cells exhibit a limited lifespan, resulting in reduced in vivo persistence. In preclinical studies, investigations have focused on the engineering of NK cells with CAR to target tumor cells and augment their persistence and cytotoxic activity. Autocrine production of IL-15 can partially ameliorate the issues of short persistence and metabolic dysfunction in the microenvironment during CAR-NK cell therapy. C-C motif chemokine ligand 21 (CCL-21) plays a pivotal role as a chemokine for T cell recruitment and activation. In B-cell lymphoma, CD19 CAR-NK cells co-expressing IL-15 and CCL-21 (15 × 21 CAR-NK) showed superior cytotoxicity and cytokine secretion functions, and were proven to recruit more T cells and work with T cells to eliminate lymphoma cells. From a mechanistic perspective, 15 × 21 CAR-NK cells displayed a pronounced enrichment in the PI3K/AKT/mTOR signaling cascade, which correlated with an augmented capacity for anti-apoptotic ability and enhanced mitochondrial functionality [6] (Table 1). CAR-NK cell therapy, engineered to target novel tumor-associated antigens on cancer cells, exhibits robust anti-tumor activity. Mesothelin (MSLN) has been demonstrated to be expressed in a subset of AML patients, including those diagnosed with a highly aggressive subtype or relapse. Building on this finding, researchers engineered MSLN-CAR constructs into cytokine-induced memory-like (CIML) NK cells—a specialized population of NK cells pre-activated with specific cytokine cocktail to acquire enhanced anti-tumor capabilities. It has been shown that IL-12, a cytokine known to amplify NK cell cytotoxicity, can be safely and feasibly incorporated into MSLN-CAR CIML NK cells and these engineered cells significantly enhanced their potency against MSLN-expressing AML through higher cytotoxicity and IFNγ production [7] (Table 1). C-type lectin-like molecule-1 (CLL-1) is another promising target antigen in AML, garnering attention due to its strong expression on leukemia cells. In vivo studies have shown that CLL-1-CAR NK cells supported with an endogenous IL-15 or IL-2 secretion cassette were safe, effective and prolonged the median overall survival, in which IL-2 outperformed IL-15 in vivo [8] (Table 1). Compared with other hematological malignancies, the expression of G protein-coupled receptor, class C, group 5, member D (GPRC5D) is notably higher in multiple myeloma cells, positioning GPRC5D as an ideal target for cell therapy for multiple myeloma. CIB315, novel anti-GPRC5D iPSC-derived CAR-NK product with enhanced cytotoxicity and persistence, could avoid daratumumab-induced fratricide and support killing of multiple myeloma cells for multiple rounds [9] (Table 1).

Table 1 Preclinical studies of CAR-NK presented at ASH 2024

Diseases	NK cell source	Target	Reference
Acute myeloid leukemia	PB	MSLN	[7]
Acute myeloid leukemia	PB	CLL-1	[8]
Acute myeloid leukemia	PB	CD19	[11]
B-cell lymphoma	NK92 cell line	CD19; IL-15; CCL-21	[6]
Multiple myeloma	iPSC	GPRC5D	[9]

CCL-21: C-C motif chemokine ligand 21; CIML: cytokine-induced memory-like; CLL-1: C-type lectin-like molecule-1; GPRC5D: G protein-coupled receptor, class C, group 5, member D; IL-15: interleukin-15; iPSC: induced-pluripotent stem cells; MSLN: mesothelin; PB: peripheral blood

Clinical trials

Several clinical trials of CAR-NK cell therapy have demonstrated encouraging efficacy and reassuring safety. TAK-007, an allogeneic, umbilical cord blood-derived, off-the-shelf CD19 CAR-NK cell therapy product, has shown promising early efficacy and a favorable safety profile in a phase 2 trial for heavily pretreated patients with relapsed/refractory large B-cell lymphoma (LBCL) or indolent non-Hodgkin lymphoma (iNHL) (NCT05020015). In the trial, 78% of iNHL patients ($n = 9$) responded to TAK-007, with 56% achieving complete responses. 50% of LBCL patients ($n = 14$) responded

Table 2 Clinical trials of CAR-NK presented at ASH 2024

Product	NK cell source	Clinical Trial ID	Clinical Stage	Diseases	Patient number	ORR	CR	CRS	ICANS	Reference
TAK-007	Cord blood	NCT05020015	Phase 2	LBCL	17 [1]	/	800 M, 21% [2]	Grade 1($n=2$);	None	[10]
				iNHL	9	/	56% [3]	Grade 2($n=1$)		
WU-NK-101	PB	NCT05470140	Phase 1	AML	9	DL ≥ 2, 50% [4]	/	Grade 1($n=4$)	None	[12]
NKX019	PB	NCT06518668	Phase 1	SLE	/	/	/	/	/	[13]

[1] As of the data cut-off date (February 23, 2024), 18 patients with LBCL were enrolled in the study, but 1 LBCL patient in expansion withdrew due to an adverse event before TAK-007 administration

[2] The study included a dose escalation phase evaluating 2 dose levels (2×10^8 [200 M] and 8×10^8 [800 M] CD19 CAR-NK cells). However, no responses were seen in the 3 LBCL patients who received 200 M TAK-007. 7 of 14 (50%) LBCL patients who received 800 M TAK-007 had a response, including 3 (21%) complete responses

[3] Among 9 iNHL patients, 7 (78%) had a response including 5 (56%) complete responses

[4] Patients received WU-NK-101 treatment at dosage levels of 300 million cells (DL1), 900 million (DL2), and 1.8 billion (DL3), with three patients at each DL. No responses were noted at DL1. At DL ≥ 2 groups, the objective response rate was 50%

AML: acute myeloid leukemia; CAR-NK: chimeric antigen receptor-engineered natural killer; CR: complete response; CRS: cytokine release syndrome; DL:dosage level; ICANS: immune effector cell-associated neurotoxicity syndrome; iNHL: indolent non-Hodgkin lymphoma; LBCL: large B-cell lymphoma; NK: natural killer; OR: objective response; ORR: objective response rate; PB: peripheral blood; SLE: systemic lupus erythematosus

to the dosage level of 800 million cells, with complete responses observed in 21% of LBCL cases. The safety profile of TAK-007 therapy was superior to that of autologous CAR-T cell therapies, with no severe cytokine release syndrome (CRS) (Grade ≥ 3) observed within 10 days post-administration and no immune effector cell-associated neurotoxicity syndrome (ICANS) reported within 60 days post-administration [10] (Table 2).

Immune inhibitory signals within the tumor microenvironment have impeded the efficacy of CAR-NK cell therapy. Nevertheless, an available adoptive memory-like NK cell therapy, WU-NK-101 (W-NK1), has demonstrated the ability to circumvent immune suppression in AML xenograft models, by secreting a diverse array of cytokines and chemokines, which facilitated the maturation and activation of dendritic cells, promoted the migration and activation of T cells, and enhanced the maturation of macrophages [11] (Table 1). The phase 1 clinical trial of W-NK1 in patients with relapsed/refractory AML exhibited an acceptable safety profile and preliminary anti-tumor activity (NCT05470140). Patients received W-NK1 treatment at dosage levels of 300 million cells (DL1), 900 million (DL2), and 1.8 billion (DL3), with three patients at each DL. At DL ≥ 2 groups, the objective response rate was 50%, with that only one severe treatment-related adverse event occurred (Grade ≥ 3 anemia), and no severe CRS (Grade ≥ 2) or ICANS were reported [12]. (Table 2).

In addition to hematological malignancies, CD19-targeted cell therapies have demonstrated encouraging early outcomes in patients with systemic lupus erythematosus (SLE) and lupus nephritis (LN). NKX019, composed of NK cells engineered to express a humanized CAR targeting CD19, is under investigation in a phase 1 study in adult patients with refractory SLE, with or without LN (NCT06518668). Participants will receive a calculated dose of 1 billion viable CAR-NK cells administered on days 0, 7, and 14 following single agent cyclophosphamide lymphodepletion to evaluate its safety, tolerability, pharmacokinetics, immunogenicity, and efficacy [13] (Table 2).

These studies mark a promising step forward in leveraging allogeneic CAR-NK cell therapy to improve outcomes for patients with hematological malignancies and autoimmune diseases.

Abbreviations

15×21 CAR-NK	CAR-NK co-expressing IL-15 and CCL-21
aAPC	Artificial antigen-presenting cells
AML	Acute myeloid leukemia
CAR	Chimeric antigen receptor
CAR-NK	Chimeric antigen receptor-engineered natural killer
CCL-21	C-C motif chemokine ligand 21
CIML	Cytokine-induced memory-like
CLL-1	C-type lectin-like molecule-1
CRS	Cytokine release syndrome
GPRC5D	G protein-coupled receptor, class C, group 5, member D
HLA	Human leukocyte antigen
ICANS	Immune effector cell-associated neurotoxicity syndrome
iNHL	Indolent non-Hodgkin lymphoma
iPSC	Induced-pluripotent stem cells
LBCL	Large B-cell lymphoma
LN	Lupus nephritis
mb	Membrane-bound
MSLN	Mesothelin
NK	Natural killer
PB	Peripheral blood
SCE	Single chain HLA-E
SLE	Systemic lupus erythematosus
W-NK1	WU-NK-101

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RK and TL drafted this manuscript. BL and HW prepared tables. TL and XZ revised the manuscript. XZ provided direction and guidance throughout

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Data availability
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Declarations

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Not applicable.

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The authors declare no competing interests.

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