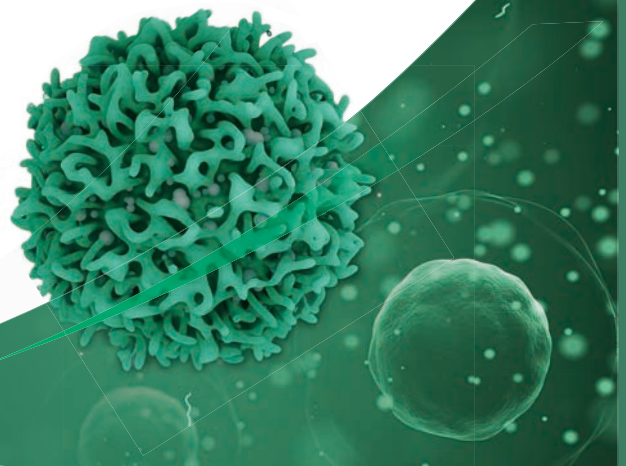
CAR-NK Therapy: An Effective Strategy for Cancer Treatment



Introduction

CAR-T therapies represent a new successful strategy for the treatment of hematological malignancies; however, recent studies have revealed some limitations, including the occurrence of graft-versus-host disease (GVHD), neurotoxicity, and cytokine release syndromes (CRS). This has prompted researchers to look for alternative, safer cells, such as natural killer (NK) cells[1]. NK cells are lymphocytes that kill tumor cells and virus-infected cells non-specifically, without prior sensitization. CAR-NK therapy involves the modification of NK cells with mature chimeric antigen receptor (CAR) technology to exploit their unique target cell recognition mechanism and broad tumor-killing ability. Figure 1 illustrates the CAR-NK therapeutic strategy[1]. Compared with CAR-T therapy, CAR-NK therapy has numerous advantages, such as lower GVHD and significantly reduced CRS toxicity and immune effector cell-associated neurotoxicity syndrome toxicity, which enhance overall safety[2, 3]. In addition, NK cells have multiple tumor recognition sites, thus potentially reducing antigenic escape failure[4].

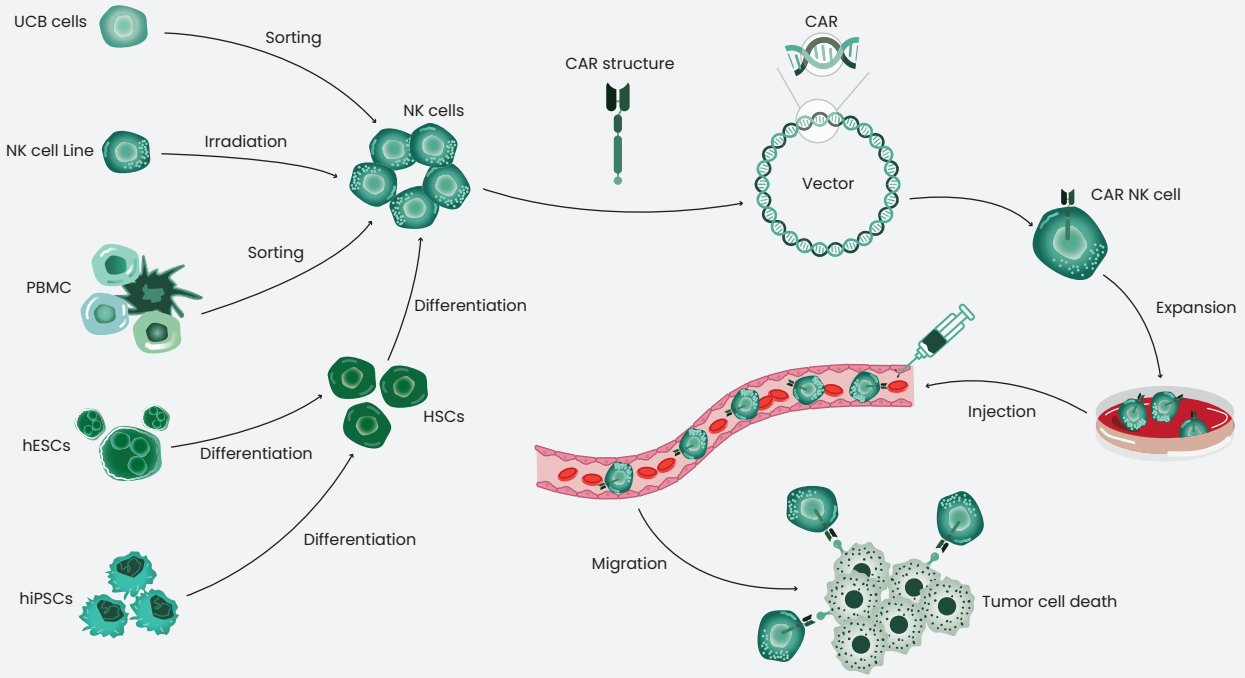


Figure 1. The flow chart of CAR-NK therapy.

www.sinobiological.com

Sino Biological US Inc. (U.S.A.)
+1-215-583-7898
order_us@sinobiologicalus.com

Sino Biological Europe GmbH (Europe)
+49(0)6196 9678656
order_eu@sinobiologicaleu.com

株式会社日本シノバイオロジカル (Japan)
044-400-1330
order@sinobiological.co.jp

Sino Biological, Inc. (Global)
+86-400-890-9989
order@sinobiological.com

CAR Molecule Design

CAR has the ability to specifically recognize target cells and activate NK cells to kill tumor cells. Therefore, the design and engineering of the CAR structure are crucial for CAR-NK therapeutic success. Similar to CAR-T, CAR is composed of an extracellular antigen binding domain, a hinge domain, a transmembrane (TM) domain, and an intracellular domain[2]. The extracellular domain contains a single-chain variable fragment (scFv) derived from monoclonal antibodies that provides the ability to specifically recognize and bind to tumor antigens. The hinge domain connects extracellular domain to TM domain[5]. The intracellular signaling domain determines the strength of the activation signal and directly affects the killing effect[6]. Table 1 summarizes the molecules of CAR structure in CAR-NK[2].

Table 1. Molecules of the CAR structure in CAR-NK

Domains	Molecules
Extracellular domain	VH-VL, VL-VH, VH-only
Hinge domain	CD8a, CD28, DAP12, IgG4, IgG2 CH2-CH3, IgG1 CH2-CH3, IgG4 CH2-CH3
TM domain	CD3ζ, CD28, CD8a, 4-1BB, DAP12, TCR ab, CD28-CD3ζ, FceRIγ, murine CD3ζ
Intracellular domain	CD3ζ, CD28, 4-1BB

Currently, there are four generations of CARs, with different structures and compositions (Figure 2). First-generation CARs contain only CD3ζ or DAP12 as signaling structural domains. Second-generation CARs express a second signaling structural domain including CD28 or 4-1BB associated with CD3ζ, and third-generation CARs include CD3ζ and two co-stimulatory signaling domains (CD28 and 4-1BB). Finally, fourth-generation CARs involving NK cells can release cytokines, such as IL-2 and IL-15, which regulate NK cells proliferation. This effectively enhanced the anti-tumor activity of NK cells against lymphoma xenografts[1,7].

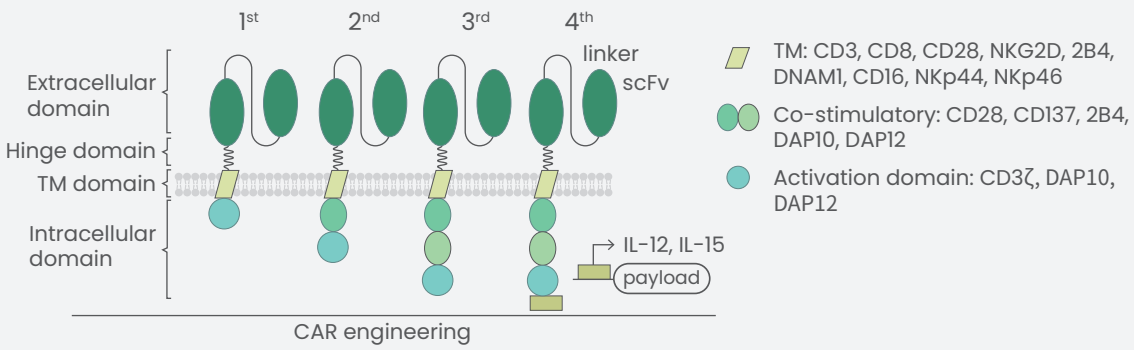


Figure 2. The four generations of CAR structure.
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Source of NK Cells

NK-92 cell lines have been widely used as a source of CAR-NK cells due to their remarkable proliferation ability *in vitro* and reduced sensitivity to repeated freeze/thaw cycles. However, as malignant cells from a NK cell lymphoma, NK92 cells have some inherent limitations including potential tumorigenicity risk, absence of CD16 and NKp44 expression, and the need for lethal irradiation prior to infusion, which hampers *in vivo* expansion potential.

Sino Biological US Inc. (U.S.A.)
+1-215-583-7898
order_us@sinobiologicalus.com

Sino Biological Europe GmbH (Europe)
+49(0)6196 9678656
order_eu@sinobiologicaleu.com

株式会社日本シノバイオロジカル (Japan)
044-400-1330
order@sinobiological.co.jp

Sino Biological, Inc. (Global)
+86-400-890-9989
order@sinobiological.com

Umbilical cord blood (UCB) presents another attractive source of NK cells with various advantages, such as off-the-shelf availability, low T cell contamination risk, and great potential for proliferation and persistence *in vivo*. To overcome volume limitations, UCB are expanded to large numbers through a variety of methods covering cytokine mixture and artificial antigen-presenting cells.

In addition, NK cells can be acquired from other sources (as shown in Figure 1), including peripheral blood (PB), cord blood (CB), peripheral blood mononuclear cells (PBMCs), and stem cells such as induced pluripotent stem cells (iPSCs) [8, 9]. Scientists are exploring the use of NK cells, and will find the best source for NK-cell immunotherapy for their research.

Preparation and Expansion of CAR-NK Cells

With advances in genetic engineering technology, several methods have been established to generate CAR-NK cells. Commonly used CAR-NK transduction systems utilize viral and non-viral vectors. Viral vectors include retroviruses, lentiviruses and adeno-associated viruses (AAV) [6], with the retroviral transduction being the most common method of delivering CAR to NK cells. Non-viral vectors include the sleeping beauty and PiggyBac transposons. Additionally, mRNA electroporation and the CRISPR/Cas9 technique can also be used.

There are several techniques to expand CAR-NK cells, and the two most commonly used approaches involve feeder cells and cytokine mixtures. Cytokines are important for the *in vivo* development and survival of NK cells. IL-21 promotes NK cell proliferation and enhances its cytotoxic function. IL-15 stimulates the expansion and cytotoxic activity of NK cells. It is also used in combination with IL-2 to expand NK cells *in vitro* [10, 11]. Sino Biological offers GMP-grade cytokines, including IL-2, IL-12, IL-15, and IL-21, and also provides comprehensive solutions for CAR-NK therapy to support cellular immunotherapy.

Clinical Trials of CAR-NK

As of August 2023, several clinical trials of solid and hematological tumors with CAR-NK therapies have been conducted worldwide. Currently, there are no FDA-approved drugs for CAR-NK and the vast majority are in preclinical phases, with some entering clinical phase I/II (Table 2).

Recently, a CAR-NK therapy was developed to target CD19 for the treatment of B-cell lymphoblastic leukemia/lymphoma in a phase I/II study (NCT05654038). This is a single-center, open-labeled, single-arm, non-randomized investigator-initiated trial to evaluate the efficacy and safety of anti-CD19 universal CAR-NK (UCAR-NK) cells therapy combined with HSCT for the treatment of B-cell hematologic malignancies. In addition, the purpose of another clinical trial (NCT05528341) is to evaluate the safety and effects of NKG2D-CAR-NK92 infusion for the treatment of relapsed/refractory solid tumors.

The clinical trials involving CAR-NK therapy have primarily focused on hematologic malignancies, including B-cell lymphoma, B-cell non-Hodgkin lymphoma (B-cell NHL), acute myeloid leukemia (AML), B-cell hematologic malignancies, acute lymphoblastic leukemia, and multiple myeloma [12-14]. CAR-NK therapy is also being tested against solid tumors, such as glioblastoma, breast cancer, small cell lung cancer, colorectal cancer, prostate cancer, and ovarian cancer [11, 15, 16]. These clinical trials have shown that CAR-NK therapies target CD19, CD7, CD22, CD33, CD70, BCMA, NKG2D, HER2, DLL3, mesothelin, ROBO1, Claudin 6, or PSMA to achieve therapeutic efficacy [13, 16].

Table 2. Clinical Trials involving CAR-NK cell therapy

Clinical trial	Target	Disease	Cell source	Status	Phase
NCT05182073	BCMA	Multiple Myeloma Myeloma	iPSCs	Recruiting	Phase 1
NCT05842707	CD19 CD70	Refractory or Relapsed B-cell Non-Hodgkin Lymphoma	CB	Recruiting	Phase 1 Phase 2
NCT02944162	CD33	Acute Myelogenous Leukemia Acute Myeloid Leukemia Acute Myeloid Leukemia With Maturation	NK-92 cell line	Unknown	Phase 1 Phase 2
NCT03940833	BCMA	Multiple Myeloma	NK-92 cell line	Unknown	Phase 1 Phase 2
NCT05654038	CD19	B-Cell Lymphoblastic Leukemia / Lymphoma	N/A	Recruiting	Phase 1 Phase 2
NCT04324996	NKG2D ACE2	COVID-19	CB	Unknown	Phase 1 Phase 2
NCT05645601	CD19	Adult Relapsed/Refractory B-cell Hematologic Malignancies	N/A	Recruiting	Phase 1
NCT05472558	CD19	B-cell Non Hodgkin Lymphoma	CB	Recruiting	Phase 1
NCT05667155	CD19, CD70	B-cell Non Hodgkin Lymphoma	CB	Recruiting	Phase 1
NCT05008536	BCMA	Multiple Myeloma, Refractory	CB	Recruiting	Early Phase 1
NCT03692637	Mesothelin	Epithelial Ovarian Cancer	PBMCs	Unknown	Early Phase 1
NCT05507593	DLL3	SCLC, Extensive Stage	N/A	Recruiting	Phase 1
NCT03415100	NKG2D	Solid Tumor	N/A	Unknown	Phase 1
NCT04847466	PD-L1	Gastroesophageal Junction (GEJ) Cancers Advanced HNSCC	N/A	Recruiting	Phase 2
NCT05528341	NKG2D	Relapsed/Refractory Solid Tumors	NK-92 cell line	Recruiting	Phase 1
NCT03940820	ROBO1	Solid Tumor	Primary NK cells	Unknown	Phase 1 Phase 2
NCT05410717	Claudin6	Stage IV ovarian cancer Testis cancer Refractory endometrial cancer	PB	Recruiting	Phase 1 Phase 2

Conclusion

CAR-NK cells have unique advantages over CAR-T cells, such as more precise killing, more cell sources, and greater efficacy against solid tumors. However, some challenges remain, such as cytotoxicity, low transfection efficiency, and storage issues. As CAR-NK therapies continue to be developed and refined, preclinical studies and preliminary clinical data indicate that CAR-NK therapy has improved safety. CAR-NK therapy lacks the human leukocyte antigen matching limitations, possesses a lower GVHD, CRS, and neurotoxicity, in addition to the potential of using allogeneic NK cells as a CAR platform for "off-the-shelf" therapy. Therefore, CAR-NK therapy holds great promise as a novel cancer treatment strategy with fewer side effects.

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order_us@sinobiologicalus.com

Sino Biological Europe GmbH (Europe)
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order_eu@sinobiologicaleu.com

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order@sinobiological.co.jp

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