

CAR-NK cell therapeutics for hematologic malignancies: hope is on the horizon

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Abstract

Chimeric antigen receptor (CAR)-T-cell therapy has achieved significant success in the treatment of hematologic malignancies. However, treatment-related toxicity and side effects remain the major drawbacks. As an important effector cell in innate immunity, natural killer (NK) cells exert strong antitumor functions and have better application prospects in the immunotherapy of hematologic malignancies. Compared with T cells, NK cells exhibit several advantages such as MHC-independent recognition. CAR-modified NK (CAR-NK) cells may exhibit a better ability of killing tumor cells. Herein, we review mainly preclinical data related to the development of CAR-NK cells in treating blood cancers.

Keywords: Hematology, Immunotherapy, NK cells

1. INTRODUCTION

In the field of cancer immunotherapy, chimeric antigen receptor (CAR)-T-cell therapy has immense significance. In fact, in vivo, the topmost player against cancer is not T cells, but natural killer (NK) cells.¹ Compared with CAR-T cells, CAR-NK cells have the following advantages for application in the immunotherapy of blood cancers:

1. allogeneic NK cells are associated with less severe graft-versus-host disease (GVHD)^{2,3};
2. mature NK cells have a shorter life span in vivo⁴; and
3. in addition to the ability of directly identifying multiple stimulatory molecules on the surface of tumor cells, NK cells can enhance the specificity of killing tumor cells through CAR modification.^{5,6}

The purpose of constructing CAR-NK cells is to create a novel strategy for activating NK cells and enhance their antitumor effects by genetic modification. CAR-NK cells inherit the basic structural framework and the transfection method of CAR-T cells. The CAR construct consists of the following three components: an extracellular antigen-recognition domain, a transmembrane domain, and an intracellular signaling domain. CAR molecules can be roughly divided into the following three

generations: first-generation CARs fused into target-specific scFv to specifically activate NK cells⁷; second-generation CARs incorporated into cytoplasmic signaling domains of T-cell costimulatory receptors; and third-generation CARs with multiple costimulatory domains⁸⁻¹⁰ (Fig. 1). In this review, we emphasize the effectiveness of CAR-NK cell therapy in preclinical and early clinical studies using CAR-NK cells.

2. ROLE OF CAR-NK CELLS IN B-ALL

Research on the application of CAR-NK cells in the treatment of relapsed and refractory acute B-cell lymphoblastic leukemia (R/R B-ALL) has just begun. Preclinical research can be roughly divided into the following two categories: CAR-NK cell therapy designed to alleviate the serious side effects caused by traditional CAR-T-cell treatment, and the other includes CAR-NK cell therapy developed for preventing relapse after CAR-T-cell treatment. Oelsner et al¹¹ transduced cytokine-induced killer (CIK) cells with a lentiviral vector encoding a CAR that carried a CD28-CD3 ζ -CD19 structure. These targeted CIK cells exhibited high cytotoxicity in vitro and in vivo. This was the first evidence showing that CAR-modified CIK cells may be used as a promising immunotherapeutic strategy for treating R/R B-ALL. Subsequently, different research groups confirmed that CAR-modified NK92 cells could exert antileukemic effects.^{12,13} Moreover, CAR-NK cells were found to be not only as effective as CAR-T cells but also less toxic.¹⁴

Being the most promising targeted therapy, CAR-T-cell therapy has attracted widespread research attention in recent years. CAR-T cells that specifically recognize the B-cell surface antigen CD19 have demonstrated significant success in clinical trials against R/R B-ALL,^{5,15-23} and two CAR-T cell products have been approved by the FDA.^{5,24} However, treatment-induced selection indicates that leukemic cells with altered or lost CD19 expression could be considered as the cause of treatment failure and relapse.^{6,25} Thus, CAR-NK92 cells were designed to express an fms-like tyrosine kinase 3 (flt3)-specific CAR(CD28-CD3 ζ) and an inducible caspase-9 (*icasp9*) suicide gene to improve safety.²⁶ Another strategy that can be used to overcome

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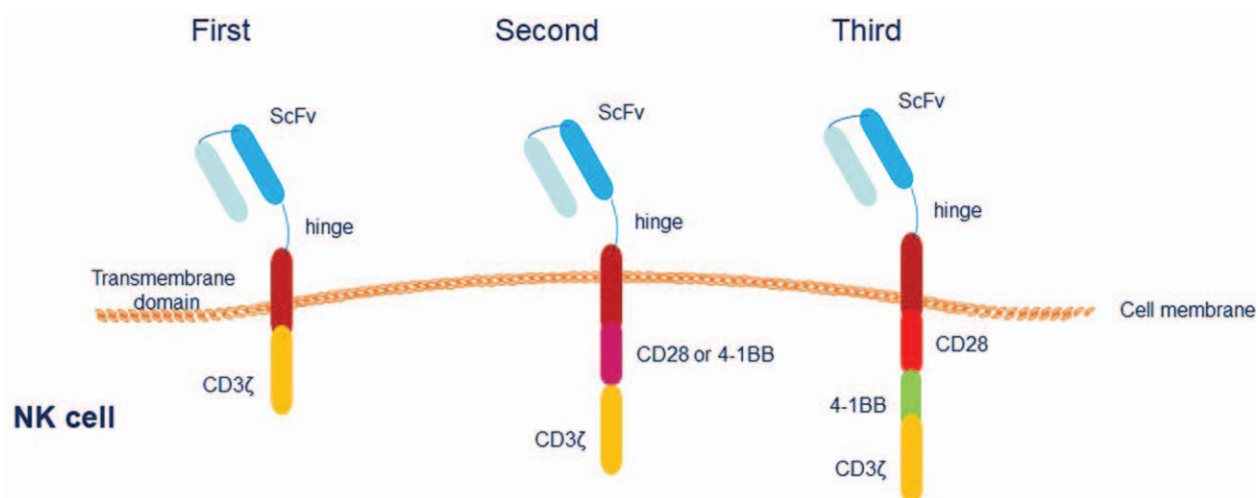


Figure 1. Structure of different generations of CARs.

antigen escape is to design CAR-NK cells that express a CAR-targeting CD22, which simultaneously secretes a CD19-specific T-cell engager to induce bystander T cells to kill leukemia cells.²⁷ Several clinical trials on CAR-NK cells are also being conducted (Table 1). Hence, the optimization of the intracellular signal domain of CAR and the antitumor effect of CAR-NK cells are aspects that have yet to be confirmed.

3. ROLE OF CAR-NK CELLS IN T-ALL

Results of basic research and clinical trials investigating the role of CAR-T cells in B-ALL are highly promising; however, there are still tremendous challenges in designing CARs for relapsed and refractory T-cell lymphocytic leukemia. As several antigens are shared by leukemia cells and normal cells, CAR T cells clear not only leukemia cells but also normal cells, even including CAR-T cells themselves.

You et al²⁸ constructed two second-generation CARs that can recognize CD7, one carrying a monovalent antibody (CD7-CAR-NK-92MI) and the other carrying a bivalent antibody (dCD7-CAR-NK-92MI). They compared the killing effects of the two types of CAR-NK cells on leukemia cells. Both the CAR-NK cell types exhibited specific antileukemic activity. Xu et al²⁹

compared the function of two intracellular domains, 4-1BB and 2B4. Both CARs exhibited specific antitumor activity against T-cell lymphoma cell lines. Analysis of the killing effects using a mouse model revealed that CAR-2B4-NK cells were more potent than CAR-4-1BB-NK cells.

4. ROLE OF CAR-NK CELLS IN B-NHL

A multicenter phase 2 clinical trial on 101 patients with relapsed and refractory non-Hodgkin's lymphoma (R/R NHL) demonstrated that the overall response rate and the complete remission rate after CAR-T-cell treatment reached 82% and 54%, respectively. The overall survival rate at 18 months was 52%.³⁰ It remains unclear why responses to CD19 CAR-T-cell therapy are more frequent and deeper in ALL than those in NHL. Purvey et al³¹ described preliminary data on CAR-NK cells at the 2017 ASH Meeting. They examined various lymphoma cell lines and primary lymphoma cells, and their experiments demonstrated that CD19 CAR-NK cells can effectively kill tumor cells. To increase the targeting specificity of amplified NK cells, Chu et al³² constructed an anti-CD20 CAR-NK cell line using peripheral blood NK cells. The anti-CD20 CAR-NK cells exhibited significantly enhanced cytotoxicity in vitro and prolonged

Table 1

Summary of CAR-NK cell clinical trials for ALL

Institution	NCT number	Costimulatory domain in CAR	Antigen targeted	Conditions	Phase	References
St Jude Children's Research Hospital, USA	NCT00995137	None	CD19	B-ALL	I	None
National University Health System, Singapore	NCT01974479	None	CD19	B-ALL	I	None
PersonGen BioTherapeutics (Suzhou) Co., Ltd. Suzhou, China	NCT02892695	CD28 and 4-1BB	CD19	B-ALL lymphosarcoma FL CLL DLBCL, MCL	I	None
M.D. Anderson Cancer Center, USA	NCT03056339	CD28	CD19	B-lymphoid malignancies B-ALL CLL NHL	I	None

B-ALL = B-lineage acute lymphoblastic leukemia (ALL); CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B cell lymphoma; FL = follicular lymphoma; MCL = mantle cell lymphoma; NHL = non-hodgkin lymphoma.

survival in xenotransplanted mice. However, the transient persistence of CAR-NK cells limits their therapeutic efficacy. To prolong the persistence time of CAR-NK cells *in vivo* and reduce treatment-related side effects, researchers have optimized the structure of CAR and introduced the induced suicide gene and *IL-15* gene. Such a novel design demonstrated remarkable survival in a lymphoma model.³³ An ongoing phase I/II trial is investigating the side effects and the best dose of CD19-CD28-zeta-2A-iCasp9-IL15-transduced cord blood NK cells (NCT03579927).

5. ROLE OF CAR-NK CELLS IN T-NHL

Peripheral T-cell lymphoma (PTCL) accounts for 10% to 15% of NHL. Compared with B-NHL, PTCL has limited treatment measures and poor prognosis.^{34–37} Because PTCL develops from mature T cells, these cells highly and commonly express CD4 antigen. Therefore, CD4 comprises an ideal target for CAR therapy. Pinz et al³⁸ constructed an anti-CD4 CAR, in which the sequence of the single variable Fc chain of the extracellular domain for CD4 antigen recognition was derived from a commercialized anti-CD4 antibody, which ensured the specificity and the high affinity of CAR for recognition of the target antigen. The application of third-generation CAR structure has been reported to prolong the life span of CAR-NK cells *in vivo*. The results revealed that CD4 CAR-NK cells could effectively inhibit the growth of lymphoma cells and prolong the survival time of mice. A more significant finding is that CD4 CAR-NK cells had no significant effect on the function of hematopoietic progenitor cells while killing tumor cells *in vivo*. Another potential target for T-cell malignancy is CD5.^{39,40} The majority of T-cell malignancies express CD5, whereas only a few lymphocyte subsets and thymocytes express CD5.^{41–43} Although the application of anti-CD5 CAR-T cells yielded good results, there is evidence of cross-reaction due to the intrinsic CD5 expression of CAR-T cells.⁴⁴ NK cells, which are CD5-negative, may be more feasible for the immunotherapy of T-cell malignancy.⁴⁵ Moot et al⁴⁶ made an interesting attempt to investigate the CD5 CAR. The CD5 on T cells was knocked out by the CRISP-Cas9 technique, and then CD5-scFv-CAR-T was constructed. To increase the affinity of CAR-NK cells to CD5, the variable lymphocyte receptor (VLR) structure was introduced. The affinity of VLR to specific antigens is known to be much higher than that of IgG antibodies. The functions of CD5-scFv-CAR-T, CD5-VLR-CAR NK92, and CD5-scFv-CAR NK92 cells were compared, which revealed that all the three types of CAR cells can recognize and kill tumor cells *in vitro*. However, the function and activity of CAR-T cells were decreased after knocking out CD5 compared with those of CAR-NK cells, suggesting that CD5 is a necessary element for maintaining the normal function of CAR-T cells. CD5-VLR-CAR NK92 and CD5-scFv-CAR NK92 cells demonstrated comparable tumor-killing activity *in vitro*; however, *in vivo*, only CD5-scFv-CAR NK92 cells prolonged the survival of mice. Although this preclinical study is preliminary, it confirmed for the first time that CAR-NK cells may be more advantageous than CAR-T cells in the treatment of T-cell malignancies. Furthermore, the antigen-recognition domain of different species may not increase the binding affinity of CARs.⁴⁷

6. ROLE OF CAR-NK CELLS IN MM

Multiple myeloma (MM) occurs in middle-aged and older people aged >45 years and poses a serious challenge to human

health. Although the treatment and prognosis of MM have been improved significantly over the past 20 years, MM still remains an incurable hematologic malignancy with a poorer overall prognosis, indicating the urgent need for new treatments. CAR-T-cell therapy for relapsed/refractory MM (R/R MM) has demonstrated remarkable success.^{48–50} MM encounters a similar dilemma as acute myeloid leukemia (AML) in CAR-NK cell treatment, the most important of which is the choice of the target antigen. Because MM cells are highly heterogeneous and the current surface markers for MM stem cells are still unclear, achieving a complete cure of MM is still difficult in clinical practice, and there is also remission after CAR-T-cell therapy. The choice of therapeutic targets is one of the key factors in the application of CAR-NK cells for treatment. The cell surface membrane protein CS1 may become a possible target for CAR-NK cell treatment. Since CS1 is ubiquitously expressed on the surface of MM cells, it is only expressed at low levels on NK cells and some T-cell subsets.^{51–53} Although Chu et al⁵⁴ demonstrated that CS1 CAR-NK cells can clear MM cells, there was also evidence of cross-reaction. Jiang et al⁵⁵ generated two NK cells carrying an anti-CD138 scFv or anti-CD19 scFv structure and demonstrated the *in vitro* antitumor activity of both these CAR-NK cells. However, they did not report the results of *in vivo* experiments, due to which it is unclear whether these CAR-NK cells are effective in mouse model. Traditional strategies used in the construction of CARs include the introduction of specific antibody sequences to mediate the recognition of cancer cells by CAR-modified cells. However, this strategy has the disadvantage of the loss of tumor cell antigen. To overcome these limitations, Leivas et al⁵⁶ constructed CAR-NK cells carrying the NKG2D receptor. The *in vitro* experiments confirmed that this novel CAR-NK cell is effective against the majority of MM cell lines. A multicenter phase I dose-escalation trial using a second-generation BCMA-targeted CAR-NK cell is ongoing, and we expect that we would obtain more information about the safety and feasibility of this strategy (NCT03940833).

7. ROLE OF CAR-NK CELLS IN AML

In 2015, AML affected approximately one million people and resulted in 147,000 deaths worldwide.^{57,58} In patients aged >65 years, therapy for AML has been unsatisfactory. Despite extensive research efforts over the years, the prognosis of AML in elderly subjects has not yet been significantly improved.⁵⁹ In preclinical models, cord blood-derived CD123 CAR NK cells exhibited antileukemic activity *in vitro*.⁶⁰ Like the preparation of CAR-T cells, the transfected CAR-NK cells are highly heterogeneous as untransfected NK cells. The presence of these cells may have an effect on the antitumor effect of CAR-NK cells. After cell sorting, CAR-NK cells were found to exhibit enhanced activity against the CD123+ AML cell line and primary human leukemia cells.⁶¹ The first-in-human clinical trial (NCT02944162) of CD33 CAR-NK92 cells in patients with AML revealed that CAR-NK92 cells can be safely used, although their effect was not obvious.⁶² In addition to the abovementioned clinical trials, there are currently two ongoing clinical trials of CAR-NK cell treatment with CD33-targeted AML (NCT02892695, NCT02944162).

8. PERSPECTIVE

Clinical research pertaining to the application of CAR-NK cells is still in the preliminary stage. The focus is still on how to reduce

the cost, improve the efficacy, and reduce the occurrence of adverse reactions. Questions that are yet to be answered include how to select the ideal target, how to define the optimal CAR structure, and how to optimize the preparation procedure of CAR-NK cells. Advancements in basic research and additional clinical trials would be helpful to address these issues.

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