

Multiple cancer treatment advances with CAR-NK

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Abstract. In the 21st century, cancer therapy has entered the era of combination therapy. The elucidation of immune system mechanism and the further development of cell culture technology pave the way for the application of immunotherapy in cancer clinical treatment. Chimeric antigen receptor technology is to form a chimeric antigen receptor by splicing the antigen binding region scFv with the intracellular part of CD3 ζ chain or Fc ϵ riy. At present, it has developed into the fourth generation of promoters that can selectively label and encode CARs amplification and suicide. In the course of years of research on CAR-T therapy, it has proved that NK cells with CAR modified have better efficacy in solid tumors and hematological tumors, with fewer side effects and stronger targeting. In recent years, CAR-modified NK cells have become a new approach to cancer in immunology. In this technology, the selection of tumor-related target proteins is very important for the therapeutic effect. Nevertheless, with the in-depth analyse of its related intrinsic machinery, the influence of microenvironment on it and the source of amplification, some issues remain to be further discussed. This article mainly summarizes the recent progress of pre-clinical and clinical trials on CAR-NK. Preclinical trials were mainly Ovarian Cancer, Glioblastoma GBM and Breast Cancer. The CAR selection and construction method of each trial were described in detail. The clinical trials section discusses several advanced trials, such as those on PD-L1 CAR-NK and NKX019, and summarizes several ongoing trials. This article reviews the progress of CAR-NK technology in recent years, aiming to summarize the advancement of CAR-NK cell, provide reference for further improving the specificity, cytotoxicity and efficacy of this method, and provide ideas for the expansion and crossover of related fields.

Keywords: Chimeric antigen receptor, Natural killer cell, CAR-NK, Immunotherapy, Cancer.

1. Introduction

The discovery and struggle of cancer run through the process of human medical development and even the whole history. From 4,600 years ago, when the ancient Egyptian physician Imhotep said in a script, "There is no cure." To 2,000 Years later, when Atosa, wife of the Persian KING Darius, removed a lump from her chest. From the "black bile" of Roman doctor Galen in the 2nd century AD to the Napoleonic family who suffered from gastric carcinoma. Many years ago, little was known about cancer. Susan Sontag wrote in her book *Illness as Metaphor and AIDS and Its Metaphors* that everyone has a dual citizenship, and illness is more troublesome citizenship. When we suffer from a disease, we become citizens of the kingdom of disease, suffering not only from physical pain, but also from psychological burden, and from society's disapproval. And when it comes to cancer, there's an added layer of fear of the unknown.

With the advent of the industrial age, cancer has greatly stepped into human vision. In the 18th century, large numbers of coal workers in England were afflicted with scrotal cancer; Cigarette and betel nut sales in the United States rose in tandem with the incidence of lung cancer in the 19th and 20th centuries. In 1900, cancer was a distant seventh after tuberculosis, pneumonia and diarrhea, but 40 years later it was the second leading cause of death after heart disease. By 2020, Worldwide, nearly 10 million deaths (or nearly 1 in 6) are caused by Cancer. Disclosed to the statistics relieved by the IARC from the WHO in 2020, there were 4.57 million new cancer patients in China in this year, with a mortality rate of nearly 66%. Nearly 3 million people died of the disease, including 1.18 million women and much more than 1.82 million men. Based on the huge population base, China has become a major cancer country [1]. The French Enlightenment thinker Jean-Jacques Rousseau said,

"Civilization keeps man away from nature, and at the cost of common diseases." It's always in the ears of medical professionals in every cancer field.

As understanding has improved, so have the ways in which cancer is diagnosed and treated. From Halstead's "radical mastectomy" to oncologist Ramon's Herceptin, which acts on breast cancer cells against the Her2 gene, from English doctor Thomas Fowler's "Fowler's solution" to interferon, interleukin-2, tumor necrosis factor... Eventually, cancer treatment came into the age of immunotherapy: rituximab, which specifically recognizes the CD20 molecule; bonatumumab, a BiTE molecule that accurately identifies cancer cells and attracts T-cell effects; ipilimumab, which reduces the risk of death from melanoma by one-third; nivolumab, which specifically binds to the PD-1 receptor. American medical scientist James Allison and Japanese medical scientist Yusuya Honjo, who made groundbreaking contributions to immunotherapy, also shared the 2018 Nobel Prize in Physiology or Medicine, which is enough to reflect the important significance of immunotherapy to the medical field. Whether it is immune checkpoint inhibitors, tumor vaccines, cellular immune cell therapy and nonspecific immunomodulator technology, there are only a handful of results available in cancer immunotherapy. The research of immunotherapy for cancer in the laboratory has made rapid progress, but the massive scientific data has not yet been fully transformed into a panacea for cancer treatment. The distance between basic research and clinical application still seems so distant.

In CAR - T cells based on years of study, based on the CAR - NK fine because of its excellent clinical effect before become a hot spot in antigen receptors technology project, this article will review the immune therapy in the CAR - research development and achievement in NK cells, from preclinical tests and clinical trials, solid tumor, blood cancer three-dimensional summarized four perspectives, to provide a foundation for further research in this field.

2. History of adoptive cell therapy-from CAR-T to CAR-NK

T cells have been a mainstay of immunotherapy research for more than a decade, but in general, T cell-based therapies are highly specific and only a small percentage of patients experience systemic and lasting improvement. CAR T cells have many limitations in cancer immunotherapy, including insufficient harvest of affected T cells, delayed production of CAR T cells, low production of CAR T cells, and innate T cell defects. And, of all these limitations, the potential for lethal toxicity like cytokine release syndrome and high cost severely limits its use in the clinic. NK cells are cytotoxic lymphocytes, which can spontaneously discover and act on malignant proliferating cells [2]. NK cells directly recognize the up-regulated bacteria-encoded ligands on stress cancer cells using a range of actuating receptors, without the need to present MHC antigens. Compared with CAR-T, CAR-NK are lower sensitive to GVHD and cytokine release syndromes, have greater cancer cell killing, graft anti-tumor potential, and are less expensive and more widely available. Nowadays, abundant preclinical evidence and several early clinical successes have proved that NK immunotherapy is an effective and feasible treatment strategy. Huntington, N.D., et al. Found that adoptively transferred human NK cells could inhibit tumor growing and metapoptosis in mice [3]. NK cells have also shown excellent results in the hematological cancers, with some clinical trials reporting improvements in nearly half of patients. However, Myers and Miller and Shimasaki et al. showed that in most trials, the metastatic therapeutic effect of individual NK cell therapy was still poor. Therefore, the research on ways to boost the therapeutic impact of CAR-NK, such as optimizing the source of NK cells and improving these function and persistence in vivo, is currently a hot direction.

3. Principle and progress of pre-clinical trials of CAR-NK cell

Recent years, CAR-NK cells have achieved remarkable results in preclinical trials in hematologic and solid tumors, and number of preclinical therapies have been shown to be beneficial for specific cancer cell killing. CAR-NK cells combine with different antigenic sites to form different costimulatory structures and exert effects on different cancer cell targets.

3.1. Ovarian Cancer

Advanced recurrence of ovarian cancer may be revealed by the action of CSC, which have high chemoresistance, self-renewal and metastasizing capacity. CSC-labeled CD133 was selected as the target of CAR structure immunotherapy for ovarian cancer, and the third generation anti-CD133-CAR vector was used to transfer to NK cells in vivo. NK92 cells expressing anti-CD133-CAR are an effective strategy for the specific eradication of ovarian CSCs, which may be used to combat ovarian cancer recurrence [4]. It has been shown that CAR-NK92 cells are highly active against cancer ovary cell lines expressing CD24 (SKOV3, OVCAR-3) and patient-derived cells. The anti-CD24 and anti-CD19 control CAR were mounted by lentiviral transduction. Finally, a double CAR assay and an anti-CD24-CD28-41BB fusion protein were used to ligate to an anti-mesothelial-CD3 ζ - CAR by 2A sequence. This CAR combination showed good activity in vitro [5]. Recent studies have identified three structures in CAR: the NKG2D transmembrane domain, 2B4 costimulatory domain and CD3 ζ signaling domain as the three regions that elicit strong NK cell effects. In ovarian cancer xenografts, NK-CAR-IPSC-NK cells considerably prevented tumour expansion and long-term living comparative to PB-NK cells, IPSC-NK cells, or T-CAR-IPSC-NK cells [6]. Folate receptor alpha (α FR) is extremely signalled in ovarian cancer with values approaching 90%. Recent studies have found that α FR- ζ , α FR-28 ζ and α FR-28b ζ third-generation anti- α FR CAR cells may effectively stop α FR-expressing tumour cells in vitro, specifically ovarian cancer cells. Furthermore, the third-generation design and α FR-28B ζ showed elevated antigenic toxicology and expansion, but low antigen-induced autophagy. NK92 engineered with anti- α FR CAR have stronger cytotoxicity to α FR-positive ovarian cancer, which should be further applied in clinical studies [7]. In a human being ovarian cancer xenograft pattern built by endoperitoneal injection of SKOV3-LUC cells (luciferiasis) modified by the luciole Luc gene, studies have found that high expression of CXCR1 can enhance the invasion effect of CAR-NK cells on cancer cells, and improve the anticancer effect of CAR-NK cells transplanted intravenously in mice through this approach [8]. A clinical trial of ovarian cancer therapy in Japan used HLA-iPSC-GPC3-CAR-expressing natural killer/innate lymphocytes. The use of pluripotent stem cells as a source helps to standardize the control of the efficacy and safety of the final product. The results of the final experiment showed that the cytotoxicity and IFN- γ secretion were significantly enhanced after the combination of drugs, and the survival rate of the mice was significantly improved. Mesothelial protein (MSLN) is often expressed in ovarian cancer. CAR-NK92 cells targeting MSLN and CD19 were constructed and found to eliminate MSLN-expressing cell lines including OVCAR-3 and SKOV3, subdermal and endoperitoneal ovarian cancer cells. MSLN-CAR-NK cells co-cultivated with OVCAR-3 and SK-OV-3 release more cytokines. The cells effectively extended the survival time of the experimental mice [9]. Other tests, such as those using iPSC and NK-92 as sources and anti-mesothelin. Mesothelin as target, also demonstrated its excellent anti-tumor potential, inhibited tumour growth and improved survival time.

3.2. Glioblastoma GBM

There is considerable potential for NK cells to treat GBM, and preclinical data on the treatment of GBM are extensive at present. First, the large underexpression of EGFR and the mutant EGFRvIII in GBM provides a direction for this study. Number of experiments targeting EGFR (EGFRvIII) and using NK-92 cell as source have shown good results. IEGFRvIII-CAR was built on the basis of DNAX-activating protein 12 (A signal adapter mixed up in activating the NK cell receptor signal transduction), named MR1.1-DAP12. Such cells were endowed with NK cell specific cytotoxicity against EGFRvIII glioblastoma cells and constituted subdermal U87-MG tumor grafts in vitro. Related experiments also found that injection of NK cells expressing MR1.1-DAP12 delayed tumor growth. Together with these experiments, we demonstrated that intravenous administration of EGFRvIII-directed DAP12.CD3 ζ inhibit the growth and extend the life of GBM tumours in heterologous mouse models of CAR-NK-YTS cell transplantation over-expressing the CXCR4 receptor. EGFR-CAR-NK cells performed well with patient-derived GB stem cells and in vitro cultured GB stem cells to increase IFN- γ secretion. Two mouse models were established by

intracranial injection of bi-specific EGFR1 and EGFRVIII (targeting CD28). Cd3 ζ was found to inhibit tumor progression and prolong the survival time. The results of this trial have wide implications for the therapy of GBM and nasopharyngeal carcinoma. Earlier studies showed that EGFRVIII-specific vaccine immunotherapy showed efficacy in GBM but its effectiveness was limited by factors such as immunological escape of antibody variable and the deficiency of EGFR wild-type favourable clones. Recent trials have shown that CAR-NK therapy with bi-specific CAR-NK constructs transduced targeting mutant and wild-type EGFR can prevent antigen loss. Variant of NK-92, directed to CD28 for electric EGFR- and EGFRVIII. Cd3 ζ was developed to prolong survival in the absence of antigen escape.

3.3. Breast cancer

In breast cancer, CAR-NK cells have shown excellent therapeutic efficacy in a variety of targeting strategies. To enhance the antitumor ability of NK-92 cells and broaden the therapeutic boundary of NK-92 cells in adoptive cell therapy, transgenic CAR-NK-92 cells expressing ErbB2 were bred in early experiments. The newly constructed CAR-CAR system consists of scFv (FRP5), partial CD8 and partial CD3 Zeta. Erbb2-targeted malignancies, including breast cancer and glioblastoma, are considered viable in this way in the future [10]. With the further study of target sites, it was found that HER-2-directed CAR-NK cells were particularly triggered when recognizing all assessed favourable tumour cells. Therefore, breast cancer cells derived from humans that can express HER-2 can be identified by this strategy, including autologous target cells, low-level McF-7, and so on. In 2015, CAR-NK cells based on ERBB2 antibody FRP5, CD28 and CD3 ζ showed a highly targeted cleavage of ErBB2-expressing tumor cells in vitro [11]. In 2016, the team constructed a mouse model to demonstrate that ERBB2-specific NK-92/5.28Z cells could be used for adoptive immunization therapy of glioma and breast cancer [12]. NK cells used for adopted cancer immunization therapy depend on cytokines such as interleukin (IL) -2, which and IL-15 belong to a usual γ -chain cytokine home, so their active effects can be superimposed. EPCAM-expressing cells exist in breast cancer cells. CAR-NK92 cells expressing EPCAM and IL-15 have stronger specific killing and cytotoxicity against breast cancer cells. This approach promotes swift segregation and continuous increase of retargeted NK cells, and its possible medical use needs to be further investigated [13]. BCBMs are often in patients with metastasizing breast carcinoma and imply a low prediction. EGFR-CAR-NK cells, OHSV-1 and united anti-BCBMs therapeutic strategies were examined in vitro and in a constructed breast cancer mice pattern. EGFR-CAR-NK92 cells with OHSV-1 therapy is a feasible treatment approach for BCBMS [14]. Tissue factor (TF) is submitted in 50 to 85% of TNBC patients. Transducer of a TF-directed CD28-41BB-CD3 ζ CAR build into NK-92MI cells while eradication the CD16 receptor to block its cytopical effect showed that, compared with control unaltered NK-92MI cells, The engineered CAR-NK cells exhibited stronger antitumor activity and active ADCC contrary to TF-expressing breast cancer cells [15].

3.4. B-cell malignancies and T cell malignancies

Original CAR-NK cell studies concentrated on B cell malignancies, and to overcome the problem that natural killer cell suppression in allogeneic hematopoiesis is limited by HLA suppressive types, transduced CD56-CD3-NK cells were tested with CAR-CD19. The results showed that the phrase of anti-CD19 receptor linked with CD3zeta overcomes NK disinclination and significantly enhances NK cell-mediated murder of leukemia cells. In order to solve the problem of resistance to CD20 specific antibody, NK-92 cells were used as sources to generate CAR-carrying transgenic NK cells composed of CD20 specific SCFV fragments, and the region connected by CD3zeta chain was utilized as the signal root. Targeting of NK cells by CD20 provides some insight into lymphoma and leukemia cells that are resistant to NK cells. Put EGFP mRNA into in vitro amplified NK cells and cleansed peripheral blood unstimulated NK cells showed good expression. Based on this approach of targeting NK cells with an mRNA targeting the CD19 receptor, it was found that in vitro extended and cleansed unstimulated NK cells expresses anti-CD19-BB-Z exhibited augmented toxicology

against CD19-positive target cells and lysis of severe lymphoblastic leukemia and descent chronic lymphocytic leukemia cells. To raise the toxic effect of NK cells to B-cell malignancies, the phrase of receptors, including CD3 ζ and 4-1BB signal molecules. (anti-CD19-BB ζ) in compassionate NK cells was detected by electroporation. The median phrase of anti-CD19-BB ζ was increased in NK cells enlarged after electroporation. NK cells articulating anti-CD19-BB ζ secreted interferon IFN- γ showed increased cytotoxicity in reaction to CD-19 target cells. To improve the prognosis of patients with relapsed/intractable CD20- B-NHL, we compared the operational activity of anti-CD20 Amended, exPBNK after nuclear transfection with anti-CD20-b-NHL mRNA in vitro and vivo. It was found that the AR (-) exPBNK handled mice considerably extended livelihood time and reducing cancer scale. These data can effectively prove that exPBNK with anti-CD20 CAR has therapeutic effect on CD20-positive hematologic malignancies. α CD19-CAR-NK92 cells recaptured the cytotoxicity of CD19-positive leukemia cells and primary leukemia cells with CD19, which were resistive to the catalytic activity of general NK92 cells. The results of this trial suggest that CAR-NK92 should be regarded as a medication choice for patients with lymphocytic malignancies. By comparison the several retroviral carrier systems, retroviral pseudotypes and transducer protocols, the α -retroviral vector was found to have the highest transduction level of 60%. NK cells happen transducing with a retroviral vector including CD19 CAR expression tape, and it was found that NK cells could selectively enhance the cytotoxicity of CD19 expressing leukemia cells and eliminate leukemia cells. Since then, retroviral vectors have become another important tool in NK cell therapy. Identification of abnormal cells by NK cells is under control by the embedding of activation and inhibition ligands and signaling cytokines such as IL-15. The cytokine induced SH2-expressing protein is an important adverse controller of IL-15 signal in NK cells, and this checkpoint may provide a direction for NK cell- mediated cancer privilege [16]. To enhance its therapeutic efficacy in B-cell malignancies, CAR-NK cells were generated by gene transfer targeting CD19 and CD3 ζ containing chimeric antigen receptors combined with CD28-CD3 ζ or CD137-CD3 ζ signal domains. The experimental results showed that no matter NK-92/63. z or NK-92/63.28.z had stronger effects on cell killing and cytokine production, and also demonstrated the possibility that NK-92/63.28.z could be used as continuously expanding cells in clinical treatment [17]. CAR containing CAR-CD19, IL-15 and iC9 was loaded into CB-NK cells to improve the proliferation and persistence of CB-NK cells. The results successfully sjowed its effectiveness in killing CD19-expressing cell lines and primary leukemia cells. Cells cultured in this protocol expanded the livelihood of a mouse heterograft example of Raji leukemia, indicating that they were effective in killing leukemia cell course and primary CLL leukemia cells in vitro [18]. In order to strengthen the anti-leukemia capacity of NK cells, two distinct antiviral carrier platforms, lentivirus and retrovirus, were compared. The two transduction enhancers were Retronectin and Vectofusin-1, respectively. RD114-TR-pseudoretroviral particle binding to Vectofusin-1 was found to be feasible, and this strategy could genetically modify PB-NK cells to create a huge number of CD19-CAR-NK cells with more cytotoxic properties [19]. Cytokine induced ML-NK cells distinguish upon actuation of interleukin-12 (IL-12), IL-15, and IL-18, showing a potent antitumoral response and alleviating leukemia. To strengthen the recognition of NK cells, ML NK cells were amended to convey anti-CD19 CAR. The results showed that the modified cells exhibited remarkably increasing interferon- γ productivity, enhanced particular-killing of NK-resistant leukemia course and main targets. The anti-tumor action of 19-CAR-ML NK cells versus autologous leukemia cells in leukemia patients has improved [20]. In order to broaden the clinical therapeutic boundaries of checkpoint targeting, the fourth generation CAR was tested in a mouse model of lymphoma by targeting CIS proteins induced by cytokines and negative regulators of IL-15 release. In this study, we found that targeting the cytokine checkpoint can promote IL-15 metabolism in CAR-NK cells without affecting their activity, which opens up new ideas for NK cell therapy.

4. Targets used in clinical trials of CAR-NK cell therapy

4.1. PD-L1 CAR-NK

In the United States, NantKwest and ImmunityBio, two major immunotherapy companies, proclaimed initial and provisional results from the PD-L1 T-HANK trial. The results of this phase, completed in 2019, showed that treatment with haNK plus the PD-L1 inhibitor Avelumab more than doubled the average continued existence rate in patients with metastatic pancreatic tumour who had not been subjected to other FDA-approved therapies. When haNK and the PD-L1 inhibitor avelumab were replaced with PD-L1 T-HANK, complete responses were achieved, and four of the five patients who did not achieve 3-month survival survived 8-16 months since receiving these expanded protocols. PD-L1 T-HANK cells are a human-derived foreign NK cell line devised to convey a CAR targeting PD-L1 obtained from the NK-92 master cell bank proprietary by NantKwest. PD-L1. T-haNK have also been conceived to convey a high-affinity variation of the receptor V158 FCγRIIA, which promotes cancer cell clearance by attaching to a monoclonal antibody previously bound to cancer cells to mediate antibody-dependent cytotoxicity (ADCC). This also means that this therapy can be used in combination with other targeted approaches. Models based on MDA-MB-231, H460 and HTB1 were constructed in NOD-SCID IL2Rγ^{−/−} (NSG) mouse. To study the antitumoral effect of PD-L1 T-Hank cells in vivo. PD-L1 T-haNK cells were found to convey PD-L1-targeted CAR and CD16. The in vitro fraction, which retained NK receptor expression and carried great substance of granzyme and perforin particles, was tested for gastric cancer, TNBC, lung cancer, and urogenital cancer cells and showed significant lytic effects. In addition, the cells showed efficacy against lung and bladder tumors and oral squamous cell tumors in mouse models in vivo[21]. Clinical studies of united immunization therapy with PD-L1 CAR-NK cells plus Pembrolizumab plus N-803 for recurring/malignant gastric or head and neck cancer are ongoing now. In these trial, 55 patients with sophisticated stomach and chief and nape cancer who were already receiving standardized therapy received PD-L1 CAR-NK cells intravenously once a week for six weeks and then once every two weeks for several weeks. Pembrolizumab was injected intravenously every 6 weeks and N-803 (an Anti-IL-15) was injected subcutaneously every 4 weeks for a total of 2 years. In the end, we obtained observational data: PD-L1 CAR-NK cell irradiation united with N-803 + Clinical response rate (CR+PR), PFS, security and tolerance, frequency of adverse events and duration of response, and duration of tumor reduction in patients with chief and nape scaly cell tumour and stomach /GEJ tumour treated with pembrolizumab.

4.2. NKG2D CAR-NK

The natural cytotoxic receptor NKG2D (CD314) is the main activating receptor. In China, multiple clinical trials of CAR-NK cell therapy are also underway: The research team of Zhejiang University is conducting experiments on NKG2D CAR-NK cells in the treatment of refractory metastatic colorectal cancer. The research team plans to measure the influence of NKG2D CAR-NK cells in the therapy of refractory metastatic colorectal cancer by first intraperitoneal infusion and second intravenous infusion in patients. At a hospital in Guangzhou, NK cells were transfected with mRNA electroporation to engender CAR-NK cells specific for cancer cells expressing the NKG2D ligand. The prepared CAR-NK cells and IL-2 supporting their livelihood were injected into the patients, and the occurrence, frequency and frequency of adverse reactions were observed, to achieve a preliminary exploration in this field.

4.3. DLL3-CAR-NK

Tianjin Medical University Cancer Hospital selected 9-18 patients with pulmonary small cell pneumonia after standardized treatment and expected survival longer than 3 months in each group. Three dose groups were set, and the patients were injected with DLL3-CAR-NK cells. The dose DLT and MTD were resolved by experimental observation, and the safety and efficacy of cell injection were evaluated in to provide an allusion for the advisable dose in phase II clinical studies. The ligand

DLL3 plays biological functions directly or indirectly by tying to Notch receptor. There are four types of Notch receptors. In contrast to else Notch ligands, the contemporary study showed that DLL3 is an inhibitory Notch ligand and promotes DLL3 expression in lung small cell lung cancer. DLL3 binds to Notch1 receptor and inhibits the activation of Notch signal. Down-regulation of Notch signal target genes HES1 and HEY1 can release tumor inhibition and promote the occurrence and development of SCLC. DLL3/Notch2 can increase the expression of cyclins CyclinD1 and CyclinD3, which suggests that DLL3/Notch2 might stimulate the expansion of SCLC cells by enhancing the phrase of CyclinD1 and CyclinD3. In addition, DLL3 and Notch receptor tying is nearly connected to else kinds of cancer. DLL3/Notch2 is implicated in the regulating of pituitary adenoma proliferation and invasion. DLL3/Notch2/Notch4 is essential for the existence and development of melanoma cells. DLL3/Notch2/Notch3 mediates the development and divergence of ovarian cancer cells and is associated with poor survival, reproduction, cell differentiation and apoptosis. The diseases that DLL3-CAR-NK cells can cope with and the mechanism of action still need to be further elucidated.

4.4. NKX019 CELL

Nkarta is sponsoring a joint clinical study of NKX019 cells administrated intramuscularly in patients with B-cell cancer in Australia. To find out the security and tolerance of a foreign CAR-NK cell experimental therapy aiming CD19, called NKX019, in patients with recurrent/intractable NHL, CLL, or B-ALL. All subjects in the trial will receive fludarabine/cyclophosphamide lymphoid clearance followed by three 3-week doses of NKX019 between 28-day cycles. NKX019 is a readily available cancer immunotherapy candidate that is devised with a CD19-directed CAR and a private, diaphragm linked IL-15 form of NK cells extracted from the peripheral blood of well donors. CD19 is a common biomarker for both normal B cells and malignant B cells. It is also a promising therapeutic target for B cell carcinoma. Preclinical studies have shown that NKX019 can target CD19 binding to generate a powerful immune response to eliminate CD19-expressing cells. Other tests have also shown that IL-15 might amplify the proliferation, persistence, even activity of NK cells in the presence of the co-stimulator OX40.

In fact, in a previous clinical trial evaluating NKX019 for recurrent/intractable B-cell lymphoma, 13 subjects included patients with multiple types of lymphoma, Five patients LBCL, three with FL, one with MZL, three with MCL, and three with B-ALL were included in the high-dose (10x10⁸ CAR-NK cells, three transfusions) group, and among the six evaluable patients: Five patients had an objective response (ORR=83%) and three had a complete response (CR=50%), including one patient with diffuse large B-cell lymphoma and one patient with mantle cell tumor. There were also no dose-limiting toxicities and fewer side effects than CAR-T therapy.

5. Conclusion

Medical researches of CAR-NK cells have shown initial results, and have shown good effects in number of animal models, but they are still in their infancy. With the increase of clinical studies year by year, the efficacy of ER2, MUC1 and PMSA targets has been preliminarily understood. In the next stage, CAR-NK cells still face serious challenges, including 1. NK cells expanded in vitro still require cytokine supplementation, and further studies on in vitro cultured cytokines are needed. 3. Several influencing factors in the immune microenvironment, such as TGF- β , adenosine, indoleamine 2, 3-dioxygenase (IDO) and PGE2, etc. ² In addition, there are specific, activity, safety and other issues that need to be further improved. This article will summarize the progress made in the preclinical stage in recent years in two dimensions: time order and clinical tumor target, and mainly describe the research history of ovarian cancer, GBM, breast cancer, T cell and B cell based malignant tumors. In addition, this paper summarizes the relevant mechanisms, definitive results and current clinical trials of PD-L1HANK cells and DLL3-CAR-NK cells. We will look forward to good results from these trails. As research progresses, CAR-NK cell therapy will continue to improve, and the discovery of

relevant mechanisms and systematic knowledge will inspire researchers, which will be the key battle for cellular immunotherapy for cancer treatment. I am honored to witness with you all.

References

- [1] Hyuna Sung, Jacques Ferlay, et al; Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians* 2021 05;71(3):209-249
- [2] Muhammad Babar Khawar, Haibo Sun; CAR-NK Cells: From Natural Basis to Design for Kill. *Frontiers in immunology* 2021;12:707542
- [3] Nicholas D Huntington, Joseph Cursons, et al; The cancer-natural killer cell immunity cycle. *Nature reviews. Cancer* 2020 08;20(8):437-454
- [4] Rüdiger Klapdor, Shuo Wang, et al; Improved Killing of Ovarian Cancer Stem Cells by Combining a Novel Chimeric Antigen Receptor-Based Immunotherapy and Chemotherapy. *Human gene therapy* 2017 10;28(10):886-896
- [5] Rüdiger Klapdor, Shuo Wang, et al; Characterization of a Novel Third-Generation Anti-CD24-CAR against Ovarian Cancer. *International journal of molecular sciences* 2019 Feb 03;20(3)
- [6] Ye Li, David L Hermanson, et al; Human iPSC-Derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-tumor Activity. *Cell stem cell* 2018 Aug 02;23(2):181-192.e5
- [7] Xiang Ao, Yu Yang, et al; Anti- α FR CAR-engineered NK-92 Cells Display Potent Cytotoxicity Against α FR-positive Ovarian Cancer. *Journal of immunotherapy (Hagerstown, Md. : 1997)* 2019 10;42(8):284-296
- [8] Yu Yang Ng, Johan C K Tay, et al; CXCR1 Expression to Improve Anti-Cancer Efficacy of Intravenously Injected CAR-NK Cells in Mice with Peritoneal Xenografts. *Molecular therapy oncolytics* 2020 Mar 27;16:75-85
- [9] Bihui Cao, Manting Liu, et al; Use of chimeric antigen receptor NK-92 cells to target mesothelin in ovarian cancer. *Biochemical and biophysical research communications* 2020 03 26;524(1):96-102
- [10] Christoph Uhrek, Torsten Tonn, et al; Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. *Blood* 2002 Aug 15;100(4):1265-73
- [11] Kurt Schönfeld, Christiane Sahm, et al; Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. *Molecular therapy : the journal of the American Society of Gene Therapy* 2015 Feb;23(2):330-8 doi:10.1038/mt.2014.219
- [12] Congcong Zhang, Michael C Burger, et al; ErbB2/HER2-Specific NK Cells for Targeted Therapy of Glioblastoma. *Journal of the National Cancer Institute* 2016 May;108(5)
- [13] Christiane Sahm, Kurt Schönfeld, et al; Expression of IL-15 in NK cells results in rapid enrichment and selective cytotoxicity of gene-modified effectors that carry a tumor-specific antigen receptor. *Cancer immunology, immunotherapy : CII* 2012 Sep;61(9):1451-61
- [14] Xilin Chen, Jianfeng Han, et al; A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases. *Oncotarget* 2016 May 10;7(19):27764-77
- [15] Zhiwei Hu; Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. *Scientific reports* 2020 02 18;10(1):2815
- [16] Rebecca B Delconte, Tatiana B Kolesnik, et al; CIS is a potent checkpoint in NK cell-mediated tumor immunity. *Nature immunology* 2016 07;17(7):816-24
- [17] Sarah Oelsner, Miriam E Friede, et al; Continuously expanding CAR NK-92 cells display selective cytotoxicity against B-cell leukemia and lymphoma. *Cytotherapy* 2017 02;19(2):235-249
- [18] E Liu, Y Tong, G Dotti, et al; Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. *Leukemia* 2018 02;32(2):520-531
- [19] Stephan Müller, Tobias Bexte, et al; High Cytotoxic Efficiency of Lentivirally and Alpharetrovirally Engineered CD19-Specific Chimeric Antigen Receptor Natural Killer Cells Against Acute Lymphoblastic Leukemia. *Frontiers in immunology* 2019;10:3123

- [20] Margery Gang, Nancy D Marin, et, al r; CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas. Blood 2020 11 12;136(20):2308-2318
- [21] Fabian KP, Padget MR, et.al. PD-L1 targeting high-affinity NK (t-haNK) cells induce direct antitumor effects and target suppressive MDSC populations. J Immunother Cancer. 2020 May;8(1): e000450.