ISSN 2959-409X

Application and progress of CAR therapy on malignances: from CAR-T to CAR-NK

Qi Zhang

Abstract:

For some malignances that are highly aggressive and metastatic are usually considered incurable. However, with the advent of immunotherapy, this therapy could be possibly effective after other three major therapies: surgery, radiation, chemotherapy. As an emerging immunotherapy, chimeric antigen receptor (CAR) T cells have been applied to treat hematologic malignances and some patients achieved long-term remission and temporary cure. However, CAR-T cells also bring side effects such as cytokine release syndrome (CRS), neurotoxicity, etc. Moreover, CAR-T cells did not perform well in solid tumors, for the reason that CAR-T cells are difficult to break through external barrier of solid tumors, at the same time maintain cytotoxicity and persistence inside. Therefore, we introduce CAR-NK cells therapy on the basis of CAR-T cells. At present all CAR-NK cell therapies are in clinical trials, its effectiveness has been initially observed. Compared with CAR-T cells, patients with hematologic malignances received CAR-NK cell therapies showed few side effects such as CRS and neurotoxicity.

Keywords: CAR-T, CAR-NK, therapy, malignances

1. Introduction

1.1. Background on CAR therapy

For some malignances that are highly aggressive and metastatic are usually considered incurable. However, with the advent of immunotherapy, this therapy could be possibly effective after other three major therapies: surgery, radiation, chemotherapy. As an emerging immunotherapy, chimeric antigen receptor (CAR) T cells have been applied to treat hematologic malignances and some patients achieved long-term remission and temporary cure. However, CAR-T cells also bring side effects such as cytokine release syndrome (CRS), neurotoxicity, etc. Moreover, CAR-T cells did not perform well in solid tumors, for the reason that CAR-T cells are difficult to break through external barrier of solid tumors, at the same time maintain cytotoxicity and persistence inside. Therefore, we introduce CAR-NK cells therapy on the basis of CAR-T cells. At present all CAR-NK cell therapies are in clinical trials, its effectiveness has been initially observed. Compared with CAR-T cells, patients with hematologic malignances received CAR-NK cell therapies showed few side effects such as CRS and neurotoxicity. These results benefit from the properties of NK cells. Scientists can design the appropriate CAR structure to enable CAR-NK cells to play a stronger role on solid tumors. This review focuses on advances in CAR-NK cell therapy on solid tumors.

2. Development of CAR therapy: CAR-T cells

2.1. CAR Structure and design

CAR is a synthetic receptor that binds to a target cell surface antigen without MHC participation and lead cytotoxic immune cells to target cells expressing that antigen. Its function is to recognize target antigens and transmit antigen signals to activate immunoreactions. Moreover, it does not require the involvement of the TCR-CD3 complex, in other words, it detours the traditional route. There are four main components of CAR: extracellular antigen binding domain, spacer or hinge region, transmembrane domain and intracellular signaling domain. The antigen-binding domain is usually composed of the variable regions of an antibody heavy (VH) and light (VL) chains and these two chains are connected by a flexible linker to form a single-chain fragment variable (scFv). In addition, scientists can design a protein or peptide to replace scFv. Compared with a TCR recognizes antigens via MHC, scFv and other synthetic structures determine target specificity and bind to target antigens(Figure1). The hinge region is the spacer region that exposes the antigen-binding domain on CAR T cell surface for binding to target antigens. The length of the hinge region depends on the location of the target antigen. Target antigens close to the cell membrane usually require a longer hinge region, whereas target antigens close to the cell surface have a shorter hinge region. The major function of the transmembrane domain is to dock CAR in the immune cell mem-

brane [1]. Some studies, however, show that this region can affect CAR activity, expression, stability, dimerization and signal transduction [2,3,4], even the transmembrane domain in the anti-HIV CAR structure can participate in the expression of CD4 on the cell surface[5]. The intracellular signaling domain is the main structure that plays a role in the structure of CAR, and there have been three generations of CAR-T. The first generation CAR T cells contain a CD3ζ signaling domain, however, its antitumor activity requires a two-step initiation to be optimal [6, 7]. Therefore, second-generation CAR-T contains a co-stimulatory domain in addition to a CD3ζ signaling structural domain to improve its function. And the third-generation CAR-T contains two co-stimulatory domains in addition to a CD3ζ signaling structural domain. The most commonly used co-stimulatory domains are CD28 and 4-1BB and are FDA-approved.CD28 and 4-1BB have been shown to favor T cell reinforcement. The combination of CD28 and CD3 contributes to the differentiation and persistence of memory T cells, increases mitochondrial biogenesis, enhances fatty acid oxidation and oxidative metabolism. CD28 was observed to cause an increase in glucose utilization and upregulation of glycolytic enzyme expression [8, 9]. 4-1BB increases mitochondrial biosynthesis and enhances fatty acid oxidative metabolism [10]. Co-stimulatory domains currently in the experimental stage include OX40, CD27 and inducible T-cell stimulator (ICOS), etc. OX40, normally induced after T cell activation, regulates Tregs glycolysis and lipid metabolism and promotes T cell expansion and generation of memory cells through a TNF receptor-associated factor 2 (TRAF2)-dependent mechanism [11]. Integration of the CD27 cytoplasmic domain into a CAR construct enhances T cell expansion, effector functions as well as survival and augments T cell persistence and anti-tumor activity in vivo [12]. ICOS is critical for the expansion and differentiation of helper T cells (Th17) [13]. Currently, in the third-generation CAR-T structures that have been designed, the combination of CD28 and 4-1BB enhances the ability of CAR to bind to antigen, increases its proliferation and central memory differentiation, and improves its in vivo activity [14].

2.2. Successful and fail experience on CAR-T therapy

Five types of CAR-T cells have been approved by the FDA. All five CAR-T cells target markers on the surface of B cells, four targeting CD19 and one targeting B cell maturation antigen (BCMA). All five cells have shown promising results in refractory or recurrent hematologic tumors such as lymphomas and leukemias of B cell origin and multiple myeloma. The first FDA-approved CAR T therapy is tisagenlecleucel (Kymriah™), based on a multicenter study of 75 pediatric and young adult patients with relapsed or refractory B cell precursor acute lymphoblastic leukemia (ALL) [15]. Within three months, this cohort achieved an overall remission rate of 81%, with 60% of patients in complete remission. Fifty five out of 75 patients (73%) had a grade 3 or 4 tisagenlecleucel-related adverse event. Grade 3 and 4 cytokine release syndrome (CRS) occurred in 21 and 25% of patients,respectively, with 35 of 75 patients (47%) being admitted to the intensive care unit (ICU) for its management [16]. Based on these results, CAR-T therapy and tisagenlecleucel have been successively approved by the FDA and used for the treatment of refractory or relapsed large B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma and DLBCL arising from follicular lymphoma.

Axicabtagene ciloleucel (Yescarta™) became the second FDA approved CAR T therapy on October 18, 2017 for large B cell lymphoma, including DLBCL, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma and DLBCL arising from follicular lymphoma, after at least two lines of systemic treatment. A multi-center ZUMA-1 trial with 101 patients showed an 82% objective response rate (54% CR) and 52% overall survival rate at 18months. Grade 3 or higher CRS occurred in about 13% of patients [17].

The third CAR T therapy, Brexucabtagene autoleucel (Tecartus™), was approved for relapsed or refractory mantle cell lymphoma by the FDA on July 24, 2020 based on a single-arm, open-label ZUMA-2 trial. In this multicenter Phase II trial with 74 patients enrolled, 68 patients took the medication. The overall response rate was 93% with 67% CR. At 12months, the overall survival was 83%. Grade 3 or higher CRS occurred in about 15% of patients [18].

Lisocabtagene maraleucel (Breyanzi™) is the most recently approved CD19-targeting CAR T therapy against relapsed or refractory large B-cell lymphoma, after two or more lines of systemic therapy, including DLBCL not otherwise specifed (including DLBCL arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B [19]. In this multicenter TRANSCEND trial with 192 evaluable patients, the objective response rate was 73%, with 53% CR, and the median duration of response was 17months. Grade 3 or higher CRS occurred in about 2% of patients.

Idecabtagene vicleucel (Abecma®) is the only FDAapproved CAR T therapy not targeting CD19. It targets BCMA on multiple myeloma (MM) cells and was approved by the FDA on March 26, 2021 for relapsed or refractory MM. Of 128 patients who received treatment,

the overall response rate was 73% with 33% CR. Grade 3 or higher CRS occurred in about 2% of patients [20].

2.3. Limitation of CAR-T therapy

2.3.1. Antigen selection

Despite the remarkable success of CAR-T cell therapy in hematologic malignancies, one of the reasons for its limited role in solid tumors is antigen selection. There are few antigens in solid tumors that are expressed only in tumor tissue and not in normal tissue. As a result, some animal experiments or clinical trials in which subjects undergoing CAR-T cell therapy experienced tumor remission have also shown significant on-target,off-tumor toxicity[21-32]. Usually, specific antigens on the surface of a tumor are generally formed by mutations in the genes controlling the expression of the antigen, which are full of randomness and diversity. Therefore, CAR-T cells are designed for such antigens. And other antigens expressed in tumor tissues are also expressed in normal tissues, which has been confirmed by many experiments.

2.3.2. Insufficient efficiency of CAR-T cell trafficking and infiltration into tumor tissue

How CAR-T cells are trafficked into solid tumor tissue after injection is also a major issue.The dense extracellular matrix1 containing large numbers of cancer-associated fibroblast forms a physical barrier that prevents CAR-T cells from entering the tumor tissue. In addition, dysregulated expression of cytokines within tumor tissues attracts suppressor immune cells to inhibit CAR-T cell function. Researchers are currently improving CAR-T cell function through a variety of approaches, such as regulating T cell migration by expressing CXCL9 on CAR-T cells, inhibiting tumor angiogenesis, and enhancing the ability of CAR-T cells to recruit T cells [33]. Expression of CXCR2 on CAR-T cells also improves migration and accumulation of CAR-T cells inside tumors, and co-expression of IL-15 and IL-18 inhibits T cell exhaustion and apoptosis [34].

2.3.3. Hostile tumor microenvironment

The tumor microenvironment (TME) contains many cells within it, and cytokines and a different pH than normal tissue can inhibit CAR-T cells. Regulatory T cells, myeloid-derived suppressor cells(MDSC), and cancer-associated fibroblasts can directly inhibit the function of CAR-T cells. Immunosuppressive cytokines, vascular endothelial growth factor, transforming growth factor (TGF-β), IL-4,IL-10 can lead to dysfunction of T cells and promote the infiltration of immunosuppressive cells. The acidic environment inside the tumor tissue is equally unfriendly for T cells to function.

2.3.4. Antigen escape

In CAR-T therapy, researchers have found that some patients experience relapse and slow progression as treatment progresses. It has been studied that mutations in the target antigen gene on the tumor surface can lead to loss of function of the target antigen. In addition, the alteration of target antigen mRNA can lead to a shift in target antigen phenotype from sensitive to resistant[34]. In addition, the target antigen is transferred to T cells during the treatment process, not only the density of target antigen on the tumor decreases, but also the T cells will kill each other[35]. There are also many studies devoted to how to prevent target antigen loss.

2.3.5. Systematic toxicity

CAR-T cells, while exerting an anti-tumor effect, also produce large side effects. T-cell activation releases large amounts of cytokines, causing cytokine release syndrome (CRS). The main manifestations are fever, hypotension, hypoxia, and multi-organ failure[36]. Cytokines can cross the blood-brain barrier, while increased levels of cytokines in the cerebrospinal fluid can lead to neurotoxicity, manifesting as aphasia, altered mental status, tremor, seizures and headaches. In addition, hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS) are the main side effects, which are characterized by fever, hepatosplenomegaly, liver function abnormalities, pancytopenia, increased levels of methemoglobin[37], increased levels of glycoferrin triphosphate, hypertriglyceridemia, and hypoglycemia, with a high mortality rate.

2.3.6. Insufficiency of CAR T cell expansion and persistence

Expansion and persistence of CAR-T cells in tumor tissues are also key to sustaining their role, especially for malignancies that require long-term treatment. In solid tumor tissues, an unfriendly tumor microenvironment leads to decreased CAR-T cell expansion and persistence. There are many ways to enhance the expansion and persistence of CAR-T cells, such as transducing genes that express promotive cytokines into CAR-T cells, which can be induced when the T cells are exposed to antigens[38]. A panel of cytokines including IL-12, IL-7, IL-15, IL-18, IL-21 and IL-23 are currently being investigated and entering early phase clinical trials[39-42].

2.3.7 Poor source and high cost

CAR-T cells are derived from a single source, mainly the patient's own peripheral blood, as the use of allogeneic sources of T cells can produce severe GVHD.After the T cells are extracted from the patient, they need to be

genetically engineered and edited in vitro to make the T cells express CAR, then expanded in vitro, and after maturation, the patient needs to undergo lymphatic clearance chemotherapy to make room for the CAR-T cells, and then finally, the CAR-T cells are removed. cells into the body. This process usually requires a wait of at least several months, during which time the patient's condition may deteriorate[43]. In addition, CAR-T injections are large, requiring at least several hundred million CAR-T cells if they are to be therapeutically effective. Finally, CAR-T treatments cost between $500,000 and $1,000,000, and very few patients can afford this treatment[44]. There are also experiments trying to use allogeneic CAR-T cells from healthy individuals, but they are not yet in commercial use. In contrast, CAR-NK cells come from a wide variety of sources, while each source has its own unique advantages, which is one of the reasons why CAR-NK cell therapies are being studied on a large scale.

3. Applications of CAR-NK Cell Therapy in Solid Tumor Treatment

3.1. CAR-NK cell source and structure

Researchers have explored different sources of NK cells for producing and expressing CAR[45]. Currently, there are four main sources of NK cells. The first one is the immortalized human cell line NK92, derived from human extracellular NK cell lymphoma. Its advantage lies in its strong ability to expand in vitro, but due to its malignant tendency, it must be irradiated before use[46]. This also reduces its survival in the peripheral blood of the recipient. In addition, NK92 cells are naturally deprived from the CD16 domain and cannot trigger ADCC, the intrinsic mechanism of NK cell anti-tumor activity[47]. NK cells can be obtained from the donor's peripheral blood, which is rich in mature NK cells that do not require HLA/KIR matching. In addition, PB-derived cells respond more efficiently and are more persistent in circulation compared to cells from other sources. NK cells can be obtained from the donor's peripheral blood, which is rich in mature NK cells that do not require HLA/KIR matching. In addition, PB-derived cells respond more efficiently and are more persistent in circulation compared to other sources.The disadvantages of PB-derived NK cells are that they do not expand easily in vitro and PB-derived NK cells are at various stages of maturation, making it difficult to standardize them[48-50]. Umbilical cord blood is a carryover source of NK cells, which has the advantage of being more capable of in vitro expansion than PB-derived NK cells, but less cytotoxic. The stimulation of immunocompetent progenitor stem cells(ipscs) are harvested from the mobilized peripheral blood of the donor or from UCB. The CAR construct is transduced into iPSCs, which are then differentiated into CAR-expressing NK cells by incubation in a cytokine cocktail of SCF, VEGF, BMP4, IL3, IL-15, IL-7 and FLT3L. This NK cell has the advantage of being able to produce a large number of homogeneous NK cells from a single iPSC[51] and the disadvantage of low cytotoxicity[52,53].

The current structure of CAR expressed by NK cells is similar to that of T cells, which consists of four regions: extracellular antigen binding domain, spacer or hinge region, transmembrane domain, and intracellular signaling domain. Unique CAR structures designed for NK cells are also currently available, the effects produced vary widely. However, these different CAR constructs exhibited varying effects on cytotoxicity and cytokine production in NK cells[54,55]. The intracellular signaling domain of the first generation CAR includes an activation domain that transmits an activation signal to activate NK cells upon binding of the activation receptor to the ligand. Currently many structures can act as activation receptors, which largely depends on the choice of ligand on the target cell. The most thoroughly studied is NKG2D, a homodimeric receptor that recognizes the stress-inducing ligands MICA, MICB, and ULBP1-6 expressed on damaged, transformed, or otherwise abnormal cells [56]. signaling through the adapter molecule DAP10 to trigger NK activation. Here DAP10 acts as an activation domain and is part of the CAR structure. In addition, other activation domains such as DAP12, CD3δ, FcεRIγ, etc. function in conjunction with specific activating receptors. The intracellular signaling domain of second-generation CARs adds a co-stimulatory domain, and CD28 and 4-1BB, which are widely used in T cells, also enhance anti-tumor effects in NK cells. However, 2B4 has been reported to have stronger anti-tumor, apoptosis-reducing, and cytokine expression-enhancing effects compared to CD28 [57]. While the intracellular signaling domains of the third-generation CARs contain multiple co-stimulatory structural domains, the fourth-generation adds to the third-generation structures expressing specific cytokines to further enhance the anti-tumor capabilities of NK cells.

3.2. Advantages of CAR-NK cells.

3.2.1. Safety:

Compared to T cells, NK cells have a shorter lifespan and rapidly die off after their effects, which prevents them from remaining in the body in large numbers to continue their side effects, as is the case with the longer-lived T cells, which continue to have side effects after their anti-tumor effects. In addition, there are more sources of NK cells, so patients can choose suitable exogenous NK cells

to receive treatment at any time without having to wait for a long period of time for expansion and cultivation. NK cells and T cells have different cytokine secretion spectra, with NK cells secreting IFN-γ and GM-CSF[58], whereas T cells primarily induce cytokines such as IL-1a, IL-1Ra, IL-2, IL-2Ra, IL-6, TNF-a, MCP-1, IL-8, IL-10, and IL-15, which are highly correlated with CRS, and can lead to severe neurotoxicity. NK cells and T cells have different cytokine secretion spectra, with NK cells secreting IFN-γ and GM-CSF, whereas T cells predominantly induce cytokines such as IL-1a, IL-1Ra, IL-2, IL-2Ra, IL-6, TNF-a, MCP-1, IL-8, IL-10, and IL-15, which are highly correlated with CRS, and can cause severe neurologic toxicity[59]. Moreover, the GVHD phenomenon is rarely observed in the CAR-NK experiments performed so far[60].

3.2.2. Dual killing mechanism:

CAR-NK cells have a dual mechanism of killing tumor cells, a CAR-independent mechanism and a CAR-dependent mechanism. In the CAR-dependent mechanism, activated NK cells lyse cells by releasing perforin and granzymes. NK cells also express transmembrane receptors, such as natural cytotoxicity receptors (NCRs), KIRs, NKG2D, or DNAM-1, etc(table1),which can induce kappa-mediated apoptosis in recognized cancer cells. The secretion of IFN-γ leads to the recruitment of macrophages and dendritic cells, thereby promoting other antitumor mechanisms[61,62]. In addition, NK cells can kill cancer cells mediated by ADCC. CD16 is key to ADCC, recognizing the Fc fragment of immunoglobulin G (IgG) that binds to epitopes on the surface of tumor cells[63]. The CAR-dependent mechanism means that researchers design special CAR structures according to the needs of different types of tumors. The most commonly used structures include CD28, CD3ζ, DAP10, DAP12, 4-1BB, 2B4, etc.

3.2.3. Multiple sources:

As mentioned before, NK cells have rich sources, and NK cells from different sources play different advantages in treating different tumors. Meanwhile, no significant GVHD and other side effects appeared in the experimental process of NK cells from different sources.

3.3. Optimization of CAR-NK cell function

3.3.1. Maximizing CAR-NK cell survival:

Unmodified NK cells can only survive for 1 week in the infused circulation[64,65]. Application of IL-2 and IL-15 after infusion of CAR-NK cells enhanced NK cell expansion and activation. When IL-2 and IL-15 were used in vitro, the function of NK cells decreased dramatically when the cytokines were withdrawn[66]. IL-15 is more when applied in vivo because IL-15 is highly selective for NK cells and does not stimulate regulatory T cells[67,68], whereas IL-2 causes systemic toxicity, such as capillary leakage syndrome, which results in rapid NK cell exhaustion and other side effects[69,70]. The use of rhIL-15 or the IL-15 engineered molecules ALT-803[71] and NKTR-255[72-74] showed minimal stimulation of CD4+ T cells and regulatory T cells and a good expansion status of NK cells, but with lower potency and also with side effects[75,76]. The investigators will attempt to transduce the interleukin gene into NK cells, prompting the NK cells to express interleukin on their own, thus eliminating the need to receive exogenous interleukin. Investigators will introduce the membrane-bound IL-15 gene (mbIL-15) into CAR-NK cells and observe an increase in survival and proliferation of NK cells, but no therapeutic effect[77]. A study showed that CISH gene expression of CIS (cytokine-inducible SH2-containing protein) inhibits the expression of IL-2 and IL-15. Introduction of membrane-bound IL-15 gene into UCB-derived CAR-NK cells knocked down for CISH gene significantly enhanced the therapeutic potential of CAR-NK cells[78]. Some studies have reported that infection with CMV increases the expansion of memory-like NK cells, and it was found that CMV caused expansion and CD94/NKG2D overexpression in a subpopulation of $CD56^{dim}$ NK cells deficient in FcεRγ, SYK, and EAT-2, while decreasing the expression of PD-1, TIGHT, and NKG1A checkpoint receptors[79,80]. Incubation of NK cells with a variety of cytokines such as IL-2, IL-15, and IL-18 mediated the formation of memory-like NK cells, and the nature and function of such NK cells were similar to those formed by CMV infection[81]. Preclinical studies have shown that implanting CAR into memory-like NK cells increases their survival and cytotoxicity[82]. In addition, memory-like CAR-NK cells mediated stronger ADCC, resisted the inhibitory effects of Treg and MDSC and survived longer in solid tumors[83]. Although CAR successfully transduced memory-like NK cells with low efficiency (15%-25%), they were able to expand in large numbers in vivo to correspond to the CD19 antigen[82]. CAR-memory-like NK cells also demonstrated stronger degranulation and IFN-γ-based responses in vitro and in mouse acute myeloid lymphoma (AML)[84].

3.3.2. Expansion and activation

Expansion and cultivation of CAR-NK cells has been a big problem. Combining CAR-NK cells with cytokines IL-2,IL-15,IL-18,IL-21, etc. can increase the expansion of CAR-NK cells in vitro, but they are quickly exhausted. One study showed that irradiated B-lymphoblasts stimulated NK cell expansion in vitro. Both early T cells and NK cells can expand, NK cells can expand 25 times, and T cells can expand 3 times. After removing CD3(+)/CD5(+)

T cells, a large number of CD16(+)/NKH-1(+) cells can be obtained[85]. Several studies have found that cultivation of immortalized K562 cell lines enhances NK cytotoxicity by 400-fold. PB-derived NK cells and modified K562 cell lines expressing 4-1BBL and mbIL-15 expand 1000-fold in the absence of T-cell co-stimulatory factors for three weeks[86]. By genetically engineering human leukocyte antigen (HLA)-A and HLA-B K562 cells, the expression of CD48, 4-1BBL and membrane-bound IL-21 was enhanced, forming a universal antigen-presenting cell that stimulates NK cells. The homologous receptor on the drug can expand CAR and non-transduced NK cells more than 900 times within 2 weeks, with high purity and strong persistence[87].

3.4. CAR-NK cell modification against solid tumors

Although NK cells have shown many advantages over T cells, they still encounter many difficulties when applied to solid tumors. The dense extracellular matrix, suppressive immune cells, and cytokines in solid tumors significantly reduce NK cell function. Therefore, it is crucial for CAR-NK cells to be modified to combat the hostile tumor microenvironment.

3.4.1. Resistance to the tumor microenvironment

NK cells found in the TME are immature and have low toxicity[88,89]. An important reason is that tumor-related cells secrete a large number of inhibitory cytokines, such as adenosine, TGFβ, IL-10, etc[90]. TGFβ can reduce the recruitment of functional NK cells. Downregulates the activating receptors of NKG2D and DNAM1 and prevents the secretion of perforin[91]. UCB-derived NK cells expressing TGFβ negative receptor II do not have this phenomenon and can maintain their killing ability against CML and other tumor cell lines[92].

3.4.2. Combat heterogeneity

Tumor heterogeneity is extremely common in solid tumors and is a major problem faced by immunotherapy. Cancer stem cells (CSCs) are highly prevalent in solid tumors and are a major contributor to drug resistance and long-term treatment[93]. In CAR-T therapy, CAR initially combines with tumor antigens to achieve a therapeutic effect. However, as time goes by, the antigen density on the tumor surface decreases, and gene mutations within the tumor lead to changes in the antigen structure. The original CAR structure becomes ineffective, and eventually leading to tumor recurrence and poor efficacy[94,95]. Due to the dual tumor immune mechanism, CAR-NK cells can mediate ADCC to kill tumor cells through CD16. To combat CSCs, researchers designed PD-L1-directed CAR-NK cells to overcome the problem of T cell escape. In addition, IFN-γ and TNFα produced by NK cells can promote the differentiation of CSCs, causing them to lose chemotherapy resistance and self-renewal capabilities. Related products are being designed[96].

3.4.3. Trafficking and infiltration

CAR-NK cells overexpressing CXCR4 can significantly increase tumor infiltration compared with other CAR-NK cells[97,98]. CXCR1 is an IL-8 receptor that can move along the IL-8 concentration gradient after activation. Various cancers, including pancreatic cancer and ovarian cancer, can secrete IL-8. Therefore, overexpressing CXCR1 receptor NKG2D.CAR-NK cells are more toxic in vivo[99]. In addition, studies have shown that the concentration of NK cells within tumors is related to the CXCL16 gradient, and NK cells have the ability to reduce the volume of tumors overexpressing CXCL16[100].

3.4.4. Intrinsic modification

Activating the NKG2D receptor induces granule-dependent and cell-mediated cytotoxicity, but activating it alone is not sufficient to produce IFNγ[101], so the researchers combined NKG2D with DAP10 to activate the downstream effects of P13K to enhance killing. The study also found that NKG2D.DAP10.CD3ζ.PBNK can also improve the anti-tumor activity of modified NK cells against a variety of tumor cells[102]. In addition, downregulation of inhibitory receptors can also enhance killing. For example, inhibitory receptors for NKG2D that inhibit NK cells can block their signal transduction and increase the toxicity of NK cells against HLA-E-expressing cancer cell lines by 40%[103].

3.5. Success in Hematological Malignancies

At the beginning, the successful cases of CAR-NK cell therapy were all tumors of the hematological system. In 2020, Liu et al. conducted a clinical trial in which 11 patients with CD19-positive hematological malignancies (non-Hodgkin lymphoma, chronic lymphocytic leukemia) received CD19-directed CAR-NK cell therapy, 8 of whom There were objective responses, and 7 patients still had complete remission without residual disease after 30 days of treatment. The study analyzed UCB-derived NK cells after retroviral transduction with CD28.CD3ζ endodomain, IL-15 and iCas9 (inducible caspase 9) safety switch and activation/expansion with K562 feeder cells and IL-2. The author speculates that the transformation efficiency is 49%. In this study, no serious adverse reactions such as CRS and GVHD occurred. The dose of CAR-NK cells used in this study reached 1*107 but did not reach the tolerated dose. The CAR-NK cells survived in the body for at least 12 months. This study also became a milestone in CAR-NK therapy, demonstrating the pow-

erful effects and low side effects of CAR-NK cells[104]. Other directed CAR-NK cells are also in the research stage. One study used baboon enveloped pseudotyped viral vectors to transduce blood-borne primary NK cells to generate CD33-directed CAR-NK cells, which can detect CD33-positive OCI in vitro. -AML2 and primary AML cells show stable expression, proliferation and stronger cytotoxicity. In addition, CD33-CAR-NK cells showed strong killing effects in the OCI-AML2 xenograft mouse model, preventing the spread of leukemia cells without side effects[105]. Another study designed a drug targeting IL-3 receptor subunit α (CD123), which is strongly expressed on the surface of acute myeloid leukemia (AML) cells. This experiment used a CD123-targeted CAR-NK92 cell line paired with an IL-15 gene cassette. The researchers conducted studies in vitro and in vivo in mice, and found that CD123-CAR-NK cells had stronger anti-AML activity in vitro and in vivo[106].

3.6. Application of CAR-NK cells in solid tumors

Currently, many in vitro and in vivo experiments are testing the role of CAR-NK cells in solid tumors, and some initial results have been achieved.

3.6.1. Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is known for its rich immune mechanisms, and its extracellular matrix accounts for 70% of the entire tumor volume. A Korean study showed that folate receptor α (FRα) and death receptor 4 (DR4) are highly expressed in PDAC, and targeting FRα and DR4/5 to CAR-NK cells can significantly enhance the efficacy of these two receptors. PDAC cell apoptosis. This experiment confirmed that FRα-directed TRAIL.CD27.CD3ζ.CAR-NK92 cells have a high killing effect on PDAC cells in vitro and in xenografted PDAC mouse models[107]. Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol-anchored cell surface protein that is abnormally overexpressed in 60-80% of pancreatic cancer patients[108] but is not found in normal pancreatic ducts[109]. Researchers evaluated the killing effect of PSCA-CAR-NK cells expressing soluble IL-15 on pancreatic cancer cells in vitro and in vivo. The results showed that this cell had a significant inhibitory effect on PSCA+ pancreatic cancer cells and no toxicity was found[110]. In addition, a study showed that the STING agonist cyclic GMP-AMP (cGAMP) can directly activate NK cells, and mesothelin-targeting CAR-NK-92 cells combined with cGAMP showed potent anti-tumor activity in a mouse model of pancreatic cancer. tumor effects[111]. ROBO-1 is an axon guidance receptor and cell adhesion receptor. A study found that CD8.CD3ζ.4-1BB.CAR-NK92 cells targeting ROBO-1 stabilized the condition of patients with pancreatic cancer for up to 5 years. Months later, the only adverse reaction was fever[112].

3.6.2. Ovarian cancer

Mesothelin (MSLN) is overexpressed in ovarian cancer. A study constructed CD19.NK92.CAR-NK cells targeting MSLN, which can kill MSLN-positive ovarian cancer cells (OVCAR-3 and SK- OV-3). Furthermore, stronger cytokine secretion was detected in MSLN.CAR-NK cells co-cultured with OVCAR-3 and SK-OV-3 compared with parental NK92 cells and CD19.CAR-NK cells. MSLN. CD19.CAR-NK cells can significantly eliminate ovarian cancer cells and prolong the survival time of mice, showing strong activity both in vivo and in vitro[113]. Not only that, researchers have constructed CAR-NK cells targeting CD24 to kill CD24-positive ovarian cancer cells. Researchers also constructed CD24 and MSLN together to enable CAR-NK cells to exhibit excellent killing effects[114]. One study showed that αFR is expressed in 90% of ovarian cancer cells, in three among anti-αFR.CD3ζ, anti-αFR.CD28.CD3ζ and anti-αFR.CD28.4-BB.CD3ζ CAR-NK cells, the latter has the most cytotoxicity and has stronger degranulation when incubated with αFR-expressing ovarian cancer cells. effects, cytokine secretion, proliferative capacity and persistence. In a xenogeneic mouse model, treatment with anti-αFR.CD28.4-1BB.CD3ζ.CAR-NK92 significantly extended survival[115].

3.6.3. Breast cancer

Tissue factor (TF) was designed as an antigen for CAR-NK cells because 50-85% of triple-negative breast cancer molecules express TF. The experimenters designed the TF-directed CD28.4-1BB.CD3ζ.CAR construct to transduce into NK-92MI cells that were deprived of CD16 receptors and NK-92MI cells that were not deprived of CD16 receptors, respectively, as experimental groups. and control group. Studies have shown that NK92-MI cells with CD16 receptors can mediate ADCC and have stronger anti-tumor activity[116]. Epidermal cell adhesion molecule (EpCAM) is an antigen commonly found on the surface of various cancer cells. In EpCAM-positive breast cancer cells, CAR-NK92 cells targeting EpCAM and IL-15 have strong cytotoxicity[117]. Furthermore, a HER-2-targeting CD28.CD3ζCAR design expressed by NK92 cells also showed antitumor activity against breast cancer in vitro and in vivo[118].

3.6.4. Glioblastoma

Although NK cells found in glioblastoma (GBM) are inhibited by the TME, NK cells are found in 89% of glioma blasts, suggesting that NK cells can infiltrate GBM. In a trial of nine patients in which NK cells were injected

intravenously or intratumorally, four responded and three had stable disease. Although the therapeutic effect quickly disappeared due to the strong inhibitory effect of TME, this experiment in humans confirmed the huge therapeutic potential of NK cells[119]. We found that intravenous injection of EGFRvIII-directed DAP12.CD3ζ.CAR-NK-YTS cells with CXCR4 receptor overexpression inhibited GBM tumor growth and prolonged survival in a xenograft mouse model[120,121]. Several clinical trials of CAR-T cell therapy in patients with GBM have shown that high anti-tumor activity leads to rapid loss of antigens in cancer cell subpopulations and, despite initial responses, resistance to CAR-T therapy quickly develops[122-124]. If CD28. CD3ζ.CAR-NK-92 cells targeting mutant and wild-type EGFR are simultaneously constructed, compared with CAR-NK cells targeting only one EGFR alone, survival can be prolonged without antigen escape[123]. Intracranial injection of bispecific epidermal growth factor receptor and epidermal growth factor receptor vIII-directed CD28. CD3ζ.CAR-NK-92/NKL prolongs survival in a GBM xenograft mouse model and is characterized by cytotoxicity and IFN - Increased gamma secretion[124]. A number of structures have been designed as targets for CAR-NK cells, and we list these targets in Table 2(Table2).

4. CAR-Macrophage

4.1. Introduction to CAR-Macrophage

Although CAR-NK cells have many advantages over CAR-T cells, the obstacles encountered by CAR-T cells during treatment also exist in CAR-NK cells. As mentioned before, the function of NK cells is inhibited in the TME. Although there is currently a study showing that CAR-NK cells can play a role in the TME of GBM patients, the therapeutic effect is short-lived due to the inhibitory effect. In short, CAR-T cells and CAR-NK cells are strongly inhibited in the TME[41], requiring researchers to continuously design more complex CAR structures to arm immune cells, which undoubtedly increases the difficulty and cost of design and manufacturing. As an emerging product, CAR-Macrophage shows great advantages in TME. First, in many malignant tumors, macrophages infiltrate in large numbers[125], and the hypoxic environment induces tumor cells and stroma to produce cytokines, such as CCL2 (C-C matrix chemokine ligand 2), CXCL12 (C-X-C matrix chemokine ligand 12), CSF1 (colony stimulating factor 1) and vascular endothelial growth factor to recruit macrophages[126]. After local macrophages are recruited into the hypoxic TME, soluble cytokine receptors are downregulated, locking macrophages in the TME. In addition, macrophages can sense the hypoxic environment and related metabolites and automatically migrate into the TME. Secondly, the environment of the TME can cause T cell exhaustion, but is favored by macrophages. There are two types of macrophages, pro-inflammatory M1 and anti-inflammatory M2[127]. M2 is the main immunosuppressive cell group in the TME and inhibits the functions of other immune cells, but has stronger phagocytic ability than M1[128]. In addition, macrophages can make phenotypic changes in response to external stimuli and have strong phenotypic plasticity. CAR The CAR in macrophages has the same structure as the CAR in CAR T cells, with an extracellular antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular domain. They differ in their intracellular signaling domains. In addition to the FDA-approved CD28 and 4-1BB, CD3ζ can also be used, with domains containing immunoreceptor tyrosine-based activation motifs (ITAMs) [129-131]. In addition to CD3ζ, other ITAM-containing intracellular domains, such as the γ subunit of Fc receptor (FcRγ) and multi-epidermal growth factor-like domain protein 10 (Megf10), have also been used, which can induce phagocytosis similar to CD3ζ[129,130]. Researchers are currently designing suitable targets for CAR-Macrophage to maximize its phagocytosis and killing effects, and relevant clinical trials are also gradually underway.

5. Conclusion

As a treatment modality that has been approved by the FDA, CAR-T cells have demonstrated a powerful therapeutic effect in hematological malignancies. However, due to its high toxicity, single source, high cost and strong inhibition in the TME, its application in solid tumors is limited. Compared with CAR-T cells, CAR-NK cells have the advantages of less toxicity, wide sources, diverse anti-tumor mechanisms, and relatively weak inhibition in the TME, showing broad prospects for future treatment. CAR-Macrophage can fully adapt to the complex environment of TME and exert anti-tumor effects. The mixed use of multiple CAR series cells may achieve better therapeutic effects in the future.

References:

1. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. J Hematol Oncol. 2021 May 1;14(1):73.

2. Zhang T, Wu MR, Sentman CL. An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy in vivo. J Immunol. 2012 Sep 1;189(5):2290-9.

3. Bridgeman JS, Hawkins RE, Bagley S, Blaylock M, Holland M, Gilham DE. The optimal antigen response of chimeric antigen receptors harboring the CD3zeta transmembrane

domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. J Immunol. 2010 Jun 15;184(12):6938-49.

4. Muller YD, Nguyen DP, Ferreira LMR, Ho P, Raffin C, Valencia RVB, Congrave-Wilson Z, Roth TL, Eyquem J, Van Gool F, Marson A, Perez L, Wells JA, Bluestone JA, Tang Q. The CD28-Transmembrane Domain Mediates Chimeric Antigen Receptor Heterodimerization With CD28. Front Immunol. 2021 Mar 23;12:639818.

5. Zenere G, Wu C, Midkiff CC, Johnson NM, Grice CP, Wimley WC, Kaur A, Braun SE. Extracellular domain, hinge, and transmembrane determinants affecting surface CD4 expression of a novel anti-HIV chimeric antigen receptor (CAR) construct. bioRxiv [Preprint]. 2023 Oct 26:2023.10.25.563930.

6. Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, Qian X, James SE, Raubitschek A, Forman SJ, Gopal AK, Pagel JM, Lindgren CG, Greenberg PD, Riddell SR, Press OW. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008 Sep 15;112(6):2261-71.

7. Hege KM, Bergsland EK, Fisher GA, Nemunaitis JJ, Warren RS, McArthur JG, Lin AA, Schlom J, June CH, Sherwin SA. Safety, tumor trafficking and immunogenicity of chimeric antigen receptor (CAR)-T cells specific for TAG-72 in colorectal cancer. J Immunother Cancer. 2017 Mar 21;5:22.

8. Pellegrino M, Del Bufalo F, De Angelis B, et al. Manipulating the metabolism to improve the efficacy of CAR T-cell immunotherapy[J]. Cells, 2020, 10(1): 14.

9. Menk AV, Scharping NE, Rivadeneira DB, Calderon MJ, Watson MJ, Dunstane D, Watkins SC, Delgoffe GM. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med. 2018 Apr 2;215(4):1091-1100.

10. Kawalekar O U, O'Connor R S, Fraietta J A, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells[J]. Immunity, 2016, 44(2): 380-390.

11. Pacella I, Procaccini C, Focaccetti C, Miacci S, Timperi E, Faicchia D, Severa M, Rizzo F, Coccia EM, Bonacina F, Mitro N, Norata GD, Rossetti G, Ranzani V, Pagani M, Giorda E, Wei Y, Matarese G, Barnaba V, Piconese S. Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth. Proc Natl Acad Sci U S A. 2018 Jul 10;115(28):E6546-E6555.

12. Peperzak V, Veraar EA, Keller AM, Xiao Y, Borst J. The Pim kinase pathway contributes to survival signaling in primed CD8+ T cells upon CD27 costimulation. J Immunol. 2010 Dec 1;185(11):6670-8.

13. Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, Cloer C, Kishton RJ, Gao X, Youngblood B, Do M, Li MO, Locasale JW, Rathmell JC, Chi H. mTORC1 and mTORC2 Kinase Signaling and Glucose Metabolism Drive Follicular Helper T Cell Differentiation. Immunity. 2016 Sep 20;45(3):540-554.

14. Drent E, Poels R, Ruiter R, van de Donk NWCJ, Zweegman S, Yuan H, de Bruijn J, Sadelain M, Lokhorst HM, Groen RWJ, Mutis T, Themeli M. Combined CD28 and 4-1BB Costimulation Potentiates Affinity-tuned Chimeric Antigen Receptor-engineered T Cells. Clin Cancer Res. 2019 Jul 1;25(13):4014-4025.

15. HIGHLIGHTS OF PRESCRIBING INFORMATION-tisagenlecleucel 2020 [updated 12/2020. Available from: https://www.fda.gov/media/107296/ download

16. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, Qayed M, De Moerloose B, Hiramatsu H, Schlis K, Davis KL, Martin PL, Nemecek ER, Yanik GA, Peters C, Baruchel A, Boissel N, Mechinaud F, Balduzzi A, Krueger J, June CH, Levine BL, Wood P, Taran T, Leung M, Mueller KT, Zhang Y, Sen K, Lebwohl D, Pulsipher MA, Grupp SA. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018 Feb 1;378(5):439-448.

17. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM, Stiff PJ, Friedberg JW, Flinn IW, Goy A, Hill BT, Smith MR, Deol A, Farooq U, McSweeney P, Munoz J, Avivi I, Castro JE, Westin JR, Chavez JC, Ghobadi A, Komanduri KV, Levy R, Jacobsen ED, Witzig TE, Reagan P, Bot A, Rossi J, Navale L, Jiang Y, Aycock J, Elias M, Chang D, Wiezorek J, Go WY. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med. 2017 Dec 28;377(26):2531-2544.

18. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. N Engl J Med

19. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, Mehta A, Purev E, Maloney DG, Andreadis C, Sehgal A, Solomon SR, Ghosh N, Albertson TM, Garcia J, Kostic A, Mallaney M, Ogasawara K, Newhall K, Kim Y, Li D, Siddiqi T. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020 Sep 19;396(10254):839-852.

20. Ma S, Li X, Wang X, Cheng L, Li Z, Zhang C, Ye Z, Qian Q. Current Progress in CAR-T Cell Therapy for Solid Tumors. Int J Biol Sci. 2019 Sep 7;15(12):2548-2560.

21. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009 Jul 16;114(3):535-46.

22. Castellarin M, Sands C, Da T, Scholler J, Graham K, Buza E,

Fraietta JA, Zhao Y, June CH. A rational mouse model to detect on-target, off-tumor CAR T cell toxicity. JCI Insight. 2020 Jul 23;5(14):e136012.

23. Smith JB, Lanitis E, Dangaj D, Buza E, Poussin M, Stashwick C, Scholler N, Powell DJ Jr. Tumor Regression and Delayed Onset Toxicity Following B7-H4 CAR T Cell Therapy. Mol Ther. 2016 Nov;24(11):1987-1999.

24. Labanieh L, Majzner RG, Klysz D, Sotillo E, Fisher CJ, Vilches-Moure JG, Pacheco KZB, Malipatlolla M, Xu P, Hui JH, Murty T, Theruvath J, Mehta N, Yamada-Hunter SA, Weber EW, Heitzeneder S, Parker KR, Satpathy AT, Chang HY, Lin MZ, Cochran JR, Mackall CL. Enhanced safety and efficacy of protease-regulated CAR-T cell receptors. Cell. 2022 May 12;185(10):1745-1763.e22.

25. Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, Gratama JW, Stoter G, Oosterwijk E. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol. 2006 May 1;24(13):e20-2.

26. Lamers CHJ et al. Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CARengineered T-cells–a completed study overview. Biochem. Soc. Trans 44, 951–959 (2016).

27. Feng K-C et al. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. J. Hematol. Oncol 10, 4 (2017).

28. Guo Y et al. Phase I study of chimeric antigen receptor-modified T cells in patients with EGFR-positive advanced biliary tract cancers. Clin. Cancer Res 24, 1277–1286 (2018).

29. Liu Y et al. Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: a phase I clinical trial. Cytotherapy 22, 573–580 (2020).

30. Feng K et al. Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. Protein Cell 9, 838–847 (2018).

31. Thistlethwaite FC, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ, Byatte AJ, Kirillova N, Valle JW, Sharma SK, Chester KA, Westwood NB, Halford SER, Nabarro S, Wan S, Austin E, Hawkins RE. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. Cancer Immunol Immunother. 2017 Nov;66(11):1425-1436.

32. Qi C et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. Nat. Med 28, 1189–1198 (2022).

33. Tian Y, Wen C, Zhang Z, Liu Y, Li F, Zhao Q, Yao C, Ni K, Yang S, Zhang Y. CXCL9-modified CAR T cells improve immune cell infiltration and antitumor efficacy. Cancer Immunol Immunother. 2022 Nov;71(11):2663-2675.

34. Rasche L, Vago L, Mutis T. Tumour Escape from CAR-T Cells. 2022 Feb 7. In: Kröger N, Gribben J, Chabannon C, Yakoub-Agha I, Einsele H, editors. The EBMT/EHA CAR-T Cell Handbook [Internet]. Cham (CH): Springer; 2022. Chapter 4. PMID: 36122074.

35. Choi BD, Yu X, Castano AP, Bouffard AA, Schmidts A, Larson RC, Bailey SR, Boroughs AC, Frigault MJ, Leick MB, Scarfò I, Cetrulo CL, Demehri S, Nahed BV, Cahill DP, Wakimoto H, Curry WT, Carter BS, Maus MV. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat Biotechnol. 2019 Sep;37(9):1049-1058.

36. Schirrmacher V. Cancer Vaccines and Oncolytic Viruses Exert Profoundly Lower Side Efects in Cancer Patients than Other Systemic Therapies: A Comparative Analysis. Biomedicines. 2020;8(3).

37. Brown, C.; Alizadeh, D.; Starr, R.; Weng, L.; Wagner, J.R.; Naranjo, A.; Ostberg, J.R.; Blanchard, M.S.; Kilpatrick, J.; Simpson, J.; et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N. Engl. J. Med. 2016, 375, 2561–2569.

38. Pietrobon V, Todd LA, Goswami A, Stefanson O, Yang Z, Marincola F. Improving CAR T-Cell Persistence. Int J Mol Sci. 2021 Oct 7;22(19):10828.

39. Chmielewski M., Abken H. TRUCKS, the fourth-generation CAR T cells: Current developments and clinical translation. Adv. CELL GENE Ther. 2020;3

40. Santomasso B., Bachier C., Westin J., Rezvani K., Shpall E.J. The Other Side of CAR T-Cell Therapy: Cytokine Release Syndrome, Neurologic Toxicity, and Financial Burden. Am. Soc. Clin. Oncol. Educ. Book. 2019;39:433–444.

41. Pan K, Farrukh H, Chittepu VCSR, Xu H, Pan CX, Zhu Z. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. J Exp Clin Cancer Res. 2022 Mar 31;41(1):119.

42. Hawkins E.R., D'Souza R.R., Klampatsa A. Armored CAR T-Cells: The Next Chapter in T-Cell Cancer Immunotherapy. Biol. Targets Ther. 2021;15:95–105.

43. Lin H, Cheng J, Mu W, Zhou J, Zhu L. Advances in Universal CAR-T Cell Therapy. Front Immunol. 2021 Oct 6;12:744823.

44. Chen YJ, Abila B, Mostafa Kamel Y. CAR-T: What Is Next? Cancers (Basel). 2023 Jan 21;15(3):663.

45. Liu S, Galat V, Galat Y, Lee YKA, Wainwright D, Wu J. NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol. 2021 Jan 6;14(1):7.

46. Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M, Nowakowska P, Bönig H, Köhl U, Kloess S, Köhler S, Holtgreve-Grez H, Jauch A, Schmidt M, Schubert R, Kühlcke K, Seifried E, Klingemann HG, Rieger MA, Tonn T, Grez M, Wels WS. Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. Mol Ther. 2015 Feb;23(2):330-8.

47. ATCC NK92 Cell Line Characteristic. Available online:

https://www.lgcstandards-atcc.org/products/all/CRL-2407.aspx?geo_ country=pl#characteristics
48. Béziat V, Descours B, Parizot C, Debré P, Vieillard V. NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. PLoS One. 2010 Aug 6;5(8):e11966.
49. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: an important NK cell subset. Immunology. 2009 Apr;126(4):458-65.
50. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-Derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-tumor Activity. Cell Stem Cell. 2018 Aug 2;23(2):181-192.e5.
51. Knorr, D.A.; Ni, Z.; Hermanson, D.; Hexum, M.K.; Bendzick, L.; Cooper, L.J.N.; Lee, D.A.; Kaufman, D.S. Clinical-Scale Derivation of Natural Killer Cells From Human Pluripotent Stem Cells for Cancer Therapy DAVID. Stem Cells Transl. Med. 2013, 2, 274–283.
52. Hermanson, D.L.; Bendzick, L.; Pribyl, L.; McCullar, V.; Vogel, R.I.; Miller, J.S.; Geller, M.A.; Kaufman, D.S. Induced Pluripotent Stem Cell-Derived Natural Killer Cells for Treatment of Ovarian Cancer. Stem Cells 2016, 34, 93–101.
53. Wrona E, Borowiec M, Potemski P. CAR-NK Cells in the Treatment of Solid Tumors. Int J Mol Sci. 2021 May 31;22(11):5899.Zheng L, Ren L, Kouhi A. A humanized Lym-1 CAR with novel DAP10/DAP12 signalling domains demonstrates reduced tonic signalling and increased anti-tumour activity in B Cell Lymphoma models. Clin Cancer Res.
54. Kotanides H, Sattler RM, Lebron MB. Characterization of 7A5: A Human CD137 (4-1BB) Receptor Binding Monoclonal Antibody with Differential Agonist Properties That Promotes Antitumor Immunity
55. Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK cells: A promising cellular immunotherapy for cancer. EBioMedicine. 2020 Sep;59:102975.
56. Xu Y, Liu Q, Zhong M. 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell malignancies.
57. Xu Y, Liu Q, Zhong M, Wang Z, Chen Z, Zhang Y, Xing H, Tian Z, Tang K, Liao X, Rao Q, Wang M, Wang J. 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell malignancies. J Hematol Oncol. 2019 May 16;12(1):49. Klingemann H. Are natural killer cells superior CAR drivers? Oncoimmunology. 2014 Apr 15;3:e28147.
58. Klingemann H. Are natural killer cells superior CAR drivers? Oncoimmunology. 2014;3:e28147.
59. Valeri A, García-Ortiz A, Castellano E, Córdoba L, Maroto-Martín E, Encinas J, Leivas A, Río P, Martínez-López J. Overcoming tumor resistance mechanisms in CAR-NK cell therapy. Front Immunol. 2022 Aug 3;13:953849.
60. Lupo KB, Matosevic S. Natural killer cells as allogeneic effectors in adoptive cancer immunotherapy. cancers (basel) 2019;11(6)
61. Sun C, Sun H, Zhang C, Tian Z. NK cell receptor imbalance and NK cell dysfunction in HBV infection and hepatocellular carcinoma. Cell Mol Immunol. 2015;12(3):292–302.
62. Sun C, Sun HY, Xiao WH, Zhang C, Tian ZG. Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy. Acta Pharmacol Sin. 2015;36(10):1191–1199.
63. Wu J, Mishra HK, Walcheck B. Role of ADAM17 as a regulatory checkpoint of CD16A in NK cells and as a potential target for cancer immunotherapy. J Leukoc Biol. 2019;105(6):1297–1303.
64. Geller, M.A.; Cooley, S.; Judson, P.L.; Ghebre, R.; Carson, L.F.; Argenta, P.A.; Jonson, A.L.; Panoskaltsis-Mortari, A.; Curtsinger, J.; McKenna, D.; et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy 2011, 13, 98–107.
65. Miller, J.S.; Soignier, Y.; Panoskaltsis-Mortari, A.; McNearney, S.A.; Yun, G.H.; Fautsch, S.K.; McKenna, D.; Le, C.; DeFor, T.E.; Burns, L.J.; et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2005, 105, 3051–3057.
66. Pfefferle A, Jacobs B, Haroun-Izquierdo A, Kveberg L, Sohlberg E, Malmberg KJ. Deciphering natural killer cell homeostasis. Front Immunol (2020) 11:812.
67. Jacobs B, Pfefferle A, Clement D, Berg-Larsen A, Saetersmoen ML, Lorenz S, et al.. Induction of the bim short splice variant sensitizes proliferating nk cells to il-15 withdrawal. J Immunol (2019) 202(3):736–46.
68. Fehniger TA. Mystery solved: Il-15. J Immunol (2019) 202(11):3125–6.
69. Assier E, Jullien V, Lefort J, Moreau JL, Di Santo JP, Vargaftig BB, et al.. Nk cells and polymorphonuclear neutrophils are both critical for il-2-Induced pulmonary vascular leak syndrome. J Immunol (2004) 172(12):7661–8.
70. MacDonald A, Wu TC, Hung CF. Interleukin 2-based fusion proteins for the treatment of cancer. J Immunol Res (2021) 2021:7855808.
71. Conlon KC, Potter EL, Pittaluga S, Lee CR, Miljkovic MD, Fleisher TA, et al.. Il15 by continuous intravenous infusion to adult patients with solid tumors in a phase I trial induced dramatic nk-cell subset expansion. Clin Cancer Res an Off J Am Assoc Cancer Res (2019) 25(16):4945–54.
72. Margolin K, Morishima C, Velcheti V, Miller JS, Lee SM, Silk AW, et al.. Phase I trial of alt-803, a novel recombinant Il15 complex, in patients with advanced solid tumors. Clin Cancer Res an Off J Am Assoc Cancer Res (2018) 24(22):5552–61.
73. Foltz JA, Hess BT, Bachanova V, Bartlett NL, Berrien-Elliott MM, McClain E, et al.. Phase I trial of n-803, an Il15 receptor agonist, with rituximab in patients with indolent non-Hodgkin lymphoma. Clin Cancer Res an Off J Am Assoc Cancer Res (2021) 27(12):3339–50.

74. Romee R, Cooley S, Berrien-Elliott MM, Westervelt P, Verneris MR, Wagner JE, et al.. First-in-Human phase 1 clinical study of the il-15 superagonist complex alt-803 to treat relapse after transplantation. Blood (2018) 131(23):2515–27.
75. Shah N, Perales MA, Turtle CJ, Cairo MS, Cowan AJ, Saeed H, et al.. Phase I study protocol: Nktr-255 as monotherapy or combined with daratumumab or rituximab in hematologic malignancies. Future Oncol (2021) 17(27):3549–60.
76. Miyazaki T, Maiti M, Hennessy M, Chang T, Kuo P, Addepalli M, et al.. Nktr-255, a novel polymer-conjugated rhil-15 with potent antitumor efficacy. J Immunother Cancer (2021) 9(5).
77. Liu, E.; Tong, Y.; Dotti, G.; Shaim, H.; Savoldo, B.; Mukherjee, M.; Orange, J.; Wan, X.; Lu, X.; Reynolds, A.; et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent anti-tumor activity.
78. Daher M, Basar R, Gokdemir E, Baran N, Uprety N, Nunez Cortes AK, Mendt M, Kerbauy LN, Banerjee PP, Shanley M, Imahashi N, Li L, Lim FLWI, Fathi M, Rezvan A, Mohanty V, Shen Y, Shaim H, Lu J, Ozcan G, Ensley E, Kaplan M, Nandivada V, Bdiwi M, Acharya S, Xi Y, Wan X, Mak D, Liu E, Jiang XR, Ang S, Muniz-Feliciano L, Li Y, Wang J, Kordasti S, Petrov N, Varadarajan N, Marin D, Brunetti L, Skinner RJ, Lyu S, Silva L, Turk R, Schubert MS, Rettig GR, McNeill MS, Kurgan G, Behlke MA, Li H, Fowlkes NW, Chen K, Konopleva M, Champlin RE, Shpall EJ, Rezvani K. Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells. Blood. 2021 Feb 4;137(5):624-636.
79. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, Han H, Chiang SC, Foley B, Mattsson K, Larsson S, Schaffer M, Malmberg KJ, Ljunggren HG, Miller JS, Bryceson YT. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity. 2015 Mar 17;42(3):443-56.
80. Lee, J.; Zhang, T.; Hwang, I.; Kim, A.; Nitschke, L.; Kim, M.; Scott, J.M.; Kamimura, Y.; Lanier, L.L.; Kim, S. Epigenetic Modification and Antibody-Dependent Expansion of Memory-like NK Cells in Human Cytomegalovirus-Infected Individuals. Immunity 2015, 42, 431–442.
81. Romee, R.; Schneider, S.E.; Leong, J.W.; Chase, J.M.; Keppel, C.; Sullivan, R.P.; Cooper, M.A.; Fehniger, T.A. Cytokine activation induces human memory-like NK cells. Blood 2012, 120, 4751–4760
82. Gang, M.; Marin, N.D.; Wong, P.; Neal, C.C.; Marsala, L.; Foster, M.; Schappe, T.; Meng, W.; Tran, J.; Schaettler, M.; et al. CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas. Blood 2020.
83. Sarhan, D.; Cichocki, F.; Zhang, B.; Yingst, A.; Spellman, S.R.; Cooley, S.; Verneris, M.R.; Blazar, B.R.; Miller, J.S. Adaptive NK Cells with Low TIGIT Expression Are Inherently Resistant to Myeloid-Derived Suppressor Cells. Cancer Res. 2016, 76, 5696–5706.
84. Sarhan, D.; Hippen, K.L.; Lemire, A.; Hying, S.; Luo, X.; Lenvik, T.; Curtsinger, J.; Davis, Z.; Zhang, B.; Cooley, S.; et al. Adaptive NK Cells Resist Regulatory T-cell Suppression Driven by IL37. Cancer Immunol. Res. 2018, 6, 766–775.
85. Perussia, B.; Ramoni, C.; Anegon, I.; Cuturi, M.C.; Faust, J.; Trinchieri, G. Preferential proliferation of natural killer cells among peripheral blood mononuclear cells cocultured with B lymphoblastoid cell lines.
86. Fernández, A.; Navarro-Zapata, A.; Escudero, A.; Matamala, N.; Ruz-Caracuel, B.; Mirones, I.; Pernas, A.; Cobo, M.; Casado, G.; Lanzarot, D.; et al. Optimizing the Procedure to Manufacture Clinical-Grade NK Cells for Adoptive Immunotherapy. Cancers 2021, 13, 577.
87. Liu, E.; Ang, S.O.T.; Kerbauy, L.; Basar, R.; Kaur, I.; Kaplan, M.; Li, L.; Tong, Y.; Daher, M.; Ensley, E.L.; et al. GMP-Compliant Universal Antigen Presenting Cells (uAPC) Promote the Metabolic Fitness and Antitumor Activity of Armored Cord Blood CAR-NK Cells. Front. Immunol. 2021, 12, 626098
88. Zhang, Q.-F.; Yin, W.-W.; Xia, Y.; Yi, Y.-Y.; He, Q.-F.; Wang, X.; Ren, H.; Zhang, D.-Z. Liver-infiltrating CD11b− CD27− NK subsets account for NK-cell dysfunction in patients with hepatocellular carcinoma and are associated with tumor progression. Cell. Mol. Immunol. 2017, 14, 819–829
89. Castaneda, D.C.; Dhommée, C.; Baranek, T.; Dalloneau, E.; Lajoie, L.; Valayer, A.; Arnoult, C.; Demattéi, M.-V.; Fouquenet, D.; Parent, C.; et al. Lack of FcRn Impairs Natural Killer Cell Development and Functions in the Tumor Microenvironment. Front. Immunol. 2018, 9, 2259.
90. Bruno, A.; Mortara, L.; Baci, D.; Noonan, D.M.; Albini, A. Myeloid Derived Suppressor Cells Interactions With Natural Killer Cells and Pro-angiogenic Activities: Roles in Tumor Progression. Front. Immunol. 2019, 10, 771.
91. Lazarova, M.; Steinle, A. Impairment of NKG2D-Mediated Tumor Immunity by TGF-β. Front. Immunol. 2019, 10, 2689.
92. Yvon, E.S.; Burga, R.; Powell, A.; Cruz, C.R.; Fernandes, R.; Barese, C.; Nguyen, T.; Abdel-Baki, M.S.; Bollard, C.M. Cord blood natural killer cells expressing a dominant negative TGF-β receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy 2017, 19, 408–418.
93. Kailayangiri, S.; Altvater, B.; Wiebel, M.; Jamitzky, S.; Rossig, C. Overcoming Heterogeneity of Antigen Expression for Effective CAR T Cell Targeting of Cancers. Cancers 2020, 12, 1075.
94. Töpfer, K.; Kempe, S.; Müller, N.; Schmitz, M.; Bachmann, M.; Cartellieri, M.; Schackert, G.; Temme, A. Tumor Evasion from T Cell Surveillance. J. Biomed. Biotechnol. 2011, 2011, 1–19.
95. Williams, J.B.; Li, S.; Higgs, E.F.; Cabanov, A.; Wang, X.; Huang, H.; Gajewski, T.F. Tumor heterogeneity and clonal cooperation influence the immune selection of IFN-γ-signaling mutant cancer cells. Nat. Commun. 2020, 11, 1–14.

96. Atashzar, M.R.; Baharlou, R.; Karami, J.; Abdollahi, H.; Rezaei, R.; Pourramezan, F.; Moghaddam, S.H.Z. Cancer stem cells: A review from origin to therapeutic implications. J. Cell. Physiol. 2020, 235, 790–803.
97. Lanier, L.L. DAP10- and DAP12-associated receptors in innate immunity. Immunol. Rev. 2009, 227, 150–160.
98. Müller, N.; Michen, S.; Tietze, S.; Töpfer, K.; Schulte, A.; Lamszus, K.; Schmitz, M.; Schackert, G.; Pastan, I.; Temme, A. Engineering NK Cells Modified with an EGFRvIII-specific Chimeric Antigen Receptor to Overexpress CXCR4 Improves Immunotherapy of CXCL12/SDF-1α-secreting Glioblastoma. J. Immunother. 2015, 38, 197–210
99. Ng, Y.Y.; Tay, J.C.; Wang, S. CXCR1 Expression to Improve Anti-Cancer Efficacy of Intravenously Injected CAR-NK Cells in Mice with Peritoneal Xenografts. Mol. Ther. Oncolytics 2020, 16, 75–85.
100. Lee, J.; Kang, T.H.; Yoo, W.; Choi, H.; Jo, S.; Kong, K.; Lee, S.-R.; Kim, S.-U.; Kim, J.-S.; Cho, D.; et al. An antibody designed to improve adoptive NK-cell therapy inhibits pancreatic cancer progression in a murine model. Cancer Immunol. Res. 2018, 7, 219–229.
101. Watzl, C.; Long, E.O. Signal transduction during activation and inhibition of natural killer cells. Curr. Protoc. Immunol. 2010.
102. Billadeau, D.D.; Upshaw, J.L.; A Schoon, R.; Dick, C.J.; Leibson, P.J. NKG2D-DAP10 triggers human NK cell–mediated killing via a Syk-independent regulatory pathway. Nat. Immunol. 2003, 4, 557–564.
103. Figueiredo, C.; Seltsam, A.; Blasczyk, R. Permanent silencing of NKG2A expression for cell-based therapeutics. J. Mol. Med. 2009, 87, 199–210.
104. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, Nassif Kerbauy L, Overman B, Thall P, Kaplan M, Nandivada V, Kaur I, Nunez Cortes A, Cao K, Daher M, Hosing C, Cohen EN, Kebriaei P, Mehta R, Neelapu S, Nieto Y, Wang M, Wierda W, Keating M, Champlin R, Shpall EJ, Rezvani K. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. N Engl J Med. 2020 Feb 6;382(6):545-553.
105. Albinger N, Pfeifer R, Nitsche M, Mertlitz S, Campe J, Stein K, Kreyenberg H, Schubert R, Quadflieg M, Schneider D, Kühn MWM, Penack O, Zhang C, Möker N, Ullrich E. Primary CD33-targeting CAR-NK cells for the treatment of acute myeloid leukemia. Blood Cancer J. 2022 Apr 13;12(4):61.
106. Morgan MA, Kloos A, Lenz D, Kattre N, Nowak J, Bentele M, Keisker M, Dahlke J, Zimmermann K, Sauer M, Heuser M, Schambach A. Improved Activity against Acute Myeloid Leukemia with Chimeric Antigen Receptor (CAR)-NK-92 Cells Designed to Target CD123. Viruses. 2021 Jul 14;13(7):1365.
107. Lee YE, Ju A, Choi HW, Kim JC, Kim EE, Kim TS, Kang HJ, Kim SY, Jang JY, Ku JL, Kim SC, Jun E, Jang M. Rationally designed redirection of natural killer cells anchoring a cytotoxic ligand for pancreatic cancer treatment. J Control Release. 2020 Oct 10;326:310-323.
108. Wente MN, Jain A, Kono E, et al. Prostate stem cell antigen is a putative target for immunotherapy in pancreatic cancer. Pancreas 2005;31:119–25.
109. Maitra A, Adsay NV, Argani P, et al. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. Mod Pathol 2003;16:902–12.
110. Teng KY, Mansour AG, Zhu Z, Li Z, Tian L, Ma S, Xu B, Lu T, Chen H, Hou D, Zhang J, Priceman SJ, Caligiuri MA, Yu J. Off-the-Shelf Prostate Stem Cell Antigen-Directed Chimeric Antigen Receptor Natural Killer Cell Therapy to Treat Pancreatic Cancer. Gastroenterology. 2022 Apr;162(4):1319-1333.
111. Da Y, Liu Y, Hu Y, Liu W, Ma J, Lu N, Zhang C, Zhang C. STING agonist cGAMP enhances anti-tumor activity of CAR-NK cells against pancreatic cancer. Oncoimmunology. 2022 Mar 21;11(1):2054105.
112. Li, C.; Yang, N.; Li, H.; Wang, Z. Robo1-specific chimeric antigen receptor natural killer cell therapy for pancreatic ductal adenocarcinoma with liver metastasis. J. Cancer Res. Ther. 2020, 16, 393–396.
113. Cao, B.; Liu, M.; Wang, L.; Liang, B.; Feng, Y.; Chen, X.; Shi, Y.; Zhang, J.; Ye, X.; Tian, Y.; et al. Use of chimeric antigen receptor NK-92 cells to target mesothelin in ovarian cancer. Biochem. Biophys. Res. Commun. 2020, 524, 96–102.
114. Klapdor, R.; Wang, S.; Morgan, M.; Dörk, T.; Hacker, U.; Hillemanns, P.; Buning, H.; Schambach, A. Characterization of a Novel Third-Generation Anti-CD24-CAR against Ovarian Cancer. Int. J. Mol. Sci. 2019, 20, 660.
115. Ao, X.; Yang, Y.; Li, W.; Tan, Y.; Guo, W.; Ao, L.; He, X.; Wu, X.; Xia, J.; Xu, X.; et al. Anti-αFR CAR-engineered NK-92 Cells Display Potent Cytotoxicity Against αFR-positive Ovarian Cancer. J. Immunother. 2019, 42, 284–296.
116. Hu, Z. Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. Sci. Rep. 2020, 10, 2815.
117. Zhang, Q.; Zhang, H.; Ding, J.; Liu, H.; Li, H.; Li, H.; Lu, M.; Miao, Y.; Li, L.; Zheng, J. Combination Therapy with EpCAM-CAR NK-92 Cells and Regorafenib against Human Colorectal Cancer Models. J. Immunol. Res. 2018, 2018, 4263520
118. Schönfeld, K.; Sahm, C.; Zhang, C.; Naundorf, S.; Brendel, C.; Odendahl, M.; Nowakowska, P.; Bönig, H.; Köhl, U.; Kloess, S.; et al. Selective Inhibition of Tumor Growth by Clonal NK Cells Expressing an ErbB2/HER2-Specific Chimeric Antigen Receptor. Mol. Ther. 2015
119. Ishikawa, E.; Tsuboi, K.; Saijo, K.; Harada, H.; Takano, S.; Nose, T.; Ohno, T. Autologous natural killer cell therapy for human recurrent malignant glioma. Anticancer Res. 2004, 24, 1861–1871.
120. Lanier, L.L. DAP10- and DAP12-associated receptors in innate immunity. Immunol. Rev. 2009, 227, 150–160.

121. Müller, N.; Michen, S.; Tietze, S.; Töpfer, K.; Schulte, A.; Lamszus, K.; Schmitz, M.; Schackert, G.; Pastan, I.; Temme, A. Engineering NK Cells Modified with an EGFRvIII-specific Chimeric Antigen Receptor to Overexpress CXCR4 Improves Immunotherapy of CXCL12/SDF-1α-secreting Glioblastoma. J. Immunother. 2015, 38, 197–210.
122. Brown, C.; Alizadeh, D.; Starr, R.; Weng, L.; Wagner, J.R.; Naranjo, A.; Ostberg, J.R.; Blanchard, M.S.; Kilpatrick, J.; Simpson, J.; et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N. Engl. J. Med. 2016, 375, 2561–2569.
123. Genßler, S.; Burger, M.C.; Zhang, C.; Oelsner, S.; Mildenberger, I.; Wagner, M.; Steinbach, J.P.; Wels, W.S. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. Oncoimmunology 2016, 5, e1119354
124. Han, J.; Chu, J.; Chan, W.K.; Zhang, J.; Wang, Y.; Cohen, J.B.; Victor, A.; Meisen, W.H.; Kim, S.-H.; Grandi, P.; et al. CAR-Engineered NK Cells Targeting Wild-Type EGFR and EGFRvIII Enhance Killing of Glioblastoma and Patient-Derived Glioblastoma Stem Cells. Sci. Rep. 2015, 5, 11483.
125. van Ravenswaay Claasen HH, Kluin PM, Fleuren GJ. Tumor infiltrating cells in human cancer. On the possible role of CD16+ macrophages in antitumor cytotoxicity. Lab Invest. 1992;67(2):166–174.
126. Henze AT, Mazzone M. The impact of hypoxia on tumor-associated macrophages. J Clin Invest. 2016;126(10):3672–3679.
127. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol. 2000;164(12):6166–6173.
128. 95. Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell. 2015;27(4):462–472.
129. Morrissey MA, Williamson AP, Steinbach AM, Roberts EW, Kern N, Headley MB, et al. Chimeric antigen receptors that trigger phagocytosis. Elife. 2018;7.
130. Klichinsky M, Ruella M, Shestova O, Lu XM, Best A, Zeeman M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947–953.
131. Niu Z, Chen G, Chang W, Sun P, Luo Z, Zhang H, et al. Chimeric antigen receptor-modified macrophages trigger systemic anti-tumour immunity. J Pathol. 2021;253(3):247–257.

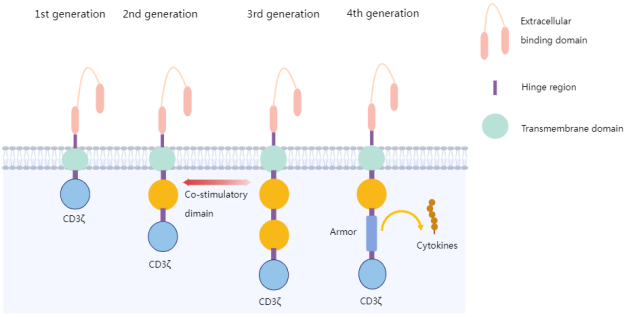

Figure 1: Basic structure of CAR-T.

The basic structure of CAR-T consists of four parts, extracellular antigen binding domain, hinge region, transmembrane domain, and intracellular signaling domain. The function of the extracellular antigen-binding domain is to recognize and bind specific antigens, the function of the hinge region is to expose the antigen-binding domain to the cell surface, the function of the transmembrane region is to dock the CAR to the immune cells, and the intracellular signaling domain is mainly responsible for transducing signals to activate the immune cells to attack the target cells. Four generations of CAR-T cells have been developed, and each generation of CAR-T cells differs in the structure of the intracellular signaling domain. The first generation CAR-T cell intracellular signaling domain includes a CD3ζ, the second generation CAR-T cell includes a CD3ζ and a co-stimulatory domain, the third generation CAR-T cell includes a CD3ζ and multiple co-stimulatory domains, and the fourth generation CAR-T cell adds other structures on top of this that can assist the CAR-T cell in releasing cytokines to fight against TME, etc. CAR-T. CAR-NK, CAR-Macrophage have similar structures.

Table 1 Common CAR-independent anti-tumor activity receptors in NK cells and their characteristics

Receptor	Ligands	Impact	Function
CD16	Fc domain of IgG	Activating	ADCC initiation
DNAM1	CD112,CD155	Activating	Cellular adhesion promotion
KIR	HLA class Ⅰ	Activating/Inhibitory	Regulate the killing of malignant tumors by NK cells and certain T cell subsets
NKG2A	HLA-E	Inhibitory	Expression of inhibitory signals in CD8+ T cells or CD56hiNK cells
NKG2D	HLA-E	Activating	Express activation signals in NK cells and T cells
NKG2C	HLA-E	Activating	Forms a complex with CD94 and sends activation signal through DAP12
NKp30	HA/HS.GAGs	Activating	Constitutively expressed in NK cells and T cells, triggering cytotoxic responses by specific ligands
NKp44	HA/HS.GAGs	Activating	Found only on activated NK cells. Associates with the DA12 homodimer for cytokine release ignition.
NKp46	HA/HS.GAGs	Activating	Triggers cytotoxic reaction.
PD-1	PDL-1,PDL-2	Inhibitory	Expressed on many immune cells, inhibiting immune cell activity
TIGHT	CD112,CD113,CD155	Inhibitory	Expressed in T cells and NK cells, can promote immune cell exhaustion and tumor escape

Table 2 Structures as targets for CAR-NK cells

Target	Tumor	Description
CD19	ALL	Major markers of B lymphocytes
CD7	ALL	Belongs to the immunoglobulin superfamily and is highly expressed on thymocytes and T cells
CD5	ALL	Expressed on the surface of human T cells and B cells, but its role as a B cell marker has not yet been fully agreed upon
FLT3	ALL	FLT3 is expressed on normal hematopoietic stem/progenitor cells, and its ligand (FL) is expressed as a membrane-bound or soluble form by bone marrow stroma cells
CD33	AML	CD33 is a sialoadhesin molecule and a member of the immunoglobulin supergene family. It is expressed by myeloid stem cells (CFU-GEMM, CFU-GM, CFU-G, and E-BFU), myeloblasts and monoblasts, monocytes/macrophages, granulocyte precursors (with
CD123	AML	CD123, the α chain of the interleukin 3 receptor, is a cytokine receptor that is overexpressed in multiple hematolymphoid neoplasms, including acute myeloid leukemia, blastic plasmacytoid dendritic cell neoplasm, acute lymphoblastic leukemia,
CD4	AML	CD4 is a glycoprotein molecule on the surface of immune cells
HER-2	Breast cancer	HER2-positive breast cancer is a breast cancer that tests positive for a protein called human epidermal growth factor receptor 2 (HER2). This protein promotes the growth of cancer cells.
EpCAM	Breast cancer	It is a transmembrane glycoprotein that mediates calcium-independent homotypic intercellular adhesion of epithelial cells. EpCAM is also involved in cell signaling, migration, proliferation and differentiation. Additionally, EpCAM has oncogenic
TF	Breast cancer	Highly expressed in triple-negative breast cancer cells
EGFR	Breast cancer	EGFR is highly expressed in patients with breast cancer brain metastases
NKG2D	Breast cancer	Activate the killing activity of NK cells
CD19	Chronic lymphocytic leukemia(CLL)	Major markers of B lymphocytes
EpCAM	Colorectal cancer	It is a transmembrane glycoprotein that mediates calcium-independent homotypic intercellular adhesion of epithelial cells. EpCAM is also involved in cell signaling, migration, proliferation and differentiation. Additionally, EpCAM has oncogenic
NKG2D	Colorectal cancer	Activate the killing activity of NK cells
CEA	Colorectal cancer	CEA expression is highly expressed in colorectal cancer cells
GD2	Ewing sarcoma	GD2 is a ganglioside diisoglutarate expressed on tumors of neuroectodermal origin, including human neuroblastoma and melanoma
HER-2	Gastric cancer	
EGFRⅦ	Glioblastoma	Approximately 20–40% of EGFR-amplified tumors harbor the EGFR variant III mutant (EGFRvIII),this mutant form shows constitutive activation in the absence of ligand to activate the tumor-promoting signaling pathways
EGFR	Glioblastoma	
HER-2	Glioblastoma	
CD73	Glioblastoma	CD73 is an ectonucleotidase overexpressed on tumor cells that suppresses anti-tumor immunity
ROBO1	Glioma and Neuroblastoma	A member of the immunoglobulin gene superfamily and encodes an integral membrane protein that functions in axon guidance and neuronal precursor cell migration. This receptor is activated by SLIT-family proteins, resulting in a repulsive effect on glioma
GPC3	Hepatocellular cancer (HCC)	Glypican-3 (GPC3) is an HSPG that is highly expressed in hepatocellular carcinoma, where it can attract Wnt proteins to the cell surface and promote cell proliferation
NKG2D	Hepatocellular cancer (HCC)	
c-MET	liver cancer	The overexpression of c-MET has been observed in various solid malignancies, such as liver , breast , lung and colorectal cancer
NKG2D	Lung cancer	
B7-H3	Lung cancer	A member of the B7 family of immune checkpoint proteins, is highly expressed in cancer cells and activated tumor-infiltrating immune cells, and helps cancer cells to evade the surveillance of cytotoxic T-cells and natural killer cells
CD19	Lymphoma	
CD4	Lymphoma	
GPA7	Melanoma	TCR-like antibody GPA7 can guide NK cells to target intracellular antigen gp100 and enhance anti-melanoma activity
CD138	Multiple Myeloma	It acts as a receptor for the extracellular matrix through its extracellular domain, mediating MM development and proliferation
CS1	Multiple Myeloma	CS1 is highly, and nearly ubiquitously, expressed on MM cells, while expression remains very low on NK cells, some T-cell subsets, and normal B cells, and almost undetectable on myeloid cells and the maiority of healthy tissues
BCMA	Multiple Myeloma	B cell maturation antigen, highly expressed in multiple myeloma cells
GD2	Neuroblastoma	
B7-H3	Neuroblastoma	
aFR	Ovarian cancer	Folate receptor alpha (αFR) is overexpressed in 90% of ovarian cancers
HER-2	Ovarian cancer	
Mesothelin	Ovarian cancer	Highly expressed in ovarian cancers
GPC3	Ovarian cancer	
Mesothelin	Pancreatic cancer	
ROBO1	Pancreatic cancer	
PSMA	Prostate	Prostate-specific membrane antigen (PSMA), which is overexpressed in prostatic neoplastic cells
HER-2	Renal cell carcinoma (RCC)	
EGFR	Renal cell carcinoma (RCC)	
PSCA	Ladder carcinoma	Prostate stem cell antigen, upregulate in the majority of prostate cancers
HLA-G	Kidney renal clear cell carcinoma, Kidney renal papillary cell carcinoma, Pancreatic ductal adenocarcinoma, Thyroid cancer	
CD20	Lymphoma, Leukemia cells	CD20 is a protein that is expressed on the surface of B cells, starting at the pre-B cell stage and on mature B cells in the bone marrow and in the periphery
NKG2D	Osteosarcoma	