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Review

Chimeric antigen receptor (CAR)-modified NK cells against cancer: Opportunities and challenges

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ABSTRACT

NK cells may have great potential in tumor immunotherapy because they can kill tumor cells directly and quickly. Chimeric antigen receptor is a fusion protein composed of extracellular antigen recognition domain, transmembrane domain and intracellular signal domain. Rapid development of CAR-modified T cells has made tremendous achievements in the treatment of malignancies, especially hematological malignancies. However, there are many deficiencies in clinical application of CAR-T cell therapy. Car-modified NK cells have attracted much attention because they may avoid these shortcomings. At present, preclinical and clinical studies have shown that CAR-NK cell therapy may play significant anti-tumor role and it is safer than CAR-T cell therapy. Nevertheless, CAR-NK cell therapy still faces some challenges, such as the expansion and activation of primary NK cells in vitro, the difficulty to store and ship NK cell products and the low transduction efficiency. Thus further research is still needed to optimize CAR-NK cell therapy. Building better CAR-NK cells is important to improve the treatment efficacy and combination therapy offers a novel direction of NK-cell based immunotherapy.

1. Introduction

Natural killer (NK) cells, which were discovered over 40 years ago [1], are large granular lymphocytes circulating in most tissues, specifically clearing target cells which have been viral-infected or malignantly transformed [2,3]. NK cells can kill tumor cells quickly and directly because they can function without the need to recognize tumor-specific antigen, thus they have great potential in cancer immunotherapy [3,4]. Various NK cell receptors have been identified. They bind to homologous ligands on target tumor cells, enabling NK cells to distinguish between normal cells and transformed cells [4]. The activating NK cell receptors include members of natural cytotoxicity receptor (NCR) family (NKp30, NKp44 and NKp46), the C-type lectin family receptors (NKG2D, CD94/NKG2F, CD94/NKG2E, CD94/NKG2C and CD161),activating killer immunoglobulin receptors (KIR2DS1, KIR2DS4 and KIR2DL4), FcγRc IIIA (CD16) and costimulatory receptor DNAM-1 (CD226) [5]. KIRs are NK cell inhibitory receptors that can inhibit NK cell-mediated lysis of normal cells in organisms expressing MHC class I molecules. According to the hypothesis of “missing-self”, NK cell activation occurs in contact with malignantly transformed cells that have lost MHC class I molecules and easily lysed. Nevertheless, if targeted cells express a large number of NK stimulating ligands, NK cells can be activated without loss of MHC class I molecules [4]. KIR-ligand mismatched NK cell therapy is a promising approach against cancer via therapeutic anti-KIR monoclonal antibodies (mAbs) directed against inhibitory KIRs. These mAbs are undergoing phase II clinical trials in patients with acute lymphoblastic leukemia or multiple myeloma [6]. They block KIRs in a stable manner and cause NK-regulated cancer cell death by direct or ADCC cytotoxic mechanisms [6]. It can be imagined that the adoptive transfer of large amounts of anti-KIR-treated autologous cells may be beneficial to hematological neoplasms and even some solid tumors [5].

Several studies have shown that low activity of NK cells in peripheral blood is related to high cancer risk, suggesting a role for NK cells against cancer [7,8]. NK cells in human peripheral blood are divided into two major subgroups: $CD56^{bright}$ and $CD56^{dim}$ NK cells. $CD56^{bright}$ NK cells are usually known as cytokine-producing cells with low cytotoxicity, while $CD56^{dim}$ NK cells are known for potential cytotoxicity [9]. Since NK cells can recognize and lyse tumor cells, immunotherapy based on NK cells has been developed.

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The chimeric antigen receptor (CAR) is a fusion protein composed of an extracellular antigen recognition domain, a transmembrane region and intracellular signaling domains. As the name suggests, extracellular antigen recognition domain, which is usually a single-chain variable fragment (scFv), can recognize the specific antigen on tumor cells. The intracellular signaling domains, such as CD28, 4-1BB (CD137) and OX40, are usually designed to improve the activation and the killing effect of T cells. CAR-modified T cells can directly recognize the tumor-associated antigen (TAA) and then kill tumor cells. CAR-T cell therapy has achieved great success in hematological tumors, such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL) and lymphoma. Notably, CD19 CAR-T therapy has been reported to show complete remission (CR) rates of 90% in both children and adults who are suffering with ALL [10]. Although CAR-T cell therapy has developed rapidly, it still has some problems in clinical application. CAR-T cell therapy is not very effective in the treatment of solid tumors [11,12]. Also, most CAR-T cell therapies require autologous adoptive cell transfer because allogeneic T cells may cause graft-versus-host-disease (GVHD) unless addressing HLA barriers [13,14]. Moreover, CAR-T cell therapy may cause side effects which may threaten patients' lives, such as cytokine release syndrome (CRS). CAR-modified NK cells have been shown to overcome the above shortcomings of CAR-T cells and exert significant anti-tumor effect [15,16]. In this review, we will mainly discuss the opportunities provided by CAR-modified NK cells and the challenges faced by CAR-NK cells.

2. Sources and manufacture of NK cells for CAR-NK cell therapy

Autologous or allogeneic PBMC-derived NK cells, which can be easily collected, are usually used to prepare for adoptive transfer of NK cells. Stem cells, including umbilical cord blood (UCB), human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are also abundant sources of NK cells. In addition to donor-derived NK cells, NK cell lines are also important cell sources for CAR-NK cell therapy. The human NK cell line NK-92 shows remarkable cytotoxic effects against malignant cells in preclinical studies and clinical studies have shown that infusions of NK-92 cells are safe in cancer patients [17,18]. A recent study has indicated that human NK cell line KHYG-1 carrying CAR induces anti-tumor effect in glioblastoma (GBM) cell lines, proving that KHYG-1 may be a promising cell line option for CAR-NK therapy [19].

After obtaining them in above ways, NK cells need to be purified and modified to express a chimeric antigen receptor (CAR) to recognize specific antigens on tumor cells. The final donor-derived NK cell products show diverse purity because of different protocols. They might include contaminating T cells, B cells and monocytes. Although residual monocytes may be beneficial for treatment, residual B cells may cause lymphoma due to reactivation of Epstein-Barr virus and T cells must be completely removed to prevent GVHD [20]. The structure of CAR has gone through three generations. The first generation of CAR, which has poor effect, contains only an antigen-binding domain and a transmembrane domain, while the second generation of CAR and the third generation add one costimulatory domain or two costimulatory domains based on the first generation of CAR, respectively. NK cells, which have been purified and modified to express a CAR, are then infused into tumor patients where they can specifically recognize and lyse tumor cells.

3. Advantages of CAR-NK cell therapy

Although CAR-T cell therapy has achieved great success in the treatment of malignancies, there remain many deficiencies in CAR-T cell therapy. CAR-NK cell therapy is expected to remedy some of these deficiencies.

Cytokine release syndrome (CRS), which is a systemic inflammatory response, is associated with the activation and proliferation of CAR-T cells and is one of the serious side effects of CAR-T cell therapy [21]. The development of CAR-NK cell therapy provides the possibility of reducing this risk. CAR-T cells in vivo expansion and persistence can produce proinflammatory cytokines, such as TNFα, interleukin-1 (IL-1) and interleukin-6 (IL-6). The cytokine-production of NK cells are considered to be safer because they are mainly composed of IFN-γ and GM-CSF. In addition, CAR-NK cells infused into patients will not expand within a few weeks, thus reducing the possibility of CRS. Also, the long-term existence of CAR-T cells may lead to the risk of autoimmunity or malignant transformation, while NK cells can disappear quickly after mediating their anti-tumor effects. Although some cancer patients were treated with allogeneic CAR-T cells [22,23], most clinical studies use autologous T cells because allogeneic T cells may cause GVHD. Several clinical studies have shown that NK cells do not cause GVHD [24–26], which offer opportunities to provide off-the-shelf allogeneic products. However, there are also studies reported that NK cells may cause acute GVHD. There may be many reasons accounting for this difference [27]. First, this study used NK cells activated by IL15/4-1BBL, different from those resting NK cells or activated by IL-2, which may lead to different clinical effects. Secondly, many previous studies used MHC-mismatched NK cells to transfer to hosts that had not under allogeneic HSCT. In this case, NK cells are likely to be quickly rejected and may not expand as much as might occur in this experiment. Thirdly, previous studies used adoptive NK transfer combined with rhIL-2, possibly having a protective effect on GVHD due to expansion of regulatory populations.

4. Current status of CAR-NK cell therapy

4.1. Hematological malignancies

Preclinical studies have shown that CD19-CAR NK cells have striking efficacy against hematological malignancies and are easy to produce, which is a great progress compared to current CAR-T cell therapy [28,29]. Clinical trials of CD19-CAR T cell therapy have shown high complete responses in hematological malignancies patients [30,31]. CD19-CAR engineered NK cells are expected to exert better anti-tumor effect due to the advantages of CAR-NK cell therapy in hematological malignancies. Clinical studies have indicated that CD19-CAR modified NK cells may be a good treatment option for patients suffering from lymphoid malignancies [32]. In addition to CD19, CAR-NK cell clinical trials for lymphoma and leukemia also target CD7 (NCT02742727) and CD33 (NCT02944162). Although CAR-T cell therapy has undergone a large number of clinical trials for hematological malignancies, only several clinical CAR-NK cell therapies against

Table 1
Clinical trials of CAR-NK cell therapy against hematological malignancies.

Target	Indications	NK cells	Intracellular signaling molecules	Ref.
CD19	Leukemia and lymphoma	NK-92	CD28, 4-1BB, CD3ζ	NCT02892695
CD19	B-cell acute lymphoblastic leukemia	PBMC-derived NK cells	$CD8\alpha_{TM}$, 4-1BB, CD3ζ	NCT01974479
CD19	B-cell acute lymphoblastic leukemia	PBMC-derived NK cells	$CD8\alpha_{TM}$, 4-1BB, CD3ζ	NCT00995137
CD19	B-lymphoid malignancies	Cord blood-derived NK cells	CD28, CD3ζ, iCasp9	NCT03056339
CD7	Leukemia and lymphoma	NK-92	CD28, 4-1BB, CD3ζ	NCT02742727
CD33	CD33 + acute myeloid leukemia	NK-92	CD28, 4-1BB, CD3ζ	NCT02944162

hematological malignancies are under way (Table 1).

4.2. Solid tumors

There are also preclinical studies using CAR-NK cells for solid tumors, including glioblastoma [19,33], breast cancer [34], neuroblastoma [35], etc. The clinical trial (NCT02839954) is the first to prove that CAR-NK cells are effective in patients with solid tumors. In this trial, dual-specific NK92 cells expressing both a CAR specific for Mucin1 and a CAR specific for PD-1 were produced and the CARs contain CD27 and 4-1BB (CD137) as signaling domains. By August 2017, 10 cancer patients including lung cancer, pancreatic cancer, colon cancer and ovarian cancer have been enrolled in the treatment group. No serious adverse reactions, including cytokine storm and bone marrow suppression, were observed in these ten patients, suggesting that this treatment is safe and well tolerated.

5. Challenges faced by CAR-NK cell therapy

The uncertainty about their ability to migrate and penetrate tumor tissues is the main reason of initial reluctance to use NK cells in CAR-engineered therapy [36]. The limited time of NK cells in vivo is also a reason why not use CAR on NK cells initially [37]. While this is an advantage for safety, it will limit the efficacy of therapy. At present, the application of CAR-NK cell therapy still faces some challenges.

5.1. Expansion and activation of primary NK cells

The primary challenge of CAR-NK cell therapy is the expansion of primary NK cells in vitro. The number of NK cells from a single-donor is not enough for therapy, making the expansion and activation of NK cells very essential [20]. This process usually takes 2–3 weeks to culture NK cells in the presence of cytokines (IL-2 or in combination with IL-15 or anti-CD3 mAb) [38]. The combination of IL-2 and IL-21 were also used to make NK cells expand [39,40]. The results showed that the combination of IL-2 and IL-21 has more significant inhibition on tumor growth than using IL-2 alone [39,40]. Although irradiated K562-mb15-4-1BBL cells is useful as feeder cells in the process of the clinical-grade primary NK cells expansion, the variability of donor cell number remains a problem [41]. In addition, T cells must be completely removed before cell expansion to prevent GVHD. Since K562 is a cancer cell line, it is necessary to ensure that they are completely removed before infusion.

Expansion of NK cell can be carried out in multiple containers or bioreactors, including Teflon bags, flasks, continuous-flow devices, stirred-tank bioreactors and the Miltenyi's Prodigy system. Stirred bioreactors that allow continuous production may be the best option for expanding cells, because costs can be controlled and cells can be expanded in large quantities with less culture medium in this way [20]. After being expanded, the cells should be cryopreserved for treating tumor patients in need.

Obtaining enough cells is very important for the treatment of patients. However, due to the technical limitations of expanding a large number of cells, it is difficult to perform a dose escalation with cells for patients.

5.2. Storage and shipping of NK cell products

In order to ensure the rationality of the price of cell therapy, it is expected that cells can be produced in centralized facilities, stored through cryopreservation, shipped to the treatment site and thawed at the treatment site. Nevertheless, compared with T cells and many other human cells, NK cells are more sensitive to freezing and thawing, especially when they have been activated by cytokines. In addition to the variable survival rate during thawing, the cytotoxicity of NK cells decreased significantly after thawing [42]. Also, cytokine-activated NK cells are very sensitive to lower temperatures [43]. Because of the temperature sensitivity of NK cells, they need to be shipped at around body temperature to maintain cytotoxicity. Appropriate cell density is also important in the process of shipping. High cell concentrations may lead to the loss of cells activity possibly due to rapid utilization of medium and changes in glucose and pH. Moreover, the quality of cells is difficult to control in the shipping process.

The sensitivity of NK cells to cryopreservation makes it difficult to store and transport, which is a limitation of CAR-NK cell therapy.

5.3. CAR transduction into NK cells

It is also important to select the appropriate method to transfer CAR into NK cells. Both viral vectors and non-viral vectors have been used to introduce CARs.

Viral vectors, including viral vectors and non-viral vectors, are widely used as transduction vectors in the treatment of CAR-NK cell therapy as they can stably integrate into the genome. Although the transfection efficacy of retroviral vectors is high, it may lead to insertional mutagenesis, oncogenesis and other adverse events [44]. Lentiviral vectors have lower levels of insertion mutagenesis, however, their transfection efficiency is under 20% for NK cells derived from peripheral blood [38]. The transfection efficiency of lentivirus vectors is sufficient for NK cells derived from cord blood [45]. Nevertheless, the lentiviral transfection efficiency of NK cells derived from peripheral blood needs further improvement. Studies have shown that inhibiting intracellular antiviral defense mechanisms may enhance lentivirus transduction of NK cells, providing a practical and safe method for CAR transduction into NK cells [46].

The high risk and cost of viral vectors for clinical application have raised interest on non-viral vectors. Sleeping Beauty (SB) transposon vectors have provided a safe, effective and economically method to integrate genetic information through gene transfer vectors and they may overcome shortcomings of viral vectors [47,48]. SB transposon system has been successfully applied to CAR-T cell therapy. Both preclinical and clinical studies have shown that CAR-T cells generated with SB transposon system are safe and effective, supporting further development of this nonviral transfection method [49,50]. Nevertheless, the applicability of SB transposon system on the transduction of CAR to primary NK cells still untested because of the low transduction efficiency and serious cytotoxicity of electroporation DNA vectors to primary NK cells [44].

Transduction with mRNA for CAR-NK cells mediated killing cancer cells has also been considered to be a safe and affordable transduction option. A previous study has shown that median receptor expression 24 h after electroporation with the corresponding mRNA was 82.0% and NK cells transfected under such conditions had significant cytotoxicity in xenograft cancer model [51]. Recently, a study indicated that “on-target off-tumor” toxicity, which is a critical factor limiting the application of CAR-based immunotherapies, may be effectively avoided by transduction with mRNA [52]. Nevertheless, the anti-tumor effect of CAR-NK cells transferred by mRNA electroporation may be transient because the expression of CARs transferred in this way is no more than 3 days [53].

6. Future considerations

6.1. Building better CAR-NK cells

CAR-modified NK cells have been proved to have significant anti-tumor effects, but the building of CAR-NK cells still needs to be further explored. The aim of CAR-NK cells is to build a novel activation pathway to improve anti-tumor effects of the cells and to enhance cancer cell targeting. It is critical to examine which NK cell lines and which type of CAR structure can build better CAR-NK cells. NK-92 cells are highly cytotoxic to many kinds of cancer cells and are the most

widely used cell lines in clinic. Also, studies have shown that NK92 cell lines are safe for cancer patients [17,18].

CAR-NK cells have the basic framework including a tumor associated antigen-binding domain, a transmembrane region and an intracellular signal domain. NKG2D, which is an important activating receptor on NK cells and cytotoxic T lymphocytes, can bind to DAP10 or DAP12 (KARAP) transfer proteins to provide activation signals which can activate the cytotoxicity of NK cells. Moreover, the activation signal transmitted by DAP12 can promote NK cells to produce cytokines [54]. Preclinical study has shown that the receptor (CAR-NKG2D-DAP10-CD3ζ receptor) composed of NKG2D, DAP10 and CD3ζ can significantly enhance the cytotoxicity of NK cells towards tumors [55]. CD244 (2B4), which is a signaling lymphocyte activation molecule-related receptor, regulate potent stimulatory and costimulatory signals in NK cells and may have the potential to boost signaling in NK cells retargeted to tumor cells [56,57]. Studies have shown that CD244 has robust costimulatory roles in NK effector cells targeting CD19 or GD2, indicating that antigen-specific CD244-ζ-expressing NK cells may have great potential for adoptive immunotherapy against malignancies [57,58].

6.2. Combination therapy

Although CAR-NK cell therapy has been proved to be effectively against tumor, the long-term anti-tumor efficiency is still modest. The combination therapy offers a novel direction of NK-cell based immunotherapy. Studies have indicated that treatments that target the immune environment may be beneficial to CAR-NK cell therapy. A recent study has shown that the addition of interleukin-15 (IL-15) can lead to productive IL-15 signaling and can significantly increase cytotoxicity of tumor cells [59]. As the high expression of ligands for co-inhibitory receptors on tumor cells is a critical factor limiting CAR-NK cell function, the silencing of NK cell inhibitory receptors may be beneficial to improve the CAR-NK cell efficiency [60]. In addition, chemotherapy may also help to enhance the efficiency of CAR-NK cell therapy. Chemotherapy can not only clear the resident cell populations to create new niches for the expansion of NK cells, but also can induce genotoxic stress to enhance the cancer cell sensitivity to NK cells [61]. Clinical studies have shown that the chemotherapeutic agent can significantly improve the tumor-killing effect of CAR-NK cells [62].

Furthermore, there are many strategies which have been shown to be useful in the augment of anti-tumor efficiency of CAR-T cell therapy. Similarly, some of these strategies may also be beneficial in the CAR-NK cell therapy, such as radiotherapy and the CRISPR/Cas9 system. Radiotherapy is a common method in the treatment of malignant tumors, which exerts an anti-tumor effect by killing cancer cells directly and inducing tumor-specific immune responses. Preclinical studies have shown that the combination of CAR-T cell therapy and radiotherapy exerts a synergistic efficacy against tumor [63]. Nevertheless, the effects of radiation on NK cells remain unclear [64]. Therefore, further studies are still required to better understand the links between these two therapies. The CRISPR/Cas9 system has become an increasingly popular genetic engineering tool because of its advantages in editing the genomes of multiple organisms precisely [65]. Evidence has shown that targeting a CAR to the T-cell receptor α constant (TRAC) locus using CRISPR/Cas9 system can lead to uniform CAR expression and boost T-cell potency [66]. Similarly, CRISPR/CAS9 system may have the potential to effectively improve the efficiency and safety of CAR-NK cells by gene editing of primary NK cells and producing stably transduced NK cells.

Declaration of Competing Interest

No conflict of interest exists.

References

[1] R. Kiessling, E. Klein, H. Wigzell, “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype, Eur. J. Immunol. 5 (2) (1975) 112–117, https://doi.org/10.1002/eji.1830050208 Feb.

[2] Michael A. Caligiuri, Human natural killer cells, Blood 112 (3) (2008 Aug 1) 461–469, https://doi.org/10.1182/blood-2007-09-077438.

[3] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, S. Ugolini, Functions of natural killer cells, Nat. Immunol. 9 (5) (2008) 503–510, https://doi.org/10.1038/ni1582 May.

[4] S.S. Farag, M.A. Caligiuri, Human natural killer cell development and biology, Blood Rev. 20 (3) (2006) 123–137 May.

[5] G. Konjević, A. Vuletić, K. Mirjačić Martinović, Natural killer cell receptors: alterations and therapeutic targeting in malignancies, Immunol. Res. 64 (1) (2016) 25–35, https://doi.org/10.1007/s12026-015-8695-4 Feb.

[6] D.M. Benson Jr., M.A. Caligiuri, Killer immunoglobulin-like receptors and tumor immunity, Cancer Immunol. Res. 2 (2) (2014) 99–104 Feb.

[7] K. Imai, S. Matsuyama, S. Miyake, K. Suga, K. Nakachi, Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population, Lancet 356 (9244) (2000) 1795–1799, https://doi.org/10.1016/S0140-6736(00)03231-1 Nov 25.

[8] Orange JS1, Ballas ZK. Natural killer cells in human health and disease. Clin. Immunol.. 2006 Jan;118(1):1–10. doi: https://doi.org/10.1016/j.clim.2005.10.011.

[9] N.K. Björkström, H.G. Ljunggren, J. Michaëlsson, Emerging insights into natural killer cells in human peripheral tissues, Nat. Rev. Immunol. 16 (5) (2016) 310–320, https://doi.org/10.1038/nri.2016.34 Apr 28.

[10] S.L. Maude, N. Frey, P.A. Shaw, R. Aplenc, D.M. Barrett, N.J. Bunin, et al., Chimeric antigen receptor T cells for sustained remissions in leukemia, N. Engl. J. Med. 371 (16) (2014) 1507–1517, https://doi.org/10.1056/NEJMoa1407222.

[11] K. Feng, Y. Guo, H. Dai, Y. Wang, X. Li, H. Jia, W. Han, Chimeric antigen receptor-modified T cells for the immunotherapy of patients with EGFR-expressing advanced relapsed/refractory non-small cell lung cancer, Sci. China Life Sci. 59 (5) (2016 May) 468–479, https://doi.org/10.1007/s11427-016-5023-8.

[12] D.E. Gilham, R. Debets, M. Pule, R.E. Hawkins, H. Abken, CAR-T cells and solid tumors: tuning T cells to challenge an inveterate foe, Trends Mol. Med. 18 (7) (2012 Jul) 377–384, https://doi.org/10.1016/j.molmed.2012.04.009.

[13] M. Kalaitsidou, G. Kueberuwa, A. Schütt, D.E. Gilham, CAR T-cell therapy: toxicity and the relevance of preclinical models, Immunotherapy 7 (5) (2015) 487–497, https://doi.org/10.2217/imt.14.123.

[14] W. Qasim, C.A.R. Allogeneic, T cell therapies for leukemia, Am. J. Hematol. 94 (S1) (2019) S50–S54, https://doi.org/10.1002/ajh.25399 May.

[15] C. Zhang, P. Oberoi, S. Oelsner, A. Waldmann, A. Lindner, T. Tonn, W.S. Wels, Chimeric antigen receptor-engineered NK-92 cells: an off-the-shelf cellular therapeutic for targeted elimination of cancer cells and induction of protective anti-tumor immunity, Front. Immunol. 8 (2017) 533, https://doi.org/10.3389/fimmu.2017.00533 May 18.

[16] Boissel L, Betancur-Boissel M, Lu W, Krause DS, Van Etten RA, Wels WS, Klingemann H. Retargeting NK-92 cells by means of CD19- and CD20-specific chimeric antigen receptors compares favorably with antibody-dependent cellular cytotoxicity. Oncoimmunology. 2013 Oct 1;2(10):e26527.

[17] G. Suck, M. Odendahl, P. Nowakowska, C. Seidl, W.S. Wels, H.G. Klingemann, T. Tonn, NK-92: an ‘off-the-shelf therapeutic’ for adoptive natural killer cell-based cancer immunotherapy, Cancer Immunol. Immunother. 65 (4) (2016 Apr) 485–492, https://doi.org/10.1007/s00262-015-1761-x.

[18] T. Tonn, D. Schwabe, H.G. Klingemann, S. Becker, R. Esser, U. Koehl, M. Suttorp, E. Seifried, O.G. Ottmann, G. Bug, Treatment of patients with advanced cancer with the natural killer cell line NK-92, Cytotherapy 15 (12) (2013 Dec) 1563–1570, https://doi.org/10.1016/j.jcyt.2013.06.017.

[19] T. Murakami, T. Nakazawa, A. Natsume, F. Nishimura, M. Nakamura, R. Matsuda, K. Omoto, Y. Tanaka, Y. Shida, Y.S. Park, Y. Motoyama, I. Nakagawa, S. Yamada, K. Tamura, Y. Takeshima, Y. Takamura, T. Wakabayashi, H. Nakase, Novel human NK cell line carrying CAR targeting EGFRvIII induces antitumor effects in glioblastoma cells, Anticancer Res. 38 (9) (2018) 5049–5056, https://doi.org/10.21873/anticanres.12824 Sep.

[20] H. Klingemann, Challenges of cancer therapy with natural killer cells, Cytotherapy 17 (3) (2015 Mar) 245–249, https://doi.org/10.1016/j.jcyt.2014.09.007.

[21] X.J. Xu, Y.M. Tang, Cytokine release syndrome in cancer immunotherapy with chimeric antigen receptor engineered T cells, Cancer Lett. 343 (2) (2014) 172–178, https://doi.org/10.1016/j.canlet.2013.10.004 Feb 28.

[22] P. Kebriaei, H. Singh, M.H. Huls, M.J. Figliola, R. Bassett, S. Olivares, B. Jena, M.J. Dawson, P.R. Kumaresan, S. Su, S. Maiti, J. Dai, B. Moriarity, M.A. Forget, V. Senyukov, A. Orozco, T. Liu, J. McCarty, R.N. Jackson, J.S. Moyes, G. Rondon, M. Qazilbash, S. Ciurea, A. Alousi, Y. Nieto, K. Rezvani, D. Marin, U. Popat, C. Hosing, E.J. Shpall, H. Kantarjian, M. Keating, W. Wierda, K.A. Do, D.A. Largaespada, D.A. Lee, P.B. Hackett, R.E. Champlin, L.J. Cooper, Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells, J. Clin. Invest. 126 (9) (2016) 3363–3376, https://doi.org/10.1172/JCI86721 Sep 1.

[23] A. Ghosh, M. Smith, S.E. James, M.L. Davila, E. Velardi, K.V. Argyropoulos, G. Gunset, F. Perna, F.M. Kreines, E.R. Levy, S. Lieberman, H.V. Jay, A.Z. Tuckett, J.L. Zakrzewski, L. Tan, L.F. Young, K. Takvorian, J.A. Dudakov, R.R. Jenq, A.M. Hanash, A.C. Motta, G.F. Murphy, C. Liu, A. Schietinger, M. Sadelain, M.R. van den Brink, Donor CD19 CAR T cells exert potent graft-versus-lymphoma activity with diminished graft-versus-host activity, Nat. Med. 23 (2) (2017 Feb) 242–249, https://doi.org/10.1038/nm.4258.

[24] J.R. Passweg, A. Tichelli, S. Meyer-Monard, D. Heim, M. Stern, T. Kühne, G. Favre, A. Gratwohl, Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation, Leukemia 18 (11) (2004) 1835–1838, https://doi.org/10.1038/sj.leu.2403524 Nov.
[25] J.A. Olson, D.B. Leveson-Gower, S. Gill, J. Baker, A. Beilhack, Negrin RS. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects, Blood 115 (21) (2010) 4293–4301, https://doi.org/10.1182/blood-2009-05-222190 May 27.
[26] B.C. Shaffer, J.B. Le Luduec, C. Forlenza, A.A. Jakubowski, M.A. Perales, J.W. Young, K.C. Hsu, Phase II. Study of haploidentical natural killer cell infusion for treatment of relapsed or persistent myeloid malignancies following allogeneic hematopoietic cell transplantation, Biol. Blood Marrow Transplant. 22 (4) (2016 Apr) 705–709, https://doi.org/10.1016/j.bbmt.2015.12.028.
[27] N.N. Shah, K. Baird, C.P. Delbrook, T.A. Fleisher, M.E. Kohler, S. Rampertaap, K. Lemberg, C.K. Hurley, D.E. Kleiner, M.S. Merchant, S. Pittaluga, M. Sabatino, D.F. Stroncek, A.S. Wayne, H. Zhang, T.J. Fry, C.L. Mackall, Acute GVHD in patients receiving IL-15/4-1BBL activated NK cells following T-cell-depleted stem cell transplantation, Blood 125 (5) (2015) 784–792, https://doi.org/10.1182/blood-2014-07-592881 Jan 29.
[28] E. Liu, Y. Tong, G. Dotti, H. Shaim, B. Savoldo, M. Mukherjee, J. Orange, X. Wan, X. Lu, A. Reynolds, M. Gagea, P. Banerjee, R. Cai, M.H. Bdaiwi, R. Basar, M. Muftuoglu, L. Li, D. Marin, W. Wierda, M. Keating, R. Champlin, E. Shpall, K. Rezvani, Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity, Leukemia 32 (2) (2018 Feb) 520–531, https://doi.org/10.1038/leu.2017.226.
[29] S. Oelsner, M.E. Friede, C. Zhang, J. Wagner, S. Badura, P. Bader, E. Ullrich, O.G. Ottmann, H. Klingemann, T. Tonn, W.S. Wels, Continuously expanding CAR NK-92 cells display selective cytotoxicity against B-cell leukemia and lymphoma, Cytotherapy 19 (2) (2017 Feb) 235–249, https://doi.org/10.1016/j.jcyt.2016.10.009.
[30] B.L. Levine, Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells, Cancer Gene Ther. 22 (2) (2015 Mar) 79–84, https://doi.org/10.1038/cgt.2015.5.
[31] J. Weiland, A. Elder, V. Forster, O. Heidenreich, S. Koschmieder, J.C.D. Vormoor, 19: a multifunctional immunological target molecule and its implications for Blineage acute lymphoblastic leukemia, Pediatr. Blood Cancer 62 (7) (2015 Jul) 1144–1148, https://doi.org/10.1002/pbc.25462.
[32] A. Romanski, C. Uherek, G. Bug, E. Seifried, H. Klingemann, W.S. Wels, O.G. Ottmann, T.C.D. Tonn, 19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies, J. Cell. Mol. Med. 20 (7) (2016 Jul) 1287–1294, https://doi.org/10.1111/jcmm.12810.
[33] N. Müller, S. Michen, S. Tietze, K. Töpfer, A. Schulte, K. Lamszus, M. Schmitz, G. Schackert, I. Pastan, A. Temme, Engineering NK cells modified with an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 improves immunotherapy of CXCL12/SDF-1α-secreting glioblastoma, J. Immunother. 38 (5) (2015) 197–210, https://doi.org/10.1097/CJI.0000000000000082 Jun.
[34] C. Sahm, K. Schönfeld, W.S. Wels, Cancer Immunol. Immunother. 61 (9) (2012 Sep) 1451–1461, https://doi.org/10.1007/s00262-012-1212-x.
[35] R. Esser, T. Müller, D. Stefes, S. Kloess, D. Seidel, S.D. Gillies, C. Aperlo-Iffland, J.S. Huston, C. Uherek, K. Schönfeld, T. Tonn, N. Huebener, H.N. Lode, U. Koehl, W.S. Wels, NK cells engineered to express a GD2-specific antigen receptor display built-in ADCC-like activity against tumour cells of neuroectodermal origin, J. Cell. Mol. Med. 16 (3) (2012) 569–581, https://doi.org/10.1111/j.1582-4934.2011.01343.x Mar.
[36] M. Carlsten, R.W. Childs, Genetic manipulation of NK cells for cancer immunotherapy: techniques and clinical implications, Front. Immunol. 6 (2015) 266, https://doi.org/10.3389/fimmu.2015.00266 Jun 10.
[37] K. Rezvani, R. Rouce, E. Liu, E. Shpall, Engineering natural killer cells for cancer immunotherapy, Mol. Ther. 25 (8) (2017) 1769–1781, https://doi.org/10.1016/j.ymthe.2017.06.012 Aug 2.
[38] N. Shimasaki, E. Coustan-Smith, T. Kamiya, D. Campana, Expanded and armed natural killer cells for cancer treatment, Cytotherapy 18 (11) (2016 Nov) 1422–1434, https://doi.org/10.1016/j.jcyt.2016.06.013.
[39] O. Oberschmidt, M. Morgan, V. Huppert, J. Kessler, T. Gardlowski, N. Matthies, K. Aleksandrova, L. Arseniev, A. Schambach, U. Koehl, S. Kloess, Development of automated separation, expansion, and quality control protocols for clinical-scale manufacturing of primary human NK cells and alpharetroviral chimeric antigen receptor engineering, Hum. Gene Ther. Methods (2019), https://doi.org/10.1089/hgtb.2019.039 May 16.
[40] M. Granzin, A. Stojanovic, M. Miller, R. Childs, V. Huppert, A. Cerwenka, Highly efficient IL-21 and feeder cell-driven ex vivo expansion of human NK cells with therapeutic activity in a xenograft mouse model of melanoma, Oncoimmunology 5 (9) (2016) e1219007, , https://doi.org/10.1080/2162402X.2016.1219007.
[41] N. Shimasaki, H. Fujisaki, D. Cho, M. Masselli, T. Lockey, P. Eldridge, W. Leung, D. Campana, A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies, Cytotherapy 14 (7) (2012 Aug) 830–840, https://doi.org/10.3109/14653249.2012.671519.
[42] M.M. van Ostaijen-ten Dam, H.J. Prins, G.H. Boerman, C. Vervat, D. Pende, H. Putter, A. Lankester, M.J. van Tol, J.J. Zwaginga, M.W. Schilham, Preparation of cytokine-activated NK cells for use in adoptive cell therapy in cancer patients: protocol optimization and therapeutic potential, J. Immunother. 39 (2) (2016) 90–100, https://doi.org/10.1097/CJI.0000000000000110 Feb-Mar.
[43] U. Koehl, C. Brehm, S. Huenecke, S.Y. Zimmermann, S. Kloess, M. Bremm, E. Ullrich, J. Soerensen, A. Quaiser, S. Erben, C. Wunram, T. Gardlowski, E. Auth, T. Tonn, C. Seidl, S. Meyer-Monard, M. Stern, J. Passweg, T. Klingebiel, P. Bader, D. Schwabe, R. Esser, Clinical grade purification and expansion of NK cell products for an optimized manufacturing protocol, Front. Oncol. 3 (2013) 118, https://doi.org/10.3389/fonc.2013.00118 May 17.
[44] Y. Hu, Z.G. Tian, C. Zhang, Chimeric antigen receptor (CAR)-transduced natural killer cells in tumor immunotherapy, Acta Pharmacol. Sin. 39 (2) (2018 Feb) 167–176, https://doi.org/10.1038/aps.2017.125.
[45] L. Boissel, M. Betancur, W. Lu, W.S. Wels, T. Marino, R.A. Van Etten, H. Klingemann, Comparison of mRNA and lentiviral based transfection of natural killer cells with chimeric antigen receptors recognizing lymphoid antigens, Leuk. Lymphoma 53 (5) (2012 May) 958–965, https://doi.org/10.3109/10428194.2011.634048.
[46] T. Sutlu, S. Nyström, M. Gilljam, B. Stellan, S.E. Applequist, E. Alici, Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy, Hum. Gene Ther. 23 (10) (2012 Oct) 1090–1100, https://doi.org/10.1089/hum.2012.080.
[47] P. Kebriaei, Z. Izsvák, S.A. Narayanavari, H. Singh, Z. Ivics, Gene therapy with the sleeping beauty transposon system, Trends Genet. 33 (11) (2017 Nov) 852–870, https://doi.org/10.1016/j.tig.2017.08.008.
[48] M. Hudecek, Z. Ivics, Non-viral therapeutic cell engineering with the Sleeping Beauty transposon system, Curr. Opin. Genet. Dev. 52 (2018 Oct) 100–108, https://doi.org/10.1016/j.gde.2018.06.003.
[49] C.F. Magnani, C. Mezzanotte, C. Cappuzzello, M. Bardini, S. Tettamanti, G. Fazio, L.J.N. Cooper, G. Dastoli, G. Cazzaniga, A. Biondi, E. Biagi, Preclinical Efficacy and Safety of CD19CAR cytokine-induced killer cells transfected with sleeping beauty transposon for the treatment of acute lymphoblastic leukemia, Hum. Gene Ther. 29 (5) (2018 May) 602–613, https://doi.org/10.1089/hum.2017.207.
[50] P. Kebriaei, H. Singh, M.H. Huls, M.J. Figliola, R. Bassett, S. Olivares, B. Jena, M.J. Dawson, P.R. Kumaresan, S. Su, S. Maiti, J. Dai, B. Moriarity, M.A. Forget, V. Senyukov, A. Orozco, T. Liu, J. McCarty, R.N. Jackson, J.S. Moyes, G. Rondon, M. Qazilbash, S. Ciurea, A. Alousi, Y. Nieto, K. Rezvani, D. Marin, U. Popat, C. Hosing, E.J. Shpall, H. Kantarjian, M. Keating, W. Wierda, K.A. Do, D.A. Largaespada, D.A. Lee, P.B. Hackett, R.E. Champlin, L.J. Cooper, Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells, J. Clin. Invest. 126 (9) (2016) 3363–3376, https://doi.org/10.1172/JCI86721 Sep 1.
[51] N. Shimasaki, H. Fujisaki, D. Cho, M. Masselli, T. Lockey, P. Eldridge, W. Leung, D. Campana, A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies, Cytotherapy 14 (7) (2012 Aug) 830–840, https://doi.org/10.3109/14653249.2012.671519.
[52] C.F. Hung, X. Xu, L. Li, Y. Ma, Q. Jin, A. Viley, C. Allen, P. Natarajan, R. Shivakumar, M.V. Peshwa, L.A. Emens, Development of anti-human mesothelin-targeted chimeric antigen receptor messenger RNA-transfected peripheral blood lymphocytes for ovarian cancer therapy, Hum. Gene Ther. 29 (5) (2018 May) 614–625, https://doi.org/10.1089/hum.2017.080.
[53] L. Li, L.N. Liu, S. Feller, C. Allen, R. Shivakumar, J. Fratantoni, L.A. Wolfraim, H. Fujisaki, D. Campana, N. Chopas, S. Dzekunov, M. Peshwa, Expression of chimeric antigen receptors in natural killer cells with a regulatory-compliant non-viral method, Cancer Gene Ther. 17 (3) (2010 Mar) 147–154, https://doi.org/10.1038/cgt.2009.61.
[54] S. Gilfillan, E.L. Ho, M. Cella, W.M. Yokoyama, M.N.K.G. Colonna, 2D recruits two distinct adapters to trigger NK cell activation and costimulation, Nat. Immunol. 3 (12) (2002 Dec) 1150–1155.
[55] Y.H. Chang, J. Connolly, N. Shimasaki, K. Mimura, K. Kono, D. Campana, A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells, Cancer Res. 73 (6) (2013) 1777–1786, https://doi.org/10.1158/0008-5472.CAN-12-3558 Mar 15.
[56] K.M. Lee, J.P. Forman, M.E. McNerney, S. Stepp, S. Kuppireddi, D. Guzior, Y.E. Latchman, M.H. Sayegh, H. Yagita, C.K. Park, S.B. Oh, C. Wülfing, J. Schatzle, P.A. Mathew, A.H. Sharpe, V. Kumar, Requirement of homotypic NK-cell interactions through 2B4(CD244)/CD48 in the generation of NK effector functions, Blood 107 (8) (2006) 3181–3188. Apr 15.
[57] J. Zhang, H. Zheng, Y. Diao, Natural killer cells and current applications of chimeric antigen receptor-modified NK-92 cells in tumor immunotherapy, Int. J. Mol. Sci. 20 (2) (2019 Jan 14), https://doi.org/10.3390/ijms20020317 pii: E317.
[58] B. Altvater, S. Landmeier, S. Pscherer, J. Temme, K. Schweer, S. Kailayangiri, D. Campana, H. Juergens, M. Pule, C. Rossig, 2B4 (CD244) signaling by recombinant antigen-specific chimeric receptors costimulates natural killer cell activation to leukemia and neuroblastoma cells, Clin. Cancer Res. 15 (15) (2009) 4857–4866, https://doi.org/10.1158/1078-0432.CCR-08-2810 Aug 1.
[59] D. Sarhan, L. Brandt, M. Felices, K. Guldevall, T. Lenvik, P. Hinderlie, J. Curtsinger, E. Warlick, S.R. Spellman, B.R. Blazar, D.J. Weisdorf, S. Cooley, D.A. Vallera, B. Önfelt, J.S. Miller, 161533 TriKE stimulates NK-cell function to overcome myeloid-derived suppressor cells in MDS, Blood Adv. 2 (12) (2018) 1459–1469, https://doi.org/10.1182/bloodadvances.2017012369 Jun 26.
[60] C. Kellner, A. Günther, A. Humpe, R. Repp, K. Klausz, S. Derer, T. Valerius, M. Ritgen, M. Brüggemann, J.G. van de Winkel, P.W. Parren, M. Kneba, M. Gramatzki, M. Peipp, Enhancing natural killer cell-mediated lysis of lymphoma cells by combining therapeutic antibodies with CD20-specific immunoligands engaging NKG2D or NKp30, Oncoimmunology 5 (1) (2015 Jun 5) e1058459.
[61] J.H. Fine, P. Chen, A. Mesci, D.S. Allan, S. Gasser, D.H. Raulet, J.R. Carlyle, Chemotherapy-induced genotoxic stress promotes sensitivity to natural killer cell cytotoxicity by enabling missing-self recognition, Cancer Res. 70 (18) (2010) 7102–7113, https://doi.org/10.1158/0008-5472.CAN-10-1316 Sep 15.
[62] R. Klapdor, S. Wang, U. Hacker, H. Büning, M. Morgan, T. Dörk, P. Hillemanns, A. Schambach, Improved killing of ovarian cancer stem cells by combining a novel chimeric antigen receptor-based immunotherapy and chemotherapy, Hum. Gene Ther. 28 (10) (2017 Oct) 886–896, https://doi.org/10.1089/hum.2017.168.
[63] T. Weiss, M. Weller, M. Guckenberger, C.L. Sentman, P.N.K.G. Roth, 2D-based CAR

T cells and radiotherapy exert synergistic efficacy in glioblastoma, Cancer Res. 78 (4) (2018) 1031–1043, https://doi.org/10.1158/0008-5472.CAN-17-1788 Feb 15.
[64] S.N. Seyedin, J.E. Schoenhals, D.A. Lee, M.A. Cortez, X. Wang, S. Niknam, C. Tang, D.S. Hong, A. Naing, P. Sharma, J.P. Allison, J.Y. Chang, D.R. Gomez, J.V. Heymach, R.U. Komaki, L.J. Cooper, J.W. Welsh, Strategies for combining immunotherapy with radiation for anticancer therapy, Immunotherapy 7 (9) (2015) 967–980, https://doi.org/10.2217/imt.15.65.
[65] F. Zhang, Y. Wen, X. Guo, CRISPR/Cas9 for genome editing: progress, implications and challenges, Hum. Mol. Genet. 23 (R1) (2014) R40–R46, https://doi.org/10.1093/hmg/ddu125 Sep 15.
[66] J. Eyquem, J. Mansilla-Soto, T. Giavridis, S.J. van der Stegen, M. Hamieh, K.M. Cunanan, A. Odak, M. Gönen, M. Sadelain, Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection, Nature 543 (7643) (2017) 113–117, https://doi.org/10.1038/nature21405 Mar 2.