Translational Oncology 17 (2022) 101342

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

iNKT: A new avenue for CAR-based cancer immunotherapy

Yilin Liu [a], Gang Wang [a,b,c], Dafei Chai [a,b,c], Yuanyuan Dang [a], Junnian Zheng [b,c,*], Huizhong Li [a,b,c,*]

[a] Cancer Institute, Xuzhou Medical University, Xuzhou, Jiangsu 221002, PR China
[b] Center of Clinical Oncology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu 221002, PR China
[c] Jiangsu Center for the Collaboration and Innovation of Cancer Biotherapy, Cancer Institute, Xuzhou Medical University, Xuzhou, Jiangsu 221002, PR China

ARTICLE INFO

Keywords:
Invariant Natural Killer T (iNKT) cells
Chimeric Antigen Receptor (CAR)
Cytokines
Cancer immunotherapy

ABSTRACT

Chimeric antigen receptor (CAR) T cell is a T lymphocyte-based immunotherapy, which achieves great successes in treating blood malignancies and provides new hope to cue advanced cancer patients. Invariant natural killer T (iNKT) cells are a kind of special T lymphocytes characterized by expressing invariant TCR of Vα24Vβ11 to recognize CD1d-presented glycolipid antigens, which bridge innate and adaptive immune responses. iNKT cells themselves show strong anti-tumor effect in tumor models via CD1d-mediated killing of CD1d-positive tumor cells and immunosuppressive TAMs and MDSCs, and are closely related to the prognosis of cancer patients. iNKT cells are not restricted to polymorphic human leukocyte antigen (HLA) and can prevent Graft versus Host Disease (GvHD), which makes it to be an ideal CAR vector for allogeneic therapy. Although CAR-iNKT was developed and verified by several different teams and attracts more and more attentions, many obstacles are still needed to be resolved before obtaining CAR-iNKT therapeutics. In this review, we summarized the current status of clinical application of iNKT cells and the latest achievements of CAR-iNKT cells, which provides new insight in CAR-iNKT development and usages.

Introduction

In recent years, chimeric antigen receptor T cells (CAR-T) had achieved remarkable achievements in the treatment of hematologic malignancies, especially in patients with CD19-positive refractory or relapsed B-ALL with the complete remission (CR) rate of 90% [1–6]. In contrast, treatment of solid tumors by CAR-T are not optimistic [7]. Even though hundreds of experimental researches are exploring to make a breakthrough for solid tumors, no one can prevail over the therapeutic efficiency of CAR-T for CD19-positive B cell acute lymphoblastic leukemia. Various barriers restrict the efficacy of CAR-T cell therapy in solid tumors. Firstly, immunosuppressive tumor microenvironment in solid tumor brings tremendous challenges for CAR-T therapy, such as physical barriers, poor nutrients, metabolic products, immunosuppressive cells and checkpoint inhibitions, etc. [8]. In addition, inefficient T cell trafficking is also an obstacle for CAR-T therapy in solid tumor. Therefore, there is an urgent need to adopt alternative strategies to overcome those limitations and improve the safety, efficacy and applicability of CAR-mediated immunotherapy against a wider range of cancers.

Natural killer T (NKT) cells are kind of non-MHC-restricted T lineage cells that share morphological and functional characteristics with both T cells and NK cells [9]. Invariant nature killer T (iNKT) cells are the main subtype of human NKT cells, expressing Vα−24 and Jα−18 chains in human, which can transform into mature iNKT cells via recognizing glycolipid antigens presented by CD1d molecules [10]. α-GalCer is one of the classic ligands of iNKT cells, which helps to activate iNKT cells to produce a large amount of immunomodulatory factors and tumor killing cytokines, such as perforin and granzymes [11]. In addition, many of the cytokines secreted by iNKT cells have powerful effects on DC maturation and αβT differentiation, linking iNKT cells to adaptive defense [12]. Hence, iNKT cells are considered as a bridge between innate immune system and adaptive immune system (Fig. 1). Most importantly, iNKT cells can kill tumor associated macrophages(TAMs) and abolish the immunosuppressive activity of myeloid derived suppressor cells (MDSCs) in a CD1d-dependent manner [13]. As compared to T cells, iNKT cells express more chemokine receptors CCR1, CCR2, CCR4, CCR5, CCR6 and CXCR3, which may facilitate iNKT cells infiltration into tumor and then recruit other immune effector cells [14]. Recently, more and more researches focused on the application of iNKT cells against cancer.

* Corresponding authors.
E-mail addresses: jnzheng@xzhmu.edu.cn (J. Zheng), lhz@xzhmu.edu.cn (H. Li).

https://doi.org/10.1016/j.tranon.2022.101342
Received 7 January 2022; Accepted 7 January 2022

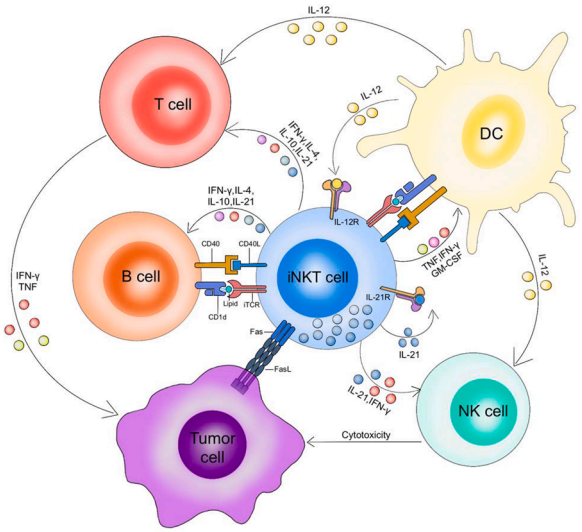

Fig. 1. iNKT cells act as a key player linking innate and adaptive immune responses through direct killing and adjuvant effects.
iNKT cells recognized endogenous and/or exogenous glycolipid antigen in the complex of CD1d molecule, which can immediately release both Th1 and Th2 types of cytokines upon activation. Meanwhile the activated iNKT cells mediate adjuvant effects, which leads to NK cells expansion, DCs maturation, involves cell-cell contacts and soluble factors. IL-12 secreted by DCs conversely stimulate more IFN-γ-producing iNKT cells. Furthermore, iNKT cells can mediate powerful antitumor response through cytolysis or directly kill tumor cells via Fas/FasL pathway.
CD40L, CD40 ligand; GM-CSF, granulocyte-macrophage colony-stimulating-factor; IFN-γ, interferon-γ; IL-12R, IL-12 receptor; IL-21R, IL-21 receptor; iTCR, invariant T cell receptor; TNF, tumor necrosis factor.

Clinical studies have found that the number and proportion of iNKT cells infiltrating in tumors are positively correlated with the overall survival rate of patients [15]. This review mainly focuses on the progress of iNKT and CAR-iNKT in pre-clinical and clinical researches, uncovers their advantages and potential usage in cancer immunotherapy.

Development of iNKT cells

NKT cells develop in the thymus from precursor cells that produce αβ T and γδ T cells [16]. NKT cells follow the growth path of traditional T cells until the positive selection stage is selected by CD1d molecules on the surface of $CD4^+CD8^+$ cortical thymocytes [17]. NKT cells are essentially T cells that differentiate in the thymus and rearrange and express T cell receptor (TCR) gene [18]. For classical NKT cells in mice, Vα14 gene fragment and Jα18 gene fragment was recombined to form NKT cell TCRα chain, and Vβ8.2, Vβ7, Vβ2 co-expression leads to CD1d dependent selection of NKT cells [19]. As NKT cells leave the thymus, majority are non-circulating residents in several tissues for a long time, including the liver, spleen, lungs and lymph nodes [20]. Interestingly, when comparing the content of NKT cells in different mouse tissues, Huh.JY found that the proportion of NKT cells in lymphocytes in adipose tissue was higher, which may be explained that adipocytes regulate the activity of NKT cells through CD1d mediated indirect contact [21]. In humans, NKT cells expressing "invariant" TCRα chain has been widely studied, which are also known as iNKT cells [19]. Recently, Zhu.Y et al. engineered hematopoietic stem cell (HSC) with iNKT TCR to generate HSC-iNKT, which resembles endogenous human iNKT cells, effectively suppressed tumor in vivo and provide the therapeutic potential for future clinical therapy [22].

Antitumoral role of iNKT cells

iNKT cells are restricted by CD1d, a non-polymorphic glycolipid-presenting molecule, expressed on professional APCs and some hepatocyte or endothelial cells. At the same time, there is no obvious upregulation during the maturation of antigen presenting cells, which limits the potential toxicity of iNKT cells in autologous or allogeneic settings [23–25]. Motohashi.S et al. conducted relevant clinical trials on the feasibility and cytotoxicity of patients with advanced or recurrent non-small cell lung cancer after in vitro activation of iNKT cells [26]. Due to the low content of NKT cells in human peripheral blood mononuclear cells, the frequency of NKT cells expanded and in vitro is still low, which requires the development of new methods to meet the number of clinical scale NKT cells [27].

A dose escalation study of α-Galcer proved that there is no dose-limiting toxicity for cancer patients [28]. Given α-Galcer-pulsed dendritic cells (DCs) effectively inhibit mouse liver and lung metastasis by specific activation of NKT [29], Chang.D.H et al. demonstrated that treatment with α-Galcer loaded DCs significantly increased the number of circulating iNKT cells and memory $CD8^+$ T cells in advanced cancer patients without serious adverse events. Preclinical studies have shown that combining α-Galcer with DCs improves the survival rate of iNKT cells in the body [30]. Several phase I clinical trials have used α-GalCer loaded monocyte derived APCs to activate iNKT cells in different types of cancer patients. Nevertheless, iNKT cell mediated cancer immunotherapy has remained challenging that a single dose can produce short-term effects, repeated infusion of iNKT cells may be required before the content is detected in the blood sample [27,31–33].

In order to enhance the activation ability of iNKT, α-GalCer combined with TLR9 stimulation showed enhanced cross-talk between DC and iNKT cells [34]. In addition, Corgnac.S et al. proved that the recombinant CD1d protein has the ability to maintain the response of iNKT cells through multiple stimulation, and the fusion of cancer cells targeting CD1d molecules with anti-tumor scFv can more effectively locate iNKT cells to tumor site [35]. iNKT cells can activate and recruit a variety of anti-tumor effector cells into the tumor microenvironment to enhance the anti-tumor immune responses. A new clinical study developed intranasal administration more effectively activate iNKT cells in the tumor microenvironment [36]. For patients with advanced or recurrent non-small cell lung cancer (NSCLC), 8 of 19 subjects were reaching disease stable [37]. The patients with head and neck squamous cell carcinoma were treated with intranasal injection of iNKT cells combined with α-GalCer pulsed DCs observed that infiltration of iNKT cells into tumor site, which is necessary for anti-tumor immune response [38]. In another phase I/II clinical trial, it has been proved that the injection of iNKT cells could increase IFN-γ-producing cells [39]. According to the different expression of CD4 and CD8, iNKT cells are divided into three subsets, and both secrete Th1 and Th2 cytokines. $CD8\alpha^+$iNKT cells are dominant in Th1 cytolytic activity, while $CD4^+$ iNKT cells release the most Th2 cytokines [40]. Furthermore it is still unclear that how these opposing functional activities are regulated [41]. Relevant reports pointed out that producing more $CD4^-$ iNKT cells is the direction of future iNKT immunotherapy [42], this undoubtedly presents a new challenge to iNKT cell immunotherapy.

iNKT protect from graft-versus-host-disease (GvHD)

In mice, iNKT cell groups include iNKT1, iNKT2, iNKT17 [18,43]. In order to observe the proliferation and migration of iNKT cells after allogeneic gene transplantation, the highly purified $CD4^+$ iNKT cells from C57 mice were injected into BALB/c mice, which were first expanded in lymphoid organs, and then migrated to GvHD target organs, but did not cause dramatic changes in body weight and significant mortality [44]. In addition, Schneidawind.D et al. verified the ability of purified $CD4^+$ iNKT cells to maintain graft-versus-tumor (GvT) effect by constructing two kinds of allogeneic hematopoietic cell transplantation (HCT) models in mice [45]. It has been reported that iNKT1 can down regulate the oxidative phosphorylation of traditional T cells, accelerate the transformation of glycolysis, and cause pro-inflammatory metabolic

state, which may lead to the risk of GvHD [46,47]. However, iNKT cells can modulate GvHD through IL-4 production, and iNKT2 and iNKT17 are potential IL-4 producers. Maas-Bauer.K et al. proved that iNKT2 and iNKT17 have immunomodulatory properties, which can effectively prevent the activation of T cells in vitro and alleviate acute GvHD in mice [48].

Human iNKT cells have not been studied to the same extent as mouse iNKT cells, most of which are differentiated by the expression of CD4 and CD8 [49]. In addition, activated iNKT cells are more likely to be described as T-cell-like through different cytokine secretion characteristics. In view of the effectiveness of donor iNKT cells in controlling aGVHD in immunodeficient mice, iNKT cells may also have similar protective effect in clinical trials. However, Coman.T et al. found that the dosage of $CD4^-$ iNKT cells is an important determinant of the severity of GvHD in the process of HLA-specific allogeneic hematopoietic stem cell transplantation (HSCT) [50]. Some clinical aHSCT experiments have proved that the release of cytokines can accelerate the occurrence of GvHD, including tumor necrosis factor (TNF-α) and IL-1 [51]. $CD4^-$ iNKT cells are different from $CD4^+$ iNKT cells in cytokines and cytotoxicity, the former contains IFN-γ and the preferential release of perforin has the characteristics of Th1 type cytokines [41]. Erkers.T et al. believe that in human iNKT cells, $CD4^+$ iNKT cells are more closely related to the production of IL-4, and the expression of KLRG1 and CD94 better defines the Th1 iNKT and cytotoxic subsets, especially the expression of CD94 is only on CD4 iNKT and will reduce the production of IL-4 [52]. Andrews.K et al. found that CD2/3/28 activated iNKT cells can change the ratio of Th1/Th2 cytokines released by iNKT cells and enhance the cytotoxicity of iNKT cells. In vitro expansion of iNKT cells through IL2 and IL-7 can be preferred expansion of $CD4^+$ iNKT cell subsets [53]. However, what exactly affects and maintains the ratio of $CD4^+$ and $CD4^-$ iNKT cells is currently unclear.

GvHD is the main source of the morbidity and mortality of heterogeneous HSCT, which is also one of the major obstacles restricting allogenic CAR-T for off-the-shelf use. Compared with T cells, iNKT cells are not restricted by MHC but presented by CD1d molecules. INKT cell immunotherapy has been shown to have the ability to prevent GvHD in mouse models. The latest experiments have found that iNKT cells can inhibit allogeneic conventional dendritic cells (cDCs) cells without affecting the presentation of plasmacytoid dendritic cells (pDCs) to microorganisms and viruses [54]. In the process of allogeneic therapy, it can reduce the activation of autologous T cells and the possibility of GvHD. However, this does not mean that immune rejection will be completely avoided. Allogeneic iNKT cells induce autologous T cells to recognize non-self pMHC and produce direct homologous recognition attack. At the same time, the release of non-self peptide after donor cell death is recognized by DCs and may be presented to naive T cells on their own MHC. After recognition stimulation, Cytotoxic T Lymphocyte (CTL) is generated to attack allogeneic cells and produce indirect rejection. To date, extensive pre-clinical and clinical evidence demonstrate an attractive role of iNKT in protecting from GvHD, which supports that iNKT-based immunotherapy could be derived from healthy donor without risk of GvHD. For the allogeneic application, GvHD should be preferred instead of producing stronger cytotoxicity, which is important for the transformation of iNKT cell therapy into anti-tumor immunotherapy and the prevention of GvHD significance.

CAR-modified iNKT cells show potent antitumor efficiency

Clinical trials related to iNKT cells still have problems such as low frequency of iNKT cells and insufficient infiltration in tumor cells [27,42]. At the same time, the majority of tumor cells are CD1d negative or CD1d expression is down regulated in the process of tumor development, which makes iNKT cells unable to directly recognize [55,56]. In fact, due to the lack of direct anti-tumor targets for the entry of iNKT cells into the body, CD1d mediated iNKT cell activation is not enough to maintain its function, and additional stimulation signals are required [57]. Therefore, it is necessary to develop new methods to improve the anti-tumor ability of iNKT cells.

Given the powerful antitumor responses through direct tumor lysis, cytokines modulation of other immune effector cells, tumor infiltration via a variety of chemokines, and inhibition of immunosuppressive cells, iNKT cell is considered as an ideal alternative for CAR strategies (Fig. 2). Autologous CAR-T cells have achieved tremendous advances in treating hematological malignancies [58–60]. Nevertheless, the personalized CAR-T therapy means labor-intensive work and increased production time as well as expensive treatment price [61]. Multiple facilities committed to develop next-generation universal or off-the-shelf CAR cell products, which can be uniformly manufactured from healthy donor and more readily available for patients [62–64]. The critical step toward the generation of universal CAR-T would be deletion endogenous TCR by gene editing. Considering allogenic iNKT cells don't cause GvHD, they provide a promising option in future universal CAR clinical studies.

The basic structure of CAR includes an extracellular antigen recognition domain, which is the single-chain Fragment variant (scFv) derived from an antibody, a transmembrane domain and the intracellular cell activation domain of CD3ζ [65]. At present, second-generation CAR construct incorporating costimulatory domain CD28 or 4–1BB in tandem with CD3ζ is most widely used [66,67]. It is expected that iNKT engineered with CAR can redirect them to desired antigen and further enhance the antitumor efficacy. Further enhancing the persistence and antitumor activity of iNKT can be achieved by providing the best stimulation signal in CAR structure, which is also crucial for the clinical efficacy of iNKT immunotherapy. Costimulatory domain composed of 4–1BB increased the proliferation of iNKT cells and the ability to kill macrophages [68]. CD28 and 4–1BB have different effects on the distribution of cytokines in iNKT cells. 4–1BB tends to release Th1 type cytokines such as IFN-γ and GM-CSF, while CD28 can induce NKT cells to produce more IL-4 and IL-10 [69]. However, there is no direct evidence for the association between costimulatory domain and Th1/2 phenotype. It is worth noting that CAR-iNKT cells containing 4–1BB costimulatory domain did not increase the production of IL-6, which is one of the important cytokines associated with cytokine release syndrome. 4–1BB may provide an effective co-stimulation for optimal expansion and function of iNKT cells [70]. At the same time, experiments have proved that 4–1BB costimulation leads to activation induced cell death (AICD) of CAR-iNKT cells, and found that the activity of death receptor dependent caspase-8 is higher than that containing CD28. In addition, CAR-iNKT co-stimulated by CD28 also showed advantages in anti-apoptotic ability [71].

Heczey et al. verified that CAR-iNKT cells effectively localized to the neuroblastoma site and showed dual antitumor activity against GD2-positive tumor cells and CD1d-positive TAMs. Moreover, they provided the first evidence that compared to CAR-T cells, CAR.GD2-NKT didn't cause GvHD disease using hu-NSG mouse model [69]. Compared with traditional CAR T cells, CAR iNKT cells showed similar cytotoxic effects on melanoma cells [72]. Multiple myeloma(MM) is a plasma cell carcinoma expressing CD1d, which can be recognized by iNKT cells. BCMA.CAR transduced iNKT cells can effectively reorient the killing function of iNKT cells to MM cells. Anti BCMA.CAR immunotherapy combined with iNKT cells has shown good early clinical efficacy, which proved the effectiveness and feasibility of CAR-iNKT in tumor treatment [70,73]. Recent study found that CAR.19-iNKT cells show enhanced dual cytotoxicity against CD1d-expressing lymphoma through targeting both CD19 by CD19.CAR and CD1d by endogenous iTCR respectively. Notably, eradication of relapsed brain lymphoma in CAR.19-iNKT cells-treated mice might be related to inherent chemotactic properties [74]. These findings demonstrate feasibility of CAR-engineered iNKT cells and their potential advantages over conventional T cells (Table 1). Unfortunately, preclinical and clinical studies showed CAR-iNKT cells are required either repeated doses or additional cytokines administration due to their short-term persistence [69]. Therefore, it is critical to optimize CAR strategies to expand its

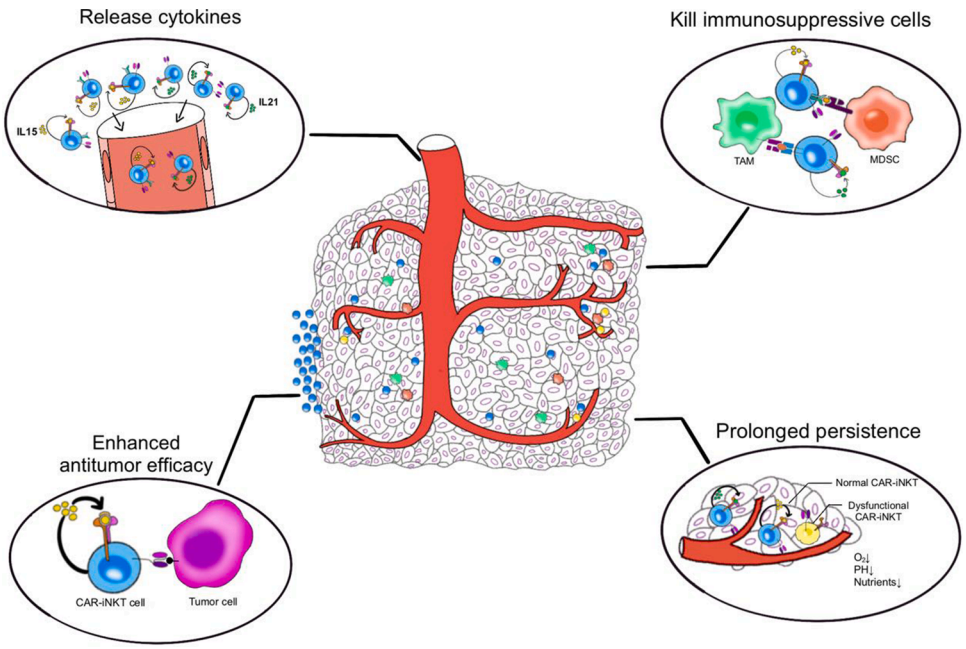

Fig. 2. The role of CAR-iNKT cells in tumor microenvironment.
After engineered with chimeric antigen receptors (CARs), CAR-iNKT cells show specific killing effect against tumor cells expressing specific targets. The evolutionary conserved potential of higher expression of chemokines facilitate CAR-iNKT cells migration to the tumor site. Most importantly, CAR-iNKT could recognize and inhibit CD1d-positive TAMs and MDSC, which promote tumor progression and protect tumors from the attack of immune effectors through buildup immune-suppressive environment. The tumor microenvironment is characterized by hypoxia, acidic conditions and nutrient deficiency. CAR-iNKT cells co-expressing IL-15 or other cytokines have the ability to improve the living environment and prolong the survival of CAR-iNKT cells.

Table 1
Pros and cons of CAR-iNKT cells and CAR-T cells.

	CAR-T	CAR-iNKT
Therapeutic target	Almost unlimited A variety of hematological and solid tumors-related targets	Limited Currently only GD2, CD19, CSPG4 and BCMA
Advantages	Easy access Good Clinical efficacy for hematological malignancies	Naturally traffic to tumor site Modulate immune-suppressive cells Do not mediate GvHD Allogeneic therapeutic potential
Challenges	Long manufacturing time Variability between personalized products Limited success in solid tumors Cytokine release syndrome	Low frequency Limited persistence
Production cost	Currently high	Expected decrease

CAR, Chimeric Antigen Receptor; MHC, Major Histocompatibility Complex; GvHD, Graft-versus-Host Disease.

application and improve longer-term efficacy.

Key cytokines augment CAR-iNKT function

Cytokines are important promoter involved in activation, proliferation, differentiation and immigration for immune effector cells including CAR-based immunotherapy [75,76]. Poor proliferation and short-term persistence of CAR-transduced effector cells leads to tumor relapse after transient remission. Many studies have reported the relationship between STAT5-related cytokines and tumor immunotherapy, such as IL-2, IL-15 [77,78]. Recent preclinical studies proved that STAT3 signaling pathway enhances the efficacy of CAR-T against patients with chronic lymphoblastic leukemia [79,80]. IL-23 can promote the proliferation of memory T cells through activating STAT3 pathway [81,82] . Ma.X et al. found that IL-23 can also provide a strong selective proliferation signal for CAR-T in tumor microenvironment [82].

IL-21

IL-21 is a member of the common γc cytokine family, which play a role in the development of innate and adaptive immune responses to pathogens and tumors [83–85]. IL-21 can activate a variety of immune cells through IL-21 receptor (IL-21R) [86]. IL-21 is mainly produced by $CD4^+$ T cells and iNKT cells. Compared with conventional $CD4^+$ T cells, iNKT cells can produce more IL-21, which can increase the production of perforin to optimize the cytotoxicity of NK cells and selectively enhance DCs to induce iNKT cells to release IFN-γ [87,88]. Previous studies have shown that IL-21 can maintain the function of activated T cells and help $CD8^+$ T cells to infiltrate tumor while effectively expanding NK cells [89]. IL-21 can selectively proliferate Tim-3-negative T cell subsets [90] and inhibit $Foxp3^+$ Treg cells, thus promote the antitumor activity of effector cells [91]. Recently, it has been reported that combination with IL-21 prolongs the efficacy of anti-CTLA-4 or anti-PD-1 therapy in the colon and breast cancer mouse models [92]. Li.Y et al. innovatively fused IL-21 to PD-1 antibody, the new fusion protein showed enhanced memory tumor-reactive T cells responses [93].

Different from T cells in peripheral blood, iNKT cells have no clear division of mature immature memory phenotype. CD62L expression is considered to be one of the indicators of memory iNKT. Preliminary analysis of immune related genes found that the expression level of IL-21R mRNA in $CD62L^+$ iNKT subgroup was significantly higher [94]. IL-21 can selectively inhibit the apoptosis of $CD62L^+$ iNKT by promoting the expression of Bcl-2, which is beneficial to the survival of iNKT cells. At the same time, IL-21 promotes the transformation of iNKT cells into Th1 type cells [95]. Recent studies have reported that IL-21 plays a prominent role in maintaining the central memory phenotype of iNKT and enhancing the anti-tumor function of iNKT cells. Moreover, IL2/IL-21 amplified CAR-iNKT significantly improves the long-term disease-free survival of the treated mice compared with the mice treated with IL-2 amplified cells [96]. IL-21 predominately induce STAT3 activation through binding motif YXXQ within IL-21R to promote memory cell formation and effector cells proliferation. A novel study armed CD19.CAR-T with truncated IL-2Rβ and YXXQ, characterized by antigen-dependent rather than constitutive activation, showed superior antitumor effects and less risk caused by excessive growth potential [79]. In view of the essential role of IL-21 in iNKT differentiation, similar strategy could be developed in CAR-iNKT cells, independent of antigen

specificity.

IL-15

IL-15 plays a critical role in the development and maintenance of memory CD8 T-cells, NK and iNKT cells [97]. Although the mechanism of memory differentiation of human iNKT has not been fully explored, the maintenance of peripheral iNKT largely depends on the steady-state proliferation mediated by IL-15 [98].

Baev.DV et al. demonstrated that $CD4^-$ iNKT cells are more responsive to IL-15 due to higher frequency of IL-15R expression than in $CD4^+$ iNKT cells, which produced more Th1 type cytokines and less Th2 type cytokines. In contrast, $CD4^+$ iNKT cells predominantly respond to IL-7 [99]. In addition, in animal models, $CD4^-$ iNKT cells showed better antitumor efficacy than $CD4^+$ iNKT cells [100]. IL-15 promotes the expression of granzyme, IFN-γ and other effector molecules in iNKT cells [101]. Liu.D et al. pointed out that TBKBP1 is an essential regulator for iNKT survival mediated by IL-15. The loss of TBKBP1 can reduce the expression of Bcl-2, Bcl-xl and other anti-apoptotic molecules. Its selective role in iNKT cells may be explained by a higher expression of TBKBP1 than in T cells [102]. The transgenic IL-15 promoted memory-like differentiation and protect iNKT from hypoxia [103]. Xu et al. reported that iNKT cells engineered with GD2-specific CAR co-expressing IL-15 exerted superior therapeutic efficacy in NB xenografts models, with reduced exhaustion, prolonged in vivo persistence, and enhanced localization to primary metastatic site [71]. Subsequently, they registered the first clinical trial (NCT03294954) to test safety and feasibility of autologous GD2.CAR.15-iNKT to treat children with neuroblastoma. Recent interim results of the first three patients demonstrated CAR-NKT could be expanded to clinical scale and well-tolerated without CRS and neurotoxicity. In addition, CAR-iNKT cells persist throughout 4-week evaluation and mediate bone metastatic lesion regression in one patient [104]. In view of systemic administration of IL-15 in cancer patients showed dose-limiting toxicities of hypotension, thrombocytopenia and elevated transaminase [105]. Therefore, the additional risk from incorporated IL-15 need to be fully evaluated.

IL-7

As above-mentioned, IL-15 show promising antitumor properties by promoting the long-term survival of $CD8^+$ T cells [106]. However, IL-7 play an adjuvant role of potentiating therapeutic efficacy by expanding polyfunctional $CD4^+$ T cells [107]. Whether they have a similar effect on iNKT or CAR-iNKT cells deserves further study. At present, a variety of strategies combination α-Galcer with homeostatic growth factor are being developed to improve antitumor efficacy of iNKT cells. Multiple schemes may lead to selective expansion of differential iNKT subpopulations. Human immature iNKT cells with IL-7 can induces obvious effect memory phenotype and regulate the expression of cytokines [108]. Compared with IL-2 alone, IL-7 further promoted the selective expansion of $CD4^+$ iNKT cells and enhanced Th2-type cytokines production [109]. The activation of IL-7R leads to a stronger signal cascade and enhances the phosphorylation of STAT-5, which may be a signal pathway of IL-7R/STAT5 interaction to further maintain the proliferation of CAR-iNKT cells.

Conclusion and future perspective

So far, numerous pre-clinical studies have confirmed the antitumor efficacy of iNKT cells against various malignancies. Clinical trials using iNKT cells have demonstrated the potential for developing off-the-shelf therapies (Table 2). However, poor expansion obviously hinder the clinical application. With the development of synthetic activators of iNKT cells with more specific and predictable effects, including Th1 or Th2-biased cytokine secretion patterns, this will greatly improve the understanding and clinical application of iNKT cells. However, compared with conventional T cells, our knowledge of iNKT cell differentiation, functionally diverse subsets, cytokine-producing inclination, survival time and metabolism feature still remains limited. Fortunately, modern high-throughput sequence techniques could provide a better understanding their properties at single-cell level.

For solid tumors, the immunosuppressive tumor microenvironment and insufficient expression of chemokine receptors greatly hinder the infiltration and weaken the efficacy of CAR-T cell therapy. iNKT cells can eliminate TAM, with enrichment in many solid tumor and significantly hamper CAR-T functions. These inherent characteristics render CAR-iNKT cells dual cytotoxicity and eliminate both tumor cells via CAR engagement and TAM via native invariant TCR.

Recently, registered clinical trials using autologous or allogenic CAR-iNKT cells are ongoing for patients B cell lymphoma, leukemia and glioblastoma. Clinical experiences with CAR-iNKT cells have demonstrated preliminary clinical effect and well-tolerated, but more clinical data are not available yet. It is hoped that iNKT cells engineered with

Table 2
Preclinical study and clinical trials of CAR-iNKT cells.

NKT cell source	Activation	Expansion	Target	CS	Generation	Cancer	Phase	Ref. or No.NCT
Healthy donors	Auto feeder cells pulsed with α-Galcer	IL-2	GD2	CD28/4–1BB/ CD28+4–1BB	Second/ third	Neuroblastoma	Preclinical	[69]
Healthy donors	Irradiated auto NKT-negative cells pulsed with α-Galcer	IL-2	CD19	4–1BB	Second	B cell lymphoma	Preclinical	[94]
Healthy donors	Anti-CD3 antibody	IL-2	CSPG4	CD28	Second	melanoma	Preclinical	[72]
Healthy donors	Irradiated auto NKT-negative cells pulsed with α-Galcer	IL-2, IL-7 and/ or IL-21	CD19	4–1BB	Second	B cell lymphoma	Preclinical	[96]
Healthy donors	Irradiated auto PBMC pulsed with CD3/28 at 1:1 beads-to-cell	IL-2 and/or IL-15	CD19	CD28/ CD28+OX40	Second/ third	B cell lymphoma	Preclinical	[74]
Healthy donors	Irradiated auto NKT-negative cells pulsed with α-Galcer	IL-2, IL-7 and/ or IL-21	GD2	CD28/4–1BB/ CD28/4–1BB	Second/ fourth	Neuroblastoma	Preclinical	[71]
Healthy donors	Irradiated auto NKT-negative cells pulsed with α-Galcer	IL-2, IL-7 and IL-15	CD38/ BCMA	CD28/4–1BB	Second	Multiple myeloma	Preclinical	[70]
Autologous iNKT			GD2	CD28	Fourth	Neuroblastoma	Phase I Recruiting	NCT03294954
Allogeneic iNKT			CD19	CD28	Fourth	B cell lymphoma	Phase I Recruiting	NCT03774654
Allogeneic iNKT			CD19	4–1BB	Fourth	B cell lymphoma	Phase I Recruiting	NCT04814004

CS, co-stimulatory domain.

more antigen-specific CAR would be evaluated in patients with an increasingly wide variety of cancers. In addition, we speculate combination CAR-iNKT cells therapy with either oncolytic virus armed with cytokines or chemokines, as well as immune checkpoint inhibitors, could further improve therapeutic efficacy through promoting their survival and migration. In conclusion, non-conventional iNKT cells represent as a novel pleiotropic effectors of CAR-based cancer immunotherapy.

CRediT authorship contribution statement

Yilin Liu: Conceptualization, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Visualization. **Gang Wang:** Supervision, Writing – review & editing, Funding acquisition. **Dafei Chai:** Supervision, Writing – review & editing. **Yuanyuan Dang:** Methodology, Investigation, Formal analysis. **Junnian Zheng:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Project administration. **Huizhong Li:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors confirm that there are no potential conflict of interest to disclose.

Acknowledgments

This project is supported by grants from the Natural Science Key Project of Jiangsu Provincial Education Department (Nos. 21KJA320007, 19KJA470001), Jiangsu Province Graduate Student Scientific Research Innovation Project (No. KYCX21_2668), The National Natural Science Foundation of China (Nos. 81871869 and 82072814), Jiangsu Province Social Development Key Projects (Nos. BE2020641, BE2020640), Youth Technology Innovation Team of Xuzhou Medical University (No. TD202003), Jiangsu Provincial Key Medical Discipline, The Project of Invigorating Health Care through Science, Technology and Education (No. ZDXKA2016014) and the Qing Lan Project of Jiangsu Province.

References

[1] J.H. Park, M.B. Geyer, R.J. Brentjens, CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date, Blood 127 (2016) 3312–3320.
[2] C.J. Turtle, L.A. Hanafi, C. Berger, M. Hudecek, B. Pender, E. Robinson, R. Hawkins, C. Chaney, S. Cherian, X. Chen, L. Soma, B. Wood, D. Li, S. Heimfeld, S.R. Riddell, D.G. Maloney, Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells, Sci. Transl. Med. 8 (2016), 355ra116.
[3] H. Dai, Z. Wu, H. Jia, C. Tong, Y. Guo, D. Ti, X. Han, Y. Liu, W. Zhang, C. Wang, Y. Zhang, M. Chen, Q. Yang, Y. Wang, W. Han, Bispecific CAR-T cells targeting both CD19 and CD22 for therapy of adults with relapsed or refractory B cell acute lymphoblastic leukemia, J. Hematol. Oncol. 13 (2020) 30.
[4] D.W. Lee, J.N. Kochenderfer, M. Stetler-Stevenson, Y.K. Cui, C. Delbrook, S. A. Feldman, T.J. Fry, R. Orentas, M. Sabatino, N.N. Shah, S.M. Steinberg, D. Stroncek, N. Tschernia, C. Yuan, H. Zhang, L. Zhang, S.A. Rosenberg, A. S. Wayne, C.L. Mackall, T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial, Lancet 385 (2015) 517–528.
[5] C.J. Turtle, L.A. Hanafi, C. Berger, T.A. Gooley, S. Cherian, M. Hudecek, D. Sommermeyer, K. Melville, B. Pender, T.M. Budiarto, E. Robinson, N. N. Steevens, C. Chaney, L. Soma, X. Chen, C. Yeung, B. Wood, D. Li, J. Cao, S. Heimfeld, M.C. Jensen, S.R. Riddell, D.G. Maloney, CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients, J. Clin. Invest. 126 (2016) 2123–2138.
[6] R.A. Gardner, O. Finney, C. Annesley, H. Brakke, C. Summers, K. Leger, M. Bleakley, C. Brown, S. Mgebroff, K.S. Kelly-Spratt, V. Hoglund, C. Lindgren, A. P. Oron, D. Li, S.R. Riddell, J.R. Park, M.C. Jensen, Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults, Blood 129 (2017) 3322–3331.
[7] Y. Tian, Y. Li, Y. Shao, Y. Zhang, Gene modification strategies for next-generation CAR T cells against solid cancers, J. Hematol. Oncol. 13 (2020) 54.
[8] H. Tang, J. Qiao, Y.X. Fu, Immunotherapy and tumor microenvironment, Cancer Lett. 370 (2016) 85–90.
[9] A. Bendelac, P.B. Savage, L. Teyton, The biology of NKT cells, Annu. Rev. Immunol. 25 (2007) 297–336.
[10] L. Gapin, iNKT cell autoreactivity: what is 'self' and how is it recognized? Nat. Rev. Immunol. 10 (2010) 272–277.
[11] S. Fujii, K. Shimizu, H. Hemmi, R.M. Steinman, Innate Valpha14(+) natural killer T cells mature dendritic cells, leading to strong adaptive immunity, Immunol. Rev. 220 (2007) 183–198.
[12] S.I. Fujii, K. Shimizu, Exploiting Antitumor Immunotherapeutic novel strategies by deciphering the cross talk between invariant NKT cells and dendritic cells, Front. Immunol. 8 (2017) 886.
[13] H.J. Ko, J.M. Lee, Y.J. Kim, Y.S. Kim, K.A. Lee, C.Y. Kang, Immunosuppressive myeloid-derived suppressor cells can be converted into immunogenic APCs with the help of activated NKT cells: an alternative cell-based antitumor vaccine, J. Immunol. 182 (2009) 1818–1828.
[14] C.H. Kim, E.C. Butcher, B. Johnston, Distinct subsets of human Valpha24-invariant NKT cells: cytokine responses and chemokine receptor expression, Trends Immunol. 23 (2002) 516–519.
[15] S.I. Fujii, K. Shimizu, Immune networks and therapeutic targeting of iNKT cells in cancer, Trends Immunol. 40 (2019) 984–997.
[16] L. Gapin, J.L. Matsuda, C.D. Surh, M. Kronenberg, NKT cells derive from double-positive thymocytes that are positively selected by CD1d, Nat. Immunol. 2 (2001) 971–978.
[17] D.I. Godfrey, S.P. Berzins, Control points in NKT-cell development, Nat. Rev. Immunol. 7 (2007) 505–518.
[18] C.M. Crosby, M. Kronenberg, Tissue-specific functions of invariant natural killer T cells, Nat. Rev. Immunol. 18 (2018) 559–574.
[19] D.I. Godfrey, H.R. MacDonald, M. Kronenberg, M.J. Smyth, L. Van Kaer, NKT cells: what's in a name? Nat. Rev. Immunol. 4 (2004) 231–237.
[20] S.Y. Thomas, S.T. Scanlon, K.G. Griewank, M.G. Constantinides, A.K. Savage, K. A. Barr, F. Meng, A.D. Luster, A. Bendelac, PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions, J. Exp. Med. 208 (2011) 1179–1188.
[21] J.Y. Huh, J.I. Kim, Y.J. Park, I.J. Hwang, Y.S. Lee, J.H. Sohn, S.K. Lee, A. A. Alfadda, S.S. Kim, S.H. Choi, D.S. Lee, S.H. Park, R.H. Seong, C.S. Choi, J. B. Kim, A novel function of adipocytes in lipid antigen presentation to iNKT cells, Mol. Cell. Biol. 33 (2013) 328–339.
[22] Y. Zhu, D.J. Smith, Y. Zhou, Y.R. Li, J. Yu, D. Lee, Y.C. Wang, S. Di Biase, X. Wang, C. Hardoy, J. Ku, T. Tsao, L.J. Lin, A.T. Pham, H. Moon, J. McLaughlin, D. Cheng, R.P. Hollis, B. Campo-Fernandez, F. Urbinati, L. Wei, L. Pang, V. Rezek, B. Berent-Maoz, M.H. Macabali, D. Gjertson, X. Wang, Z. Galic, S.G. Kitchen, D. S. An, S. Hu-Lieskovan, P.J. Kaplan-Lefko, S.N. De Oliveira, C.S. Seet, S.M. Larson, S.J. Forman, J.R. Heath, J.A. Zack, G.M. Crooks, C.G. Radu, A. Ribas, D.B. Kohn, O.N. Witte, L. Yang, Development of hematopoietic stem cell-engineered invariant natural killer T cell therapy for cancer, Cell Stem Cell, 25 (2019) 542–557, e549.
[23] L. Brossay, M. Chioda, N. Burdin, Y. Koezuka, G. Casorati, P. Dellabona, M. Kronenberg, CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution, J. Exp. Med. 188 (1998) 1521–1528.
[24] M. Brigl, M.B. Brenner, CD1: antigen presentation and T cell function, Annu. Rev. Immunol. 22 (2004) 817–890.
[25] S. Fujii, K. Shimizu, Y. Okamoto, N. Kunii, T. Nakayama, S. Motohashi, M. Taniguchi, NKT cells as an ideal anti-tumor immunotherapeutic, Front. Immunol. 4 (2013) 409.
[26] S. Motohashi, A. Ishikawa, E. Ishikawa, M. Otsuji, T. Iizasa, H. Hanaoka, N. Shimizu, S. Horiguchi, Y. Okamoto, S. Fujii, M. Taniguchi, T. Fujisawa, T. Nakayama, A phase I study of in vitro expanded natural killer T cells in patients with advanced and recurrent non-small cell lung cancer, Clin. Cancer Res. 12 (2006) 6079–6086.
[27] S. Motohashi, Y. Okamoto, I. Yoshino, T. Nakayama, Anti-tumor immune responses induced by iNKT cell-based immunotherapy for lung cancer and head and neck cancer, Clin. Immunol. 140 (2011) 167–176.
[28] G. Giaccone, C.J. Punt, Y. Ando, R. Ruijter, N. Nishi, M. Peters, B.M. von Blomberg, R.J. Scheper, H.J. van der Vliet, A.J. van den Eertwegh, M. Roelvink, J. Beijnen, H. Zwierzina, H.M. Pinedo, A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors, Clin. Cancer Res. 8 (2002) 3702–3709.
[29] I. Toura, T. Kawano, Y. Akutsu, T. Nakayama, T. Ochiai, M. Taniguchi, Cutting edge: inhibition of experimental tumor metastasis by dendritic cells pulsed with alpha-galactosylceramide, J. Immunol. 163 (1999) 2387–2391.
[30] D.H. Chang, K. Osman, J. Connolly, A. Kukreja, J. Krasovsky, M. Pack, A. Hutchinson, M. Geller, N. Liu, R. Annable, J. Shay, K. Kirchhoff, N. Nishi, Y. Ando, K. Hayashi, H. Hassoun, R.M. Steinman, M.V. Dhodapkar, Sustained expansion of NKT cells and antigen-specific T cells after injection of alpha-galactosyl-ceramide loaded mature dendritic cells in cancer patients, J. Exp. Med. 201 (2005) 1503–1517.
[31] F.L. Schneiders, R.J. Scheper, B.M. von Blomberg, A.M. Woltman, H.L. Janssen, A. J. van den Eertwegh, H.M. Verheul, T.D. de Gruijl, H.J. van der Vliet, Clinical experience with alpha-galactosylceramide (KRN7000) in patients with advanced cancer and chronic hepatitis B/C infection, Clin. Immunol. 140 (2011) 130–141.
[32] A. Ishikawa, S. Motohashi, E. Ishikawa, H. Fuchida, K. Higashino, M. Otsuji, T. Iizasa, T. Nakayama, M. Taniguchi, T. Fujisawa, A phase I study of alpha-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer, Clin. Cancer Res. 11 (2005) 1910–1917.

[33] J. Richter, N. Neparidze, L. Zhang, S. Nair, T. Monesmith, R. Sundaram, F. Miesowicz, K.M. Dhodapkar, M.V. Dhodapkar, Clinical regressions and broad immune activation following combination therapy targeting human NKT cells in myeloma, Blood 121 (2013) 423–430.
[34] C. Paget, T. Mallevaey, A.O. Speak, D. Torres, J. Fontaine, K.C. Sheehan, M. Capron, B. Ryffel, C. Faveeuw, M. Leite de Moraes, F. Platt, F. Trottein, Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids, Immunity 27 (2007) 597–609.
[35] S. Corgnac, R. Perret, L. Derre, L. Zhang, K. Stirnemann, M. Zauderer, D. E. Speiser, J.P. Mach, P. Romero, A. Donda, CD1d-antibody fusion proteins target iNKT cells to the tumor and trigger long-term therapeutic responses, Cancer Immunol. Immunother. 62 (2013) 747–760.
[36] A.N. Courtney, P. Thapa, S. Singh, A.M. Wishahy, D. Zhou, J. Sastry, Intranasal but not intravenous delivery of the adjuvant alpha-galactosylceramide permits repeated stimulation of natural killer T cells in the lung, Eur. J. Immunol. 41 (2011) 3312–3322.
[37] F. Ishibashi, Y. Sakairi, T. Iwata, Y. Moriya, T. Mizobuchi, H. Hoshino, S. Yoshida, H. Hanaoka, I. Yoshino, S. Motohashi, A phase I study of loco-regional immunotherapy by transbronchial injection of alpha-galactosylceramide-pulsed antigen presenting cells in patients with lung cancer, Clin. Immunol. 215 (2020), 108457.
[38] N. Kunii, S. Horiguchi, S. Motohashi, H. Yamamoto, N. Ueno, S. Yamamoto, D. Sakurai, M. Taniguchi, T. Nakayama, Y. Okamoto, Combination therapy of in vitro-expanded natural killer T cells and alpha-galactosylceramide-pulsed antigen-presenting cells in patients with recurrent head and neck carcinoma, Cancer Sci. 100 (2009) 1092–1098.
[39] S. Motohashi, K. Nagato, N. Kunii, H. Yamamoto, K. Yamasaki, K. Okita, H. Hanaoka, N. Shimizu, M. Suzuki, I. Yoshino, M. Taniguchi, T. Fujisawa, T. Nakayama, A phase I-II study of alpha-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer, J. Immunol. 182 (2009) 2492–2501.
[40] V. O'Reilly, S.G. Zeng, G. Bricard, A. Atzberger, A.E. Hogan, J. Jackson, C. Feighery, S.A. Porcelli, D.G. Doherty, Distinct and overlapping effector functions of expanded human CD4+, CD8alpha+ and CD4-CD8alpha- invariant natural killer T cells, PLoS One 6 (2011) e28648.
[41] J.E. Gumperz, S. Miyake, T. Yamamura, M.B. Brenner, Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining, J. Exp. Med. 195 (2002) 625–636.
[42] M.A. Exley, P. Friedlander, N. Alatrakchi, L. Vriend, S. Yue, T. Sasada, W. Zeng, Y. Mizukami, J. Clark, D. Nemer, K. LeClair, C. Canning, H. Daley, G. Dranoff, A. Giobbie-Hurder, F.S. Hodi, J. Ritz, S.P. Balk, Adoptive transfer of invariant NKT cells as immunotherapy for advanced melanoma: a phase I clinical trial, Clin. Cancer Res. 23 (2017) 3510–3519.
[43] I. Engel, G. Seumois, L. Chavez, D. Samaniego-Castruita, B. White, A. Chawla, D. Mock, P. Vijayanand, M. Kronenberg, Innate-like functions of natural killer T cell subsets result from highly divergent gene programs, Nat. Immunol. 17 (2016) 728–739.
[44] D.B. Leveson-Gower, J.A. Olson, E.I. Sega, R.H. Luong, J. Baker, R. Zeiser, R. S. Negrin, Low doses of natural killer T cells provide protection from acute graft-versus-host disease via an IL-4-dependent mechanism, Blood 117 (2011) 3220–3229.
[45] D. Schneidawind, A. Pierini, M. Alvarez, Y. Pan, J. Baker, C. Buechele, R. H. Luong, E.H. Meyer, R.S. Negrin, CD4+ invariant natural killer T cells protect from murine GVHD lethality through expansion of donor CD4+CD25+FoxP3+ regulatory T cells, Blood 124 (2014) 3320–3328.
[46] K.L. Hippen, E.G. Aguilar, S.Y. Rhee, S. Bolivar-Wagers, B.R. Blazar, Distinct regulatory and effector T cell metabolic demands during graft-versus-host disease, Trends Immunol. 41 (2020) 77–91.
[47] R. Kumari, S. Palaniyandi, G.C. Hildebrandt, Metabolic reprogramming-a new era how to prevent and treat graft versus host disease after allogeneic hematopoietic stem cell transplantation has begun, Front Pharmacol 11 (2020), 588449.
[48] K. Maas-Bauer, J.K. Lohmeyer, T. Hirai, T. Lopes Ramos, F.M. Fazal, U. M. Litzenburger, K.E. Yost, J.V. Ribado, N. Kambham, A. Wenokur, P.Y. Lin, M. Alvarez, M. Mavers, J. Baker, A.S. Bhatt, H. Chang, F. Simonetta, R.S. Negrin, Invariant natural killer T cell subsets have diverse graft-versus-host-disease-preventing and anti-tumor effects, Blood (2021).
[49] C.J. Montoya, D. Pollard, J. Martinson, K. Kumari, C. Wasserfall, C.B. Mulder, M. T. Rugeles, M.A. Atkinson, A.L. Landay, S.B. Wilson, Characterization of human invariant natural killer T subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11, Immunology 122 (2007) 1–14.
[50] T. Coman, J. Rossignol, M. D'Aveni, B. Fabiani, M. Dussiot, R. Rignault, J. Babdor, M. Bouille, A. Herbelin, F. Cote, I.C. Moura, O. Hermine, M.T. Rubio, Human CD4- invariant NKT lymphocytes regulate graft versus host disease, Oncoimmunology 7 (2018), e1470735.
[51] T. Teshima, R. Ordemann, P. Reddy, S. Gagin, C. Liu, K.R. Cooke, J.L. Ferrara, Acute graft-versus-host disease does not require alloantigen expression on host epithelium, Nat. Med. 8 (2002) 575–581.
[52] T. Erkers, B.J. Xie, L.J. Kenyon, B. Smith, M. Rieck, K.P. Jensen, X. Ji, M. Basina, S. Strober, R.S. Negrin, H.T. Maecker, E.H. Meyer, High-parametric evaluation of human invariant natural killer T cells to delineate heterogeneity in allo- and autoimmunity, Blood 135 (2020) 814–825.
[53] K. Andrews, A.A.J. Hamers, X. Sun, G. Neale, K. Verbist, P. Tedrick, K.E. Nichols, S. Pereira, D.E. Geraghty, A.B. Pillai, Expansion and CD2/CD3/CD28 stimulation enhance Th2 cytokine secretion of human invariant NKT cells with retained anti-tumor cytotoxicity, Cytotherapy 22 (2020) 276–290.
[54] H. Schmid, E.M. Ribeiro, K.A. Secker, S. Duerr-Stoerzer, H. Keppeler, R. Dong, T. Munz, K. Schulze-Osthoff, S. Hailfinger, C. Schneidawind, D. Schneidawind, Human invariant natural killer T cells promote tolerance by preferential apoptosis induction of conventional dendritic cells, Haematologica (2021).
[55] L.S. Metelitsa, Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans, Clin. Immunol. 140 (2011) 119–129.
[56] M.V. Dhodapkar, M.D. Geller, D.H. Chang, K. Shimizu, S. Fujii, K.M. Dhodapkar, J. Krasovsky, A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma, J. Exp. Med. 197 (2003) 1667–1676.
[57] M. Kronenberg, L. Gapin, The unconventional lifestyle of NKT cells, Nat. Rev. Immunol. 2 (2002) 557–568.
[58] T. Li, Y. Zhang, D. Peng, X. Mao, X. Zhou, J. Zhou, A good response of refractory mantel cell lymphoma to haploidentical CAR T cell therapy after failure of autologous CAR T cell therapy, J. Immunother. Cancer 7 (2019) 51.
[59] L. Roncati, B. Palmieri, Adoptive cell transfer (ACT) of autologous tumor-infiltrating lymphocytes (TILs) to treat malignant melanoma: the dawn of a chimeric antigen receptor T (CAR-T) cell therapy from autologous donor, Int. J. Dermatol. 59 (2020) 763–769.
[60] J. Cao, G. Wang, H. Cheng, C. Wei, K. Qi, W. Sang, L. Zhenyu, M. Shi, H. Li, J. Qiao, B. Pan, J. Zhao, Q. Wu, L. Zeng, M. Niu, G. Jing, J. Zheng, K. Xu, Potent anti-leukemia activities of humanized CD19-targeted Chimeric antigen receptor T (CAR-T) cells in patients with relapsed/refractory acute lymphoblastic leukemia, Am. J. Hematol. 93 (2018) 851–858.
[61] A.V. Stepanov, O.V. Markov, I.V. Chernikov, D.V. Gladkikh, H. Zhang, T. Jones, A.V. Sen'kova, E.L. Chernolovskaya, M.A. Zenkova, R.S. Kalinin, M.P. Rubtsova, A.N. Meleshko, D.D. Genkin, A.A. Belogurov, J. Xie, A.G. Gabibov, R.A. Lerner, Autocrine-based selection of ligands for personalized CAR-T therapy of lymphoma, Sci. Adv. 4 (2018) eaau4580.
[62] J.H. Cho, J.J. Collins, W.W. Wong, Universal chimeric antigen receptors for multiplexed and logical control of T cell responses, Cell 173 (2018) 1426–1438, e1411.
[63] M. Wermke, S. Kraus, A. Ehninger, R.C. Bargou, M.E. Goebeler, J.M. Middeke, C. Kreissig, M. von Bonin, J. Koedam, M. Pehl, M. Bornhauser, H. Einsele, G. Ehninger, M. Cartellieri, Proof of concept for a rapidly switchable universal CAR-T platform with UniCAR-T-CD123 in relapsed/refractory AML, Blood 137 (2021) 3145–3148.
[64] S. Depil, P. Duchateau, S.A. Grupp, G. Mufti, L. Poirot, Off-the-shelf' allogeneic CAR T cells: development and challenges, Nat. Rev. Drug Discov. 19 (2020) 185–199.
[65] J.E. Jaspers, R.J. Brentjens, Development of CAR T cells designed to improve antitumor efficacy and safety, Pharmacol. Ther. 178 (2017) 83–91.
[66] S.J. van der Stegen, M. Hamieh, M. Sadelain, The pharmacology of second-generation chimeric antigen receptors, Nat. Rev. Drug Discov. 14 (2015) 499–509.
[67] A.H. Long, W.M. Haso, J.F. Shern, K.M. Wanhainen, M. Murgai, M. Ingaramo, J. P. Smith, A.J. Walker, M.E. Kohler, V.R. Venkateshwara, R.N. Kaplan, G. H. Patterson, T.J. Fry, R.J. Orentas, C.L. Mackall, 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors, Nat. Med. 21 (2015) 581–590.
[68] S.L. Cole, K.H. Benam, A.J. McMichael, L.P. Ho, Involvement of the 4-1BB/4-1BBL pathway in control of monocyte numbers by invariant NKT cells, J. Immunol. 192 (2014) 3898–3907.
[69] A. Heczey, D. Liu, G. Tian, A.N. Courtney, J. Wei, E. Marinova, X. Gao, L. Guo, E. Yvon, J. Hicks, H. Liu, G. Dotti, L.S. Metelitsa, Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy, Blood 124 (2014) 2824–2833.
[70] R. Poels, E. Drent, R. Lameris, A. Katsarou, M. Themeli, H.J. van der Vliet, T.D. de Gruijl, N. van de Donk, T. Mutis, Preclinical evaluation of invariant natural killer T cells modified with CD38 or BCMA chimeric antigen receptors for multiple myeloma, Int. J. Mol. Sci. 22 (2021).
[71] X. Xu, W. Huang, A. Heczey, D. Liu, L. Guo, M. Wood, J. Jin, A.N. Courtney, B. Liu, E.J. Di Pierro, J. Hicks, G.A. Barragan, H. Ngai, Y. Chen, B. Savoldo, G. Dotti, L.S. Metelitsa, NKT cells coexpressing a GD2-specific chimeric antigen receptor and IL15 show enhanced in vivo persistence and antitumor activity against neuroblastoma, Clin. Cancer Res. 25 (2019) 7126–7138.
[72] B. Simon, M. Wiesinger, J. Marz, K. Wistuba-Hamprecht, B. Weide, B. Schuler-Thurner, G. Schuler, J. Dorrie, U. Uslu, The generation of CAR-transfected natural killer T cells for the immunotherapy of melanoma, Int. J. Mol. Sci. 19 (2018).
[73] A. Rotolo, V. Caputo, A. Karadimitris, The prospects and promise of chimeric antigen receptor immunotherapy in multiple myeloma, Br. J. Haematol. 173 (2016) 350–364.
[74] A. Rotolo, V.S. Caputo, M. Holubova, N. Baxan, O. Dubois, M.S. Chaudhry, X. Xiao, K. Goudevenou, D.S. Pitcher, K. Petevi, C. Kachramanoglou, S. Iles, K. Naresh, J. Maher, A. Karadimitris, Enhanced anti-lymphoma activity of CAR19-iNKT cells underpinned by dual CD19 and CD1d targeting, Cancer Cell 34 (2018) 596–610, e511.
[75] O.O. Yeku, R.J. Brentjens, Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy, Biochem. Soc. Trans. 44 (2016) 412–418.
[76] A.A. Hombach, U. Geumann, C. Gunther, F.G. Hermann, H. Abken, IL7-IL12 engineered mesenchymal stem cells (MSCs) improve A CAR T cell attack against colorectal cancer cells, Cells 9 (2020).

[77] L.V. Hurton, H. Singh, A.M. Najjar, K.C. Switzer, T. Mi, S. Maiti, S. Olivares, B. Rabinovich, H. Huls, M.A. Forget, V. Datar, P. Kebriaei, D.A. Lee, R. E. Champlin, L.J. Cooper, Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells, Proc. Natl. Acad. Sci. USA 113 (2016) E7788–E7797.
[78] J.T. Sockolosky, E. Trotta, G. Parisi, L. Picton, L.L. Su, A.C. Le, A. Chhabra, S. L. Silveria, B.M. George, I.C. King, M.R. Tiffany, K. Jude, L.V. Sibener, D. Baker, J. A. Shizuru, A. Ribas, J.A. Bluestone, K.C. Garcia, Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes, Science 359 (2018) 1037–1042.
[79] Y. Kagoya, S. Tanaka, T. Guo, M. Anczurowski, C.H. Wang, K. Saso, M.O. Butler, M.D. Minden, N. Hirano, A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects, Nat. Med. 24 (2018) 352–359.
[80] J.A. Fraietta, S.F. Lacey, E.J. Orlando, I. Pruteanu-Malinici, M. Gohil, S. Lundh, A. C. Boesteanu, Y. Wang, R.S. O'Connor, W.T. Hwang, E. Pequignot, D.E. Ambrose, C. Zhang, N. Wilcox, F. Bedoya, C. Dorfmeier, F. Chen, L. Tian, H. Parakandi, M. Gupta, R.M. Young, F.B. Johnson, I. Kulikovskaya, L. Liu, J. Xu, S.H. Kassim, M.M. Davis, B.L. Levine, N.V. Frey, D.L. Siegel, A.C. Huang, E.J. Wherry, H. Bitter, J.L. Brogdon, D.L. Porter, C.H. June, J.J. Melenhorst, Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia, Nat. Med. 24 (2018) 563–571.
[81] S.F. Ngiow, M.W. Teng, M.J. Smyth, A balance of interleukin-12 and -23 in cancer, Trends Immunol. 34 (2013) 548–555.
[82] X. Ma, P. Shou, C. Smith, Y. Chen, H. Du, C. Sun, N. Porterfield Kren, D. Michaud, S. Ahn, B. Vincent, B. Savoldo, Y. Pylayeva-Gupta, S. Zhang, G. Dotti, Y. Xu, Interleukin-23 engineering improves CAR T cell function in solid tumors, Nat. Biotechnol. 38 (2020) 448–459.
[83] R. Spolski, W.J. Leonard, IL-21 is an immune activator that also mediates suppression via IL-10, Crit. Rev. Immunol. 30 (2010) 559–570.
[84] S.M. Liu, C. King, IL-21-producing Th cells in immunity and autoimmunity, J. Immunol. 191 (2013) 3501–3506.
[85] R. Spolski, W.J. Leonard, Interleukin-21: a double-edged sword with therapeutic potential, Nat. Rev. Drug Discov. 13 (2014) 379–395.
[86] J. Bessa, M. Kopf, M.F. Bachmann, Cutting edge: IL-21 and TLR signaling regulate germinal center responses in a B cell-intrinsic manner, J. Immunol. 184 (2010) 4615–4619.
[87] J.M. Coquet, K. Kyparissoudis, D.G. Pellicci, G. Besra, S.P. Berzins, M.J. Smyth, D. I. Godfrey, IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production, J. Immunol. 178 (2007) 2827–2834.
[88] M. Maeda, Y. Yanagawa, K. Iwabuchi, K. Minami, Y. Nakamaru, D. Takagi, S. Fukuda, K. Onoe, IL-21 enhances dendritic cell ability to induce interferon-gamma production by natural killer T cells, Immunobiology 212 (2007) 537–547.
[89] J. Parrish-Novak, S.R. Dillon, A. Nelson, A. Hammond, C. Sprecher, J.A. Gross, J. Johnston, K. Madden, W. Xu, J. West, S. Schrader, S. Burkhead, M. Heipel, C. Brandt, J.L. Kuijper, J. Kramer, D. Conklin, S.R. Presnell, J. Berry, F. Shiota, S. Bort, K. Hambly, S. Mudri, C. Clegg, M. Moore, F.J. Grant, C. Lofton-Day, T. Gilbert, F. Rayond, A. Ching, L. Yao, D. Smith, P. Webster, T. Whitmore, M. Maurer, K. Kaushansky, R.D. Holly, D. Foster, Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function, Nature 408 (2000) 57–63.
[90] S. Deng, Z. Sun, J. Qiao, Y. Liang, L. Liu, C. Dong, A. Shen, Y. Wang, H. Tang, Y. X. Fu, H. Peng, Targeting tumors with IL-21 reshapes the tumor microenvironment by proliferating PD-1intTim-3-CD8+ T cells, JCI Insight 5 (2020).
[91] C. Bucher, L. Koch, C. Vogtenhuber, E. Goren, M. Munger, A. Panoskaltsis-Mortari, P. Sivakumar, B.R. Blazar, IL-21 blockade reduces graft-versus-host disease mortality by supporting inducible T regulatory cell generation, Blood 114 (2009) 5375–5384.
[92] K.E. Lewis, M.J. Selby, G. Masters, J. Valle, G. Dito, W.R. Curtis, R. Garcia, K. A. Mink, K.S. Waggie, M.S. Holdren, J.F. Grosso, A.J. Korman, M. Jure-Kunkel, S. R. Dillon, Interleukin-21 combined with PD-1 or CTLA-4 blockade enhances antitumor immunity in mouse tumor models, Oncoimmunology 7 (2017), e1377873.
[93] Y. Li, Y. Cong, M. Jia, Q. He, H. Zhong, Y. Zhao, H. Li, M. Yan, J. You, J. Liu, L. Chen, H. Hang, S. Wang, Targeting IL-21 to tumor-reactive T cells enhances memory T cell responses and anti-PD-1 antibody therapy, Nat. Commun. 12 (2021) 951.
[94] G. Tian, A.N. Courtney, B. Jena, A. Heczey, D. Liu, E. Marinova, L. Guo, X. Xu, H. Torikai, Q. Mo, G. Dotti, L.J. Cooper, L.S. Metelitsa, CD62L+ NKT cells have prolonged persistence and antitumor activity in vivo, J. Clin. Invest. 126 (2016) 2341–2355.
[95] D.A. Hildeman, Y. Zhu, T.C. Mitchell, P. Bouillet, A. Strasser, J. Kappler, P. Marrack, Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim, Immunity 16 (2002) 759–767.
[96] H. Ngai, G. Tian, A.N. Courtney, S.B. Ravari, L. Guo, B. Liu, J. Jin, E.T. Shen, E. J. Di Pierro, L.S. Metelitsa, IL-21 selectively protects CD62L(+) NKT cells and enhances their effector functions for adoptive immunotherapy, J. Immunol. 201 (2018) 2141–2153.
[97] G.C. Sim, L. Radvanyi, The IL-2 cytokine family in cancer immunotherapy, Cytokine Growth Factor Rev. 25 (2014) 377–390.
[98] E.F. Castillo, L.F. Acero, S.W. Stonier, D. Zhou, K.S. Schluns, Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation, Blood 116 (2010) 2494–2503.
[99] D.V. Baev, X.H. Peng, L. Song, J.R. Barnhart, G.M. Crooks, K.I. Weinberg, L. S. Metelitsa, Distinct homeostatic requirements of CD4+ and CD4- subsets of Valpha24-invariant natural killer T cells in humans, Blood 104 (2004) 4150–4156.
[100] N.Y. Crowe, J.M. Coquet, S.P. Berzins, K. Kyparissoudis, R. Keating, D.G. Pellicci, Y. Hayakawa, D.I. Godfrey, M.J. Smyth, Differential antitumor immunity mediated by NKT cell subsets *in vivo*, J. Exp. Med. 202 (2005) 1279–1288.
[101] L.E. Gordy, J.S. Bezbradica, A.I. Flyak, C.T. Spencer, A. Dunkle, J. Sun, A. K. Stanic, M.R. Boothby, Y.W. He, Z. Zhao, L. Van Kaer, S. Joyce, IL-15 regulates homeostasis and terminal maturation of NKT cells, J. Immunol. 187 (2011) 6335–6345.
[102] L. Zhu, X. Xie, L. Zhang, H. Wang, Z. Jie, X. Zhou, J. Shi, S. Zhao, B. Zhang, X. Cheng, S.C. Sun, TBK-binding protein 1 regulates IL-15-induced autophagy and NKT cell survival, Nat. Commun. 9 (2018) 2812.
[103] D. Liu, L. Song, J. Wei, A.N. Courtney, X. Gao, E. Marinova, L. Guo, A. Heczey, S. Asgharzadeh, E. Kim, G. Dotti, L.S. Metelitsa, IL-15 protects NKT cells from inhibition by tumor-associated macrophages and enhances antimetastatic activity, J. Clin. Invest. 122 (2012) 2221–2233.
[104] A. Heczey, A.N. Courtney, A. Montalbano, S. Robinson, K. Liu, M. Li, N. Ghatwai, O. Dakhova, B. Liu, T. Raveh-Sadka, C.N. Chauvin-Fleurence, X. Xu, H. Ngai, E. J. Di Pierro, B. Savoldo, G. Dotti, L.S. Metelitsa, Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis, Nat. Med. 26 (2020) 1686–1690.
[105] K.C. Conlon, E. Lugli, H.C. Welles, S.A. Rosenberg, A.T. Fojo, J.C. Morris, T. A. Fleisher, S.P. Dubois, L.P. Perera, D.M. Stewart, C.K. Goldman, B.R. Bryant, J. M. Decker, J. Chen, T.A. Worthy, W.D. Figg Sr., C.J. Peer, M.C. Sneller, H.C. Lane, J.L. Yovandich, S.P. Creekmore, M. Roederer, T.A. Waldmann, Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer, J. Clin. Oncol. 33 (2015) 74–82.
[106] L. Cheng, X. Du, Z. Wang, J. Ju, M. Jia, Q. Huang, Q. Xing, M. Xu, Y. Tan, M. Liu, P. Du, L. Su, S. Wang, Hyper-IL-15 suppresses metastatic and autochthonous liver cancer by promoting tumour-specific CD8+ T cell responses, J. Hepatol. 61 (2014) 1297–1303.
[107] Z.C. Ding, T. Habtetsion, Y. Cao, T. Li, C. Liu, M. Kuczma, T. Chen, Z. Hao, L. Bryan, D.H. Munn, G. Zhou, Adjuvant IL-7 potentiates adoptive T cell therapy by amplifying and sustaining polyfunctional antitumor CD4+ T cells, Sci. Rep. 7 (2017) 12168.
[108] C. de Lalla, N. Festuccia, I. Albrecht, H.D. Chang, G. Andolfi, U. Benninghoff, F. Bombelli, G. Borsellino, A. Aiuti, A. Radbruch, P. Dellabona, G. Casorati, Innate-like effector differentiation of human invariant NKT cells driven by IL-7, J. Immunol. 180 (2008) 4415–4424.
[109] A. Trujillo-Ocampo, H.W. Cho, M. Clowers, S. Pareek, W. Ruiz-Vazquez, S.E. Lee, J.S. Im, IL-7 during antigenic stimulation using allogeneic dendritic cells promotes expansion of CD45RA(-)CD62L(+)CD4(+) invariant nkt cells with th-2 biased cytokine production profile, Front. Immunol. 11 (2020), 567406.