CHAPTER

4

Successes and challenges of NKT cell immunotherapy: Breaking tolerance to cancer resistance

Zhe Li[a,*], *Derek Lee*[a,*], *Samuel Zeng*[a], *and Lili Yang*[a,b,c,d]

[a]Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, United States [b]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles (UCLA), Los Angeles, CA, United States [c]Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, United States [d]Molecular Biology Institute, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, United States

Abstract

Natural killer T (NKT) cells comprise a small population of αβ T lymphocytes. They bridge the innate and adaptive immune systems and mediate strong and rapid responses to many diseases, including cancer. This has made NKT cells attractive agents for cell-based cancer therapies. Various approaches have been applied in clinical trials to target NKT cells, and the recent advances in chimeric antigen receptor (CAR) have also shown great potential in increasing the efficacy of NKT cells. In this chapter, we first introduce the biology and importance of NKT cells in antitumor immunity. Then, we summarize the preclinical data and clinical trials utilizing NKT cell-based immunotherapies. Finally, we discuss the challenges and future work that could be done to unleash the full potential of NKT cells for cancer immunotherapy.

Abbreviations

NKT	natural killer T
CAR	chimeric antigen receptor
TCR	T-cell receptor

*Contributed equally to this manuscript.

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MHC	major histocompatibility complex
iNKT	invariant NKT
DN	CD4-CD8-
α-GalCer	α-galactosylceramide
IL	interleukin
NKR	NK receptor
KIR	killer cell immunoglobulin-like receptor
MIC	MHC class I chain-related molecule
Th	T helper
IFN-γ	interferon γ
FasL	Fas ligand
TNFα	tumor necrosis factor α
TRAIL	TNFα-related apoptosis-inducing ligand
DCs	dendritic cells
TME	tumor microenvironment
MOAs	mechanisms of actions
TAMs	tumor-associated macrophages
MDSCs	myeloid-derived suppressor cells
APCs	antigen presenting cells
CRS	cytokine release syndrome
GvHD	graft-versus-host disease
HSC	hematopoietic stem cell
iPSC	induced pluripotent stem cell
allo-HCT	allogeneic hematopoietic cell transplantation
GvT	graft-versus-tumor
TLI	total lymphoid irradiation
ATG	antithymocyte globulin

Conflict of interest

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Biology of NKT cells

NKT cells are a unique subset of αβ T lymphocytes characterized by their expression of both a αβ T-cell receptor (TCR) and NK lineage markers. They derive from the same lymphoid precursor pool as T cells and mature in the thymus [1,2]. However, unlike conventional T cells, which recognize peptide antigens, NKT cells respond to lipid antigens presented by the nonclassical monomorphic major histocompatibility complex (MHC) molecule CD1d. Despite their small numbers in vivo (∼0.1%–1% in mouse blood and ∼0.01%–1% in human blood), NKT cells are some of the first cells to be activated during an immune response and can rapidly produce copious amounts of cytokines and chemokines, thereby functioning as a "bridge" linking the innate and adaptive immune responses [3].

Subtypes of NKT cells

NKT cells are generally divided into two major subsets based on their TCR expression. Type I NKT cells, or invariant NKT (iNKT) cells, are the most prevalent type of NKT cells and express a restricted TCR recombinant comprising a semi-invariant TCR α chain

(Vα14-Jα18 in the mouse and Vα24-Jα18 in humans) paired to a limited repertoire of Vβ chains (Vβ 2, 7, or 8.2 in mice and Vβ 11 in humans) [2,3]. iNKT cells can be further divided based on their CD4 and CD8 expressions. Human iNKT cells include $CD4^+CD8^-$ ($CD4^+$), $CD4^-CD8^+$ ($CD8^+$), and $CD4^-CD8^-$ (DN) populations, whereas mice only have $CD4^+$ and DN populations [2]. For both mice and humans, iNKT cells are also functionally defined by their ability to recognize the prototypic antigen α-galactosylceramide (α-GalCer) [4,5]. α-GalCer is a strong activator of iNKT cells and has been widely used to study iNKT cell biology. In contrast to type I NKT cells, type II NKT cells express more diverse TCRs and are therefore also called variant NKT cells [6–8]. Importantly, type II NKT cells do not respond to α-GalCer. Given this and the lack of specific markers, study of this subpopulation has been more challenging. Currently, no experimental tools are available to identify and analyze the entire population of type II NKT cells.

From studies of animal models and humans, both subsets of NKT cells have been shown to play a role in diverse immune settings, particularly tumor immunity [9,10]. Although their functions sometimes coordinate, in most cases, they counteract each other. Compared to type II NKT cells, type I NKT cells are better characterized and known to possess a superior antitumor activity. Thus, hereafter this chapter will focus on discussing the role and current status of type I NKT cells in cancer immunotherapy.

Activation of iNKT cells

There are three main mechanisms to activate iNKT cells: (1) via TCR/CD1d/lipid stimulation, (2) via cytokine stimulation, and (3) via activating NK receptor stimulation [11–14]. Potent glycolipid antigens (e.g., α-GalCer) presented by CD1d can directly activate iNKT cells in a TCR-dependent manner without the need for costimulation or cytokine receptor engagement [3]. However, in a more physiological context, iNKT cells are stimulated by microbial or self-lipid antigens that are weakly immunogenic [15]. In this case, iNKT cell activation requires a second activation signal from proinflammatory cytokines. Interestingly, cytokines alone (e.g., IL-12, IL-18, IL-23, and IL-25) are sometimes sufficient to activate iNKT cells even in the absence of TCR engagement. Several studies have found that iNKT cells release IFN-γ when stimulated by IL-12 during viral infections [16,17]. The remarkable capacity of iNKT cells to respond to cytokines is due to their elevated expression of functional receptors at baseline. iNKT cells also express activating and inhibitory NK receptors (NKR) and killer cell immunoglobulin-like receptors (KIR) on their cell surface, so they can become activated when the summation of stimulatory signals overcomes the inhibitory signals. Activating NKRs recognize a variety of MHC-like molecules and cellular targets frequently referred to as "stress proteins." For example, DNAM-1 recognizes the poliovirus receptor and Nectin-2; the NKG2D receptor recognizes MHC class I-like molecules (MIC) A and B and unique long-binding proteins. Inhibitory NKRs and KIRs often bind to HLA molecules [14].

Effector functions of iNKT cells

Unlike conventional T cells, which emerge from the thymus "naïve," iNKT cells leave the thymus fully mature and able to perform their effector functions immediately without priming [2,18,19]. The most significant effector function of activated NKT cells is secreting

cytokines, including T helper (Th)1-like (IFN-γ), Th2-like (IL-4, IL-13), Th17-like (IL-17, IL-22), and regulatory (IL-10) cytokines [2,20]. Which cytokines are produced is dependent on the mechanisms of cell activation, the location, and the iNKT cell subsets. For example, the activation of iNKT cells with the potent agonist α-GalCer leads to the production of both Th1- and Th2-like cytokines, whereas activation involving both recognition of the endogenous ligand/CD1d complexes and cytokine costimulation leads to the polarized production of Th1-like IFN-γ, but not Th2-like cytokines [15,21]. In addition, both human $CD8^+$ and DN iNKT cells have been found to predominately express Th1 cytokines, whereas the human $CD4^+$ iNKT cells predominately express Th2 cytokines, although this distinction is less apparent with the mouse iNKT cells [22,23]. Interestingly, subsets expressing different cytokines, transcription factors, and surface markers appear to be acquired during thymic development rather than as a result of peripheral experience [2,24].

In addition to cytokines, iNKT cells also produce cytolytic proteins such as perforin and granzyme B, and surface molecules involved in cytotoxicity such as Fas ligand (FasL) and tumor necrosis factor α (TNFα)-related apoptosis-inducing ligand (TRAIL) [25,26]. Given their ability to produce an array of effector molecules, iNKT cells can profoundly influence many other cell types, including dendritic cells (DCs), macrophages, neutrophils, NK cells, and T and B cells, thereby orchestrating immune responses during infection, autoimmune disease, allergy, and cancer [2].

Mechanisms of iNKT cell-based cancer immunotherapy

Recent progress in understanding the activation and effector functions of iNKT cells has led to an increased appreciation of their roles in health and disease. In particular, the antitumor potential of iNKT cells has made them extremely attractive agents for developing cancer immunotherapy [10,12,19,27]. There are compelling evidences suggesting the significant role of iNKT cells in tumor surveillance. In humans, the frequency of iNKT cells is decreased in patients with solid tumors (including melanoma, colon, lung, breast, and head-and-neck cancers) and hematologic malignancies (including leukemia, multiple myeloma, and myelodysplastic syndromes), and increased iNKT cell numbers are associated with a better prognosis [14,28,29]. In addition to correlative human data, the role of iNKT cells in exerting antitumor activity has been well characterized using murine models of tumors [28,30]. Direct tumor killing, cytokine-mediated regulation of effector cells, and the modulation of immunosuppressive tumor microenvironment (TME) are currently considered as the three major mechanisms of action (MOAs) of iNKT cells in combating cancer [10].

Direct cytotoxicity against tumor cells (Fig. 1A)

iNKT cells are known to exert a direct tumor-killing effect against $CD1d^+$ tumors, mainly through the production of cytolytic molecules such as perforin and granzyme B [25,31,32], and the interaction of death-inducing receptors including Fas and TRAIL receptors [26,33,34]. The CD1d expression level in tumor cells has been demonstrated as an important determinant for iNKT cell-mediated cytotoxicity. The correlation between reduced CD1d

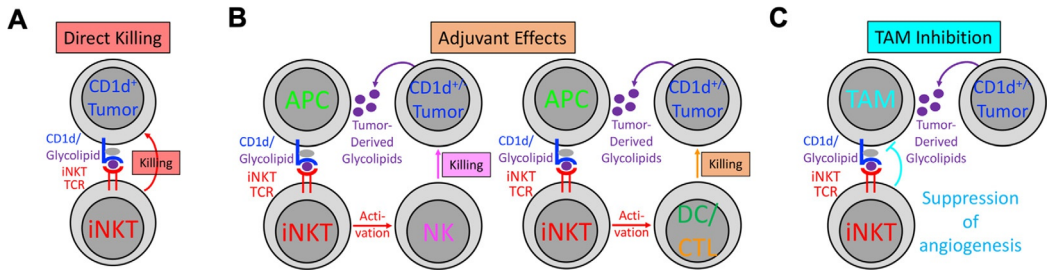

FIG. 1 Mechanisms utilized by iNKT cells to attack tumor cells. (A) iNKT cells can directly recognize and kill CD1d + tumor cells. (B) iNKT cells can exhibit adjuvant effects to enhance NK cell and DC/CTL cell-mediated killing of tumor cells. (C) iNKT cells can inhibit TAMs thereby targeting tumor through suppressing angiogenesis and modifying tumor immunosuppressive microenvironment. *APC*, antigen-presenting cell; *NK*, natural killer cell; *DC*, dendritic cell; *CTL*, cytotoxic T lymphocyte; *TAM*, tumor-associated macrophage.

expression and enhanced tumor progression has been reported in a variety of tumor models [35–41], indicating that CD1d downregulation may be an important cause of tumor evasion from iNKT cell-mediated immunosurveillance.

Aside from recognition of CD1d, more than half of all iNKT cells also express NK-activating receptors, such as NKG2D, enabling direct cytotoxicity against tumors expressing corresponding ligands [22,42].

Regulation of antitumor effector cells (Fig. 1B)

In addition to their direct killing capability, iNKT cells are known to potentiate their antitumor effects by enhancing the immunogenic activities of a variety of immune cell subsets [43]. As mentioned above, iNKT cells can rapidly produce various Th1 and Th2 cytokines upon activation, leading to reciprocal activation and modulation of both innate and adaptive immune cells [2,44–47]. Notably, one of the major contributions of iNKT cells to immune surveillance is linked to DC maturation. Most DCs found in the TME are immature, and maturation of DCs is essential to initiate a sufficient T cell-mediated response [48]. Indeed, activation of iNKT cells causes the upregulation of the IL-12 receptor and CD40L and induces the maturation of DCs via a CD40-CD40L interaction. Mature DCs then express higher costimulatory molecules CD40, CD80, CD86, and CD70 and release more IL-12, which in turn further activate iNKT cells and amplify IFN-γ responses, leading to a positive feedback loop for Th1 immunity [49–51]. Furthermore, this maturation of DCs induces the transactivation of NK cells and contributes to the priming of CD8 T cells [52,53], allowing the establishment of both innate and adaptive immune responses to eliminate MHC-negative and -positive tumor cells, respectively.

Modulation of immunosuppressive TME (Fig. 1C)

iNKT cells can also augment their antitumor efficacy by counteracting immunosuppressive cells in the TME. The immunosuppressive microenvironment not only promotes tumor growth and migration, but also helps the tumor cells evade the surveillance of the host

immunity and resists immunotherapy [54,55]. Tumor-associated macrophages (TAMs) constitute a significant portion of cell populations in the TME and serve as major tumor-promoting immune cells [56]. It has been shown that iNKT cells can kill CD1d-expressing TAMs in primary human neuroblastoma samples, in part by relieving the immunosuppressive TME and limiting metastases [57]. In addition to TAMs, iNKT cells can also alter the numbers and effects of myeloid-derived suppressor cells (MDSCs), also known to be a population of myeloid cells with immunosuppressive properties [58]. In a model of influenza A virus infection, adoptive transfer of iNKT cells reduced the immunosuppressive activity of MDSCs [59]. In another study using murine tumor models, iNKT cells facilitated the conversion of immunosuppressive MDSCs into immunogenic antigen-presenting cells (APCs), eliciting successful antitumor immunity and providing the basis for alternative cell-based vaccines [60].

Advantages of iNKT cell-based immunotherapy

T cells engineered with chimeric antigen receptors (CARs) represent a novel class of immunotherapeutics that have shown promising clinical results and have obtained the FDA approval for treating blood cancers such as acute lymphoblastic leukemia and B cell lymphoma [61]. These CAR-T cells can respond quickly to CAR-specific tumor antigenic stimulation and rapidly produce cytokines to enable an effective antitumor response. However, there are several major drawbacks of CAR-T cell therapy, including serious side effects such as the cytokine release syndrome (CRS), lethal neurotoxicity, and graft-versus-host disease (GvHD) caused by allogeneic CAR-T cells [62,63]. In addition, the therapy is also less effective against solid tumors than against blood cancers [64].

In contrast, a major benefit of iNKT cell therapy is that iNKT cells have limited capacity to cause GvHD, and instead, is associated with reduced GvHD in clinical trials [65–68]. The presence of iNKT cells after hematopoietic stem cell transfer is predictive for survival with a reduction in GvHD [65–68]. Additionally, iNKT cells interact with the monomorphic CD1d [69–73] and are, therefore, not MHC restricted, making them highly suitable for "off-the-shelf" therapy [4,74,75].

The use of iNKT cells has mechanistic benefits beyond safety. iNKT cells can attack tumor cells through multiple mechanisms. Their TCRs can directly recognize $CD1d^+$ tumors, using either perforin, granzyme B, Fas ligand, or TNF-α-mediated cytotoxic pathways. iNKT cells can also have indirect antitumoral effects by targeting $CD1d^+$ tumor-associated macrophages and tumor-promoting myeloid cells [41,57,76]. In addition, iNKT cells express NK receptors such as NKG2D enabling direct cytotoxicity against tumors expressing NKG2D ligands [22,42]. Another advantage of iNKT cells is that they are an ideal vector to target nonlymphoid tumors: their interaction with tissue chemokines CCL2 and CCL20 allows them to migrate into nonlymphoid tissues [77,78]. In the tumor microenvironment, most DCs are immature. It has been shown that iNKT cells can promote the maturation of DCs through CD40-CD40L and CD1d/lipid antigen-TCR interactions [79]. Mature DCs can activate NK cells and iNKT cells by expressing costimulatory molecules (CD40, CD80, CD86) and cytokines, further enhancing the antitumor effects of these cells [80].

Several preclinical and clinical studies have reported using ex vivo stimulation and loading of autologous DCs with α-GalCer prior to administration of DC vaccines [81–92]. A detailed analysis showed that injecting these vaccines into mice can augment the frequency of iNKT cells and circulating IFN-γ-producing cells in a solid tumor preclinical model [84]. In humans, α-GalCer-loaded DCs can increase the infiltration of lymphocytes into the tumor microenvironment and promote iNKT cell-induced immune memory upon secondary administration [87]. In a phase I/II trial, Chang et al. intravenously injected monocyte-derived mature DCs loaded with α-GalCer into five patients with advanced myeloma [89]. In all patients, the frequency of iNKT cells increased over a 100-fold and the number of iNKT cells stayed above baseline for 3 months. Elevated IFN-γ levels were also detected. These data demonstrate the feasibility of using α-GalCer-loaded DCs to improve iNKT-based therapies, including iNKT cells, in clinical settings.

Current status of iNKT cell-based immunotherapy

iNKT cells can induce strong antitumor immune responses in preclinical mouse models and in clinical trials via in vivo stimulation or adoptive transfer approach. These cells can also be activated by administering α-GalCer-loaded DCs. Activated iNKT cells increase IFN-γ production and cytokine secretion, which further enhances activation of other immune cells such as NK cells, T cells, and B cells. Multiple clinical trials based on α-GalCer-DC transfer have demonstrated the clinically relevant antitumor effects and the safety of this approach. In addition to α-GalCer-based therapy, there are other approaches to utilize iNKT cells for cancer treatments. These include using autologous and allogeneic transfer of iNKT cells, CAR-iNKT cells, hematopoietic stem cell (HSC)-derived iNKT cells, and induced pluripotent stem cell (iPSC)-derived iNKT cells. Numerous clinical trials utilizing these methods are summarized in Table 1.

Autologous transfer approach

The autologous adoptive transfer approach seeks to increase iNKT cell numbers in cancer patients by harvesting patients' own PBMCs, expanding the PBMC iNKT cells by stimulation with α-GalCer and/or cytokines, and then infusing the enriched iNKT cell population back into the patient. This approach has been shown to be more effective in expanding iNKT cells than I.V. administration of α-GalCer [93–96]. In a preclinical study, adoptive transfer of IL-12-activated iNKT cells prevented hepatic metastasis of melanoma in mice [84]. This study also suggested the involvement of direct cytotoxic mechanisms rather than cytokine-mediated immune responses at the effector phase of the iNKT cell-mediated antitumor activity.

Motohashi et al. performed a phase I study with autologous activated iNKT cell therapy for non-small cell lung cancer patients [93]. No severe side effects were observed during this study and the number of IFN-γ-producing cells in the blood increased in some patients after the administration of activated iNKT cells. This study demonstrated the safety and feasibility of this approach, although no patient developed a partial or complete response. Subsequent adoptive iNKT cell-based clinical trials began treating patients with α-GalCer-loaded DCs in addition to iNKT cells [94–96]. In these studies, the treatments did not cause major adverse

TABLE 1 Clinical studies utilizing iNKT cell-based immunotherapies.

Year	Treatment	Tumor type	Safety	Clinical outcome	Reference
Direct α-GalCer injection					
2002	α-GalCer	Solid tumor	No severe side effects	Stable disease (7/24)	[86]
Ex-vivo generated dendritic cell loaded with α-GalCer					
2004	α-GalCer-loaded CD1d + immature DCs	Metastatic malignancy	No severe side effects	1. Decreased serum tumor markers (2/12) 2. Developed necrosis of tumor-infiltrating bone marrow (1/12) 3. Inflammatory response at tumor sites	[87]
2005	α-GalCer-loaded IL-2/ GM-CSF-cultured PBMCs	Non-small cell lung cancer	No severe side effects	Stable disease (3/11)	[88]
2005	α-GalCer-loaded mature DCs	Advanced cancer	No severe side effects	Decreased M spike levels in serum and urine (3/5)	[89]
2008	α-GalCer-loaded antigen-presenting cell (APCs)	Head-and-neck squamous cell carcinoma	No severe side effects	1. Partial response (1/9) 2. Stable disease (7/9)	[90]
2009	α-GalCer-loaded immature DCs	Non-small cell lung cancer	No severe side effects	Stable disease (5/17)	[91]
2011	α-GalCer-loaded immature DCs	Metastatic solid tumor	No severe side effects	1. Stable disease (6/10) 2. Reduction in tumor volume (3/10) 3. Tumor inflammation (9/12)	[131]
2012	α-GalCer-loaded IL-2/ GM-CSF-cultured PBMCs	Non-small cell lung cancer	No severe side effects	N/A	[92]
Adoptive transfer of autologous* ex vivo-*expanded iNKT cells					
2006	Ex vivo-expanded iNKT cells with autologous α-GalCer-loaded PBMCs	Non-small cell lung cancer	No severe side effects	1. No tumor regression 2. Stable disease (2/9)	[93]
2009	Ex vivo-expanded iNKT cells with autologous α-GalCer-loaded PBMCs	Head-and-neck squamous cell carcinoma	Severe side effects (1); mild symptoms (7)	1. Partial response (3/8) 2. Stable disease (4/8) 3. Progressive disease (1/8)	[94]
2011	Ex vivo-expanded iNKT cells (intra-arterial) and autologous α-GalCer-pulsed PBMCs (via nasal submucosal)	Head-and-neck squamous cell carcinoma	No severe side effects	1. Objective tumor regression (5/10) 2. Stable disease (5/10) 3. Antitumor effects (8/10)	[95]

TABLE 1 Clinical studies utilizing iNKT cell-based immunotherapies—cont'd

Year	Treatment	Tumor type	Safety	Clinical outcome	Reference
2017	Ex vivo-expanded iNKT cells	Advanced melanoma	No severe side effects	1. Patients deceased (3/9) 2. Patients progressed (3/9)	[96]
CAR-iNKT cells					
2020	Autologous GD2 CAR-iNKT cells overexpressed IL-15	Neuroblastoma	No severe side effects	1. Reduction of tumor volume (2/5) 2. Near complete response (1/5) 3. Plan to increase dosage level	[97] (Kuur Therapeutics)
2020	Allogeneic CD19 CAR-iNKT cells overexpressed IL-15	B-cell malignancies	N/A	N/A	N/A (Kuur Therapeutics)
Stem cell-derived iNKT cells					
2020	*In vitro*-differentiated iPSC-derived iNKT cells	Head-and-neck cancers	N/A	N/A	[98] (BrightPath Bio)

effects: only one patient had a grade 3 toxicity in a phase II trial of 10 patients [95]. Increased numbers of iNKT cells were seen after several infusions, but the effects were transient in the same phase II study. At the end of this trial, some patients had no evidence of disease, some had partial responses, and some died of disease [95]. These clinical trials demonstrated that the adoptive transfer of autologous iNKT cells was feasible, well tolerated, and had some beneficial clinical effects. However, further studies are clearly needed to improve clinical outcomes.

A variation on this approach, the Yang laboratory sought to increase circulating iNKT cells through infusion of TCR-engineered HSCs [99,100]. Smith et al. demonstrated the feasibility of dramatically increasing iNKT cells in mice through TCR gene engineering of autologous HSCs [99]; Zhu et al. utilized a BLT (human bone marrow-liver-thymus engrafted NOD/SCID/$\gamma c^{-/-}$ mice) humanized mouse model to support the engraftment of engineered human HSCs and give rise to human iNKT cells [100]. iNKT TCR-engineered HSCs could generate a clonal population of iNKT cells. These HSC-iNKT cells closely resembled endogenous iNKT cells, could deploy multiple mechanisms to attack tumor cells, and effectively suppressed tumor growth in vivo in multiple tumor mouse models [100]. Preclinical safety studies showed no toxicity or tumorigenicity of the HSC-iNKT cell therapy [100].

Allogeneic transfer approach

Allogeneic hematopoietic cell transplantation (allo-HCT) is another powerful tool for treating hematological malignancies, but the development of GvHD remains a significant

clinical challenge. Preclinical studies have demonstrated iNKT cells can simultaneously prevent GvHD while retaining the graft-versus-tumor (GvT) effect following allo-HCT. Murine studies demonstrated that selective depletion of host conventional T cells through fractionated total lymphoid irradiation (TLI) and antithymocyte globulin (ATG), and thereby selectively enriching host iNKT cells, prior to allo-HCT, attenuated GvHD through host iNKT cell elevation of IL-4 secretion and polarization of donor T cells toward a Th2 cytokine pattern. This, in turn, inhibited donor T-cell early expansion and infiltration of GvHD target organs [101]. Likewise, the addition of donor or third-party $CD4^+$ iNKT cells to the allograft was found to prevent GvHD by inhibiting T-cell proliferation, promoting Th2-biased cytokine response, and expanding donor MDSCs [102,103]. Notably, enrichment of the iNKT cells, whether host, donor, or third party, did not abrogate donor T-cell GvT [101–103].

The protective role of human iNKT cells against GvHD has also been highlighted by several clinical studies. Nonmyeloablative conditioning with TLI/ATG prior to allo-HCT was associated with a higher iNKT/T cell ratio, increased IL-4 production, decreased incidence of GvHD, and retained GvT effect [104,105]. Patients with acute GvHD were found to have reduced numbers of total iNKT cells [65], whereas enhanced iNKT cell reconstitution following allo-HCT positively correlated with reduction in GvHD without loss of GvT effect [67]. Separately, low $CD4^-$ iNKT cell numbers in donor graft were associated with clinically significant GvHD in patients receiving HLA-identical allo-HCT [106]. Thus, increasing the iNKT cell numbers of allograft that contains few iNKT cells may provide an attractive strategy for suppressing GvHD while preventing leukemia relapse. Human $CD4^+$ and $CD4^-$ iNKT cells likely mediate distinct effects that collectively result in a beneficial effector response for transplant recipients. $CD4^-$ iNKT cells are Th1 biased—secreting more IFN-γ than IL-4 and preferentially expressing perforin—is thought to both promote GvT and suppress GvHD. Supporting this notion are in vitro studies demonstrating that $CD4^-$ iNKT cells display direct cytotoxicity against CD1d-expressing mature myeloid DCs [106]. Human iNKT cells also express KIRs, including KIRDL4, KIR3DL2, and KIR2DL1 [107]. In an allogeneic setting, donor $CD4^-$ iNKT cells may kill host APCs in a TCR-CD1d and KIR-dependent manners to downregulate GvHD. $CD4^+$ iNKT cells, on the other hand, can ameliorate GvHD through the production of IL-4 [22], polarizing pathogenic donor T cells toward an antiinflammatory Th2 response, and promoting the expansion of regulatory T cells [108,109].

CAR-iNKT approach

iNKT cells as a platform for CAR immunotherapy represent a novel approach, as the addition of a CAR increases their tumor-targeting specificity, in vivo persistence, and potentially tumor infiltrating capability [110]. The Metelitsa group provides the first evidence for the feasibility of engineering iNKT cells with CAR and expanding them to a clinical scale. GD2-CARs and CD19-CARs were inserted into human iNKT cells and tested against neuroblastoma and $CD19^+$ lymphoma in vitro and in preclinical murine models [111,112]. CAR-iNKT cells were capable of killing target tumor cells without causing GvHD, whereas conventional CAR-T cells cleared the tumor but recipients also succumbed to GvHD. Furthermore, the endogenous TCR of CAR-iNKT cells remained functional, such that they were also able to kill $CD1d^+$ tumor cells [111]. A subsequent study published by the same group

coexpressed IL-15 with GD2-CAR in iNKT cells [97]. Coexpression of IL-15 reduced expression of exhaustion markers in CAR-iNKT cells and enhanced in vivo persistence and localization to tumor sites. Owing to their results, the first-in-human CAR-iNKT cell clinical trial is underway [97]. In their interim report consisting of three patients, the group observed no dose-limiting toxicities, expansion of CAR-iNKT cells in vivo, and localized to tumors [113]. One patient also demonstrated an objective response with regression of bone metastatic lesions. These initial results suggest that CAR-iNKT cells can be expanded to clinical scale and safely applied to treat patients with cancer. Another study reported by the Karadimitris group further demonstrated that CD19-CAR iNKT cells can be activated by CD1d and CD19-dependent stimulation, which provided a dual-targeting strategy for CD19-CAR-iNKT cells against CD1d-expressing lymphomas in vitro and in vivo [114].

iPSC-iNKT approach

Adoptive CAR T-cell therapy is currently limited to autologous administration. Having to make individualized CAR T-cell therapy is expensive, time consuming, and labor intensive [74]. In addition, many cancer patients are nonideal candidates owing to the chemotherapy or irradiation they received beforehand that could negatively impact the quality of their T cells. The use of donor-derived allogeneic CAR therapy provides an opportunity to treat cancer patients with "off-the-shelf" cell products. However, the risk of GvHD remains a major concern for allogeneic CAR-T cell therapy due to HLA mismatches between the donor and patient [74,115]. Advances in gene editing have allowed scientists to remove the endogenous TCR from allogeneic CAR-T cells, lowering the chances of developing GvHD. However, 100% endogenous TCR removal is not guaranteed. Because having less than 1% of TCR-expressing T cells is sufficient to cause GvHD, patients receiving these products remain at risk for developing GvHD [74,115]. Although NK cells, iNKT cells, and γδ T cells may serve as better candidates for allogeneic adoptive transfer therapy, cell products derived from donors still have residual conventional αβ T cells.

To overcome these issues, allogeneic cell therapy generated from iPSCs could be an alternative approach. iPSCs can be stored as master cell banks, continuously differentiating and generating cells for treatments, which avoid using primary lymphoid cells from patients or healthy donors as the cell source [116]. This approach standardizes manufacturing procedures and provides a solution to donor-to-donor variations, cell number, and dosage limitations of the cell products. iPSCs can also be genetically modified by gene-editing technology prior to the differentiation step. Genetically engineered cell clones can be isolated and expanded as stable cell sources in which all clones contain 100% purity of desirable characteristics [116].

It has been demonstrated that T cells and NK cells can be successfully differentiated from iPSCs in vitro [116–123]. Recently, the Kaneko group reported the generation of human iNKT cells from iPSCs [124]. They reprogrammed iNKT cells to pluripotency and then redifferentiated the cells into iNKT cells in vitro. The iPSC-derived iNKT cells showed TCR-dependent proliferation and IFN-γ production after α-GalCer stimulation. In vitro cytotoxicity against the tumor cell line K562 (leukemia) and U937 (lymphoma) were also observed [124]. In a separate study, the Fujii group also generated functional iPSC-iNKT cells and then

tested them in vitro and in vivo [98]. These cells possessed a significant antitumor activity in a K562 xenograft mouse model, and activated human NK cells by adjuvant effect [98]. The data from this preclinical study also led to the first clinical trial using iPSC-derived iNKT cells to treat patients with head-and-neck cancer. This phase I trial is expected to last for 2 years and involves 4–18 patients.

Challenges and perspectives

Recent progress in our understanding of iNKT cells has paved the way for iNKT cell-based cancer therapies. However, promising findings in preclinical studies have not yet convincingly translated to similar outcomes in human clinical trials. In fact, many challenges limit the overall performance of iNKT cell-based immunotherapies, including insufficient antitumor activity, limited in vivo persistence, and immunosuppressive TME. Various approaches are currently being explored to address these concerns.

The addition of CARs to iNKT cells to more potently direct iNKT cell-mediated cytotoxicity against refractory tumors holds promise to revolutionize cancer treatment. This approach has been demonstrated to enhance antitumor efficacy against neuroblastoma in preclinical studies and is under further investigation in clinical trials. Compared to conventional CAR-T therapy, CAR-iNKT cells are less likely to cause GvHD. However, iNKT are also highly inflammatory and may induce the cytokine release/storm syndrome (CRS) as a side effect to rapid tumor lysis. Indeed, CRS is a common concern, especially for all CAR-T therapies. So far, the fast-evolving CAR-T cell therapy has accumulated valuable clinical experiences on managing CRS (e.g., anti-IL-6 antibody treatment) that can be adapted for guiding the iNKT cell-based therapies.

Expansion and persistence of iNKT cells following infusion appear to be another determinant of clinical response. Importantly, both mouse and human studies have highlighted the central role of IL-15 in iNKT cell homeostasis [125,126]. So far, transgenic expression of IL-15 in adoptively transferred iNKT cells have improved iNKT in vivo persistence without causing significant toxicity, and is therefore a potential approach for general application to maximize NKT persistence and efficacy [97].

Although iNKT cells are known to modulate immunosuppressive cells in the TME, they are frequently suppressed in cancer patients due to nutrient deprivation, hypoxia, acidity, and accumulation of toxic by-products of catabolism [55]. One strategy that can partially reverse the dysfunction of iNKT cells is through blocking inhibitory pathways such as PD-1 and CTLA-4. While these “immune checkpoints” are predominantly defined in the context of $CD8^+$ T cells, iNKT cells also showed upregulated PD-1 expression following stimulation with α-GalCer [127–129]. Indeed, blockade of PD-1 at the time of α-GalCer injection prevents the anergy of iNKT cells [127–129]. Considering the broad use of checkpoint inhibitors in the clinic, future combination treatments may synergize the antitumor efficacies of iNKT cells. Another strategy involves the blockade of inhibitory cytokines present in the TME, such as TGF-β. The use of dominant negative receptors on T cells is also being actively explored and has shown some promises [130], suggesting a potential role in enhancing iNKT cell-mediated antitumor immunity.

Furthermore, the possibility of third-party "off-the-shelf" iNKT products derived from standardized, allogeneic sources can improve the practicality of iNKT cell therapy. With increasing focus on improving the persistence and functions of iNKT cells, it is likely that iNKT cells will move to the forefront of cancer therapy over the next few years.

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References

[1] Godfrey DI, Stankovic S, Baxter AG. Raising the NKT cell family. Nat Immunol 2010;11(3):197–206.
[2] Brennan PJ, Brigl M, Brenner MB. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. Nat Rev Immunol 2013;13(2):101–17.
[3] Bendelac A, Savage PB, Teyton L. The biology of NKT cells. Annu Rev Immunol 2007;25:297–336.
[4] Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, et al. CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science 1997;278(5343):1626–9.
[5] Brossay L, Chioda M, Burdin N, Koezuka Y, Casorati G, Dellabona P, et al. CD1d-mediated recognition of an α-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. J Exp Med 1998;188(8):1521–8.
[6] Dasgupta S, Kumar V. Type II NKT cells: a distinct CD1d-restricted immune regulatory NKT cell subset. Immunogenetics 2016;68(8):665–76.
[7] Marrero I, Ware R, Kumar V. Type II NKT cells in inflammation, autoimmunity, microbial immunity, and cancer. Front Immunol 2015;6:316.
[8] Dhodapkar MV, Kumar V. Type II NKT cells and their emerging role in health and disease. J Immunol 2017;198(3):1015–21.
[9] Terabe M, Berzofsky JA. The immunoregulatory role of type I and type II NKT cells in cancer and other diseases. Cancer Immunol Immunother 2014;63(3):199–213.
[10] Nair S, Dhodapkar MV. Natural killer T cells in cancer immunotherapy. Front Immunol 2017;8:1178.
[11] Kumar A, Suryadevara N, Hill TM, Bezbradica JS, Van Kaer L, Joyce S. Natural killer T cells: an ecological evolutionary developmental biology perspective. Front Immunol 2017;8:1858.
[12] Bedard M, Salio M, Cerundolo V. Harnessing the power of invariant natural killer T cells in cancer immunotherapy. Front Immunol 2017;8:1829.
[13] Reilly EC, Wands JR, Brossay L. Cytokine dependent and independent iNKT cell activation. Cytokine 2010;51(3):227–31.
[14] Krijgsman D, Hokland M, Kuppen PJ. The role of natural killer T cells in cancer—a phenotypical and functional approach. Front Immunol 2018;9:367.
[15] Brigl M, Bry L, Kent SC, Gumperz JE, Brenner MB. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. Nat Immunol 2003;4(12):1230–7.
[16] Wesley JD, Tessmer MS, Chaukos D, Brossay L. NK cell–like behavior of Vα14i NK T cells during MCMV infection. PLoS Pathog 2008;4(7), e1000106.
[17] Tyznik AJ, Tupin E, Nagarajan NA, Her MJ, Benedict CA, Kronenberg M. Cutting edge: the mechanism of invariant NKT cell responses to viral danger signals. J Immunol 2008;181(7):4452–6.

[18] Rossjohn J, Pellicci DG, Patel O, Gapin L, Godfrey DI. Recognition of CD1d-restricted antigens by natural killer T cells. Nat Rev Immunol 2012;12(12):845–57.
[19] Wolf BJ, Choi JE, Exley MA. Novel approaches to exploiting invariant NKT cells in cancer immunotherapy. Front Immunol 2018;9:384.
[20] Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang Z-E, Gapin L, et al. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. J Exp Med 2003;198(7):1069–76.
[21] Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. J Immunol 2007;178(5):2706–13.
[22] Gumperz JE, Miyake S, Yamamura T, Brenner MB. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J Exp Med 2002;195(5):625–36.
[23] Lee PT, Benlagha K, Teyton L, Bendelac A. Distinct functional lineages of human Vα24 natural killer T cells. J Exp Med 2002;195(5):637–41.
[24] Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, et al. Development and function of invariant natural killer T cells producing TH 2-and TH 17-cytokines. PLoS Biol 2012;10(2), e1001255.
[25] Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Sato H, et al. Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Vα14 NKT cells. Proc Natl Acad Sci 1998;95(10):5690–3.
[26] Wingender G, Krebs P, Beutler B, Kronenberg M. Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178-dependent and is correlated with antigenic potency. J Immunol 2010;185(5):2721–9.
[27] Bae E-A, Seo H, Kim I-K, Jeon I, Kang C-Y. Roles of NKT cells in cancer immunotherapy. Arch Pharm Res 2019;42(7):543–8.
[28] Berzins SP, Smyth MJ, Baxter AG. Presumed guilty: natural killer T cell defects and human disease. Nat Rev Immunol 2011;11(2):131–42.
[29] Lam PY, Nissen MD, Mattarollo SR. Invariant natural killer T cells in immune regulation of blood cancers: harnessing their potential in immunotherapies. Front Immunol 2017;8:1355.
[30] Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. Nat Rev Immunol 2012;12(4):239–52.
[31] Bassiri H, Das R, Guan P, Barrett DM, Brennan PJ, Banerjee PP, et al. iNKT cell cytotoxic responses control T-lymphoma growth in vitro and in vivo. Cancer Immunol Res 2014;2(1):59–69.
[32] Dao T, Mehal WZ, Crispe IN. IL-18 augments perforin-dependent cytotoxicity of liver NK-T cells. J Immunol 1998;161(5):2217–22.
[33] Leite-de-Moraes MC, Hameg A, Arnould A, Machavoine F, Koezuka Y, Schneider E, et al. A distinct IL-18-induced pathway to fully activate NK T lymphocytes independently from TCR engagement. J Immunol 1999;163(11):5871–6.
[34] Hagihara M, Gansuvd B, Ueda Y, Tsuchiya T, Masui A, Tazume K, et al. Killing activity of human umbilical cord blood-derived TCRValpha24+ NKT cells against normal and malignant hematological cells in vitro: a comparative study with NK cells or OKT3 activated T lymphocytes or with adult peripheral blood NKT cells. Cancer Immunol Immunother 2002;51(1):1–8.
[35] Spanoudakis E, Hu M, Naresh K, Terpos E, Melo V, Reid A, et al. Regulation of multiple myeloma survival and progression by CD1d. Blood 2009;113(11):2498–507.
[36] Tiper IV, Webb TJ. Histone deacetylase inhibitors enhance CD1d-dependent NKT cell responses to lymphoma. Cancer Immunol Immunother 2016;65(11):1411–21.
[37] Nowak M, Arredouani MS, Tun-Kyi A, Schmidt-Wolf I, Sanda MG, Balk SP, et al. Defective NKT cell activation by CD1d+ TRAMP prostate tumor cells is corrected by interleukin-12 with alpha-galactosylceramide. PLoS ONE 2010;5(6), e11311.
[38] Miura S, Kawana K, Schust DJ, Fujii T, Yokoyama T, Iwasawa Y, et al. CD1d, a sentinel molecule bridging innate and adaptive immunity, is downregulated by the human papillomavirus (HPV) E5 protein: a possible mechanism for immune evasion by HPV. J Virol 2010;84(22):11614–23.
[39] Hix LM, Shi YH, Brutkiewicz RR, Stein PL, Wang C-R, Zhang M. CD1d-expressing breast cancer cells modulate NKT cell-mediated antitumor immunity in a murine model of breast cancer metastasis. PLoS ONE 2011;6(6), e20702.
[40] Yang P-M, Lin P-J, Chen C-C. CD1d induction in solid tumor cells by histone deacetylase inhibitors through inhibition of HDAC1/2 and activation of Sp1. Epigenetics 2012;7(4):390–9.
[41] Metelitsa LS. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. Clin Immunol 2011;140(2):119–29.

[42] Kuylenstierna C, Björkström NK, Andersson SK, Sahlström P, Bosnjak L, Paquin-Proulx D, et al. NKG2D performs two functions in invariant NKT cells: direct TCR-independent activation of NK-like cytolysis and co-stimulation of activation by CD1d. Eur J Immunol 2011;41(7):1913–23.
[43] Parekh VV, Lalani S, Kaer LV. The in vivo response of invariant natural killer T cells to glycolipid antigens. Int Rev Immunol 2007;26(1–2):31–48.
[44] Hermans IF, Silk JD, Gileadi U, Salio M, Mathew B, Ritter G, et al. NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. J Immunol 2003;171(10):5140–7.
[45] S-i F, Shimizu K, Smith C, Bonifaz L, Steinman RM. Activation of natural killer T cells by α-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. J Exp Med 2003;198(2):267–79.
[46] Coquet JM, Kyparissoudis K, Pellicci DG, Besra G, Berzins SP, Smyth MJ, et al. IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production. J Immunol 2007;178(5):2827–34.
[47] Chung Y, Kim B-S, Kim Y-J, Ko H-J, Ko S-Y, Kim D-H, et al. CD1d-restricted T cells license B cells to generate long-lasting cytotoxic antitumor immunity in vivo. Cancer Res 2006;66(13):6843–50.
[48] de Jong EC, Smits HH, Kapsenberg ML. Dendritic cell-mediated T cell polarization. In: Springer seminars in immunopathology. Springer; 2005. p. 289–307.
[49] Kitamura H, Iwakabe K, Yahata T, Nishimura S-I, Ohta A, Ohmi Y, et al. The natural killer T (NKT) cell ligand α-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. J Exp Med 1999;189(7):1121–8.
[50] Fujii SI, Shimizu K, Hemmi H, Steinman RM. Innate Vα14+ natural killer T cells mature dendritic cells, leading to strong adaptive immunity. Immunol Rev 2007;220(1):183–98.
[51] Cui J, Shin T, Kawano T, Sato H, Kondo E, Toura I, et al. Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. Science 1997;278(5343):1623–6.
[52] Taraban VY, Martin S, Attfield KE, Glennie MJ, Elliott T, Elewaut D, et al. Invariant NKT cells promote CD8+ cytotoxic T cell responses by inducing CD70 expression on dendritic cells. J Immunol 2008;180(7):4615–20.
[53] Semmling V, Lukacs-Kornek V, Thaiss CA, Quast T, Hochheiser K, Panzer U, et al. Alternative cross-priming through CCL17-CCR4-mediated attraction of CTLs toward NKT cell–licensed DCs. Nat Immunol 2010;11(4):313–20.
[54] Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 2018;24(5):541–50.
[55] Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science 2015;348(6230):74–80.
[56] Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity 2014;41(1):49–61.
[57] Song L, Asgharzadeh S, Salo J, Engell K, Wu H-W, Sposto R, et al. Vα24-invariant NKT cells mediate antitumor activity via killing of tumor-associated macrophages. J Clin Invest 2009;119(6):1524–36.
[58] Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 2012;12(4):253–68.
[59] De Santo C, Salio M, Masri SH, Lee LY-H, Dong T, Speak AO, et al. Invariant NKT cells reduce the immunosuppressive activity of influenza A virus–induced myeloid-derived suppressor cells in mice and humans. J Clin Invest 2008;118(12):4036–48.
[60] Ko H-J, Lee J-M, Kim Y-J, Kim Y-S, Lee K-A, Kang C-Y. 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 2009;182(4):1818–28.
[61] June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med 2018;379(1):64–73.
[62] Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. Nat Rev Clin Oncol 2018;15(1):47.
[63] Bedoya F, Frigault MJ, Maus MV. The flipside of the power of engineered T cells: observed and potential toxicities of genetically modified T cells as therapy. Mol Ther 2017;25(2):314–20.
[64] Ma S, Li X, Wang X, Cheng L, Li Z, Zhang C, et al. Current progress in CAR-T cell therapy for solid tumors. Int J Biol Sci 2019;15(12):2548.
[65] Haraguchi K, Takahashi T, Hiruma K, Kanda Y, Tanaka Y, Ogawa S, et al. Recovery of V α 24+ NKT cells after hematopoietic stem cell transplantation. Bone Marrow Transplant 2004;34(7):595–602.

[66] Pillai AB, George TI, Dutt S, Teo P, Strober S. Host NKT cells can prevent graft-versus-host disease and permit graft antitumor activity after bone marrow transplantation. J Immunol 2007;178(10):6242–51.
[67] Rubio M-T, Moreira-Teixeira L, Bachy E, Bouillié M, Milpied P, Coman T, et al. Early posttransplantation donor-derived invariant natural killer T-cell recovery predicts the occurrence of acute graft-versus-host disease and overall survival. Blood 2012;120(10):2144–54.
[68] De Lalla C, Rinaldi A, Montagna D, Azzimonti L, Bernardo ME, Sangalli LM, et al. Invariant NKT cell reconstitution in pediatric leukemia patients given HLA-haploidentical stem cell transplantation defines distinct CD4+ and CD4− subset dynamics and correlates with remission state. J Immunol 2011;186(7):4490–9.
[69] Brennan PJ, Tatituri RV, Brigl M, Kim EY, Tuli A, Sanderson JP, et al. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. Nat Immunol 2011;12(12):1202.
[70] Cox D, Fox L, Tian R, Bardet W, Skaley M, Mojsilovic D, et al. Determination of cellular lipids bound to human CD1d molecules. PLoS ONE 2009;4(5), e5325.
[71] Fox LM, Cox DG, Lockridge JL, Wang X, Chen X, Scharf L, et al. Recognition of lyso-phospholipids by human natural killer T lymphocytes. PLoS Biol 2009;7(10), e1000228.
[72] Muindi K, Cernadas M, Watts GF, Royle L, Neville DC, Dwek RA, et al. Activation state and intracellular trafficking contribute to the repertoire of endogenous glycosphingolipids presented by CD1d. Proc Natl Acad Sci 2010;107(7):3052–7.
[73] Yuan W, Kang S-J, Evans JE, Cresswell P. Natural lipid ligands associated with human CD1d targeted to different subcellular compartments. J Immunol 2009;182(8):4784–91.
[74] Torikai H, Cooper LJ. Translational implications for off-the-shelf immune cells expressing chimeric antigen receptors. Mol Ther 2016;24(7):1178–86.
[75] Shissler SC, Lee MS, Webb TJ. Mixed signals: co-stimulation in invariant natural killer T cell-mediated cancer immunotherapy. Front Immunol 2017;8:1447.
[76] Robertson FC, Berzofsky JA, Terabe M. NKT cell networks in the regulation of tumor immunity. Front Immunol 2014;5:543.
[77] Song L, Ara T, Wu H-W, Woo C-W, Reynolds CP, Seeger RC, et al. Oncogene MYCN regulates localization of NKT cells to the site of disease in neuroblastoma. J Clin Invest 2007;117(9):2702–12.
[78] Liu D, Song L, Wei J, Courtney AN, Gao X, Marinova E, et al. IL-15 protects NKT cells from inhibition by tumor-associated macrophages and enhances antimetastatic activity. J Clin Invest 2012;122(6):2221–33.
[79] Cerundolo V, Silk JD, Masri SH, Salio M. Harnessing invariant NKT cells in vaccination strategies. Nat Rev Immunol 2009;9(1):28–38.
[80] Tyznik AJ, Verma S, Wang Q, Kronenberg M, Benedict CA. Distinct requirements for activation of NKT and NK cells during viral infection. J Immunol 2014;192(8):3676–85.
[81] Kawano T, Nakayama T, Kamada N, Kaneko Y, Harada M, Ogura N, et al. Antitumor cytotoxicity mediated by ligand-activated human Vα24 NKT cells. Cancer Res 1999;59(20):5102–5.
[82] Toura I, Kawano T, Akutsu Y, Nakayama T, Ochiai T, Taniguchi M. Cutting edge: inhibition of experimental tumor metastasis by dendritic cells pulsed with α-galactosylceramide. J Immunol 1999;163(5):2387–91.
[83] Nishimura T, Kitamura H, Iwakabe K, Yahata T, Ohta A, Sato M, et al. The interface between innate and acquired immunity: glycolipid antigen presentation by CD1d-expressing dendritic cells to NKT cells induces the differentiation of antigen-specific cytotoxic T lymphocytes. Int Immunol 2000;12(7):987–94.
[84] Shin T, Nakayama T, Akutsu Y, Motohashi S, Shibata Y, Harada M, et al. Inhibition of tumor metastasis by adoptive transfer of IL-12-activated Vα14 NKT cells. Int J Cancer 2001;91(4):523–8.
[85] Smyth MJ, Crowe NY, Pellicci DG, Kyparissoudis K, Kelly JM, Takeda K, et al. Sequential production of interferon-γ by NK1. 1+ T cells and natural killer cells is essential for the antimetastatic effect of α-galactosylceramide. Blood 2002;99(4):1259–66.
[86] Giaccone G, Punt CJ, Ando Y, Ruijter R, Nishi N, Peters M, et al. A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors. Clin Cancer Res 2002;8(12):3702–9.
[87] Nieda M, Okai M, Tazbirkova A, Lin H, Yamaura A, Ide K, et al. Therapeutic activation of Vα24+ Vβ11+ NKT cells in human subjects results in highly coordinated secondary activation of acquired and innate immunity. Blood 2004;103(2):383–9.
[88] Ishikawa A, Motohashi S, Ishikawa E, Fuchida H, Higashino K, Otsuji M, et al. A phase I study of α-galactosylceramide (KRN7000)–pulsed dendritic cells in patients with advanced and recurrent non–small cell lung cancer. Clin Cancer Res 2005;11(5):1910–7.

[89] Chang DH, Osman K, Connolly J, Kukreja A, Krasovsky J, Pack M, et al. Sustained expansion of NKT cells and antigen-specific T cells after injection of α-galactosyl-ceramide loaded mature dendritic cells in cancer patients. J Exp Med 2005;201(9):1503–17.
[90] Uchida T, Horiguchi S, Tanaka Y, Yamamoto H, Kunii N, Motohashi S, et al. Phase I study of α-galactosyl-ceramide-pulsed antigen presenting cells administration to the nasal submucosa in unresectable or recurrent head-and-neck cancer. Cancer Immunol Immunother 2008;57(3):337–45.
[91] Motohashi S, Nagato K, Kunii N, Yamamoto H, Yamasaki K, Okita K, et al. A phase I-II study of α-galactosyl-ceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer. J Immunol 2009;182(4):2492–501.
[92] Nagato K, Motohashi S, Ishibashi F, Okita K, Yamasaki K, Moriya Y, et al. Accumulation of activated invariant natural killer T cells in the tumor microenvironment after α-galactosylceramide-pulsed antigen presenting cells. J Clin Immunol 2012;32(5):1071–81.
[93] Motohashi S, Ishikawa A, Ishikawa E, Otsuji M, Iizasa T, Hanaoka H, et al. 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 2006;12(20):6079–86.
[94] Kunii N, Horiguchi S, Motohashi S, Yamamoto H, Ueno N, Yamamoto S, et al. Combination therapy of in vitro-expanded natural killer T cells and α-galactosylceramide-pulsed antigen-presenting cells in patients with recurrent head and neck carcinoma. Cancer Sci 2009;100(6):1092–8.
[95] Yamasaki K, Horiguchi S, Kurosaki M, Kunii N, Nagato K, Hanaoka H, et al. Induction of NKT cell-specific immune responses in cancer tissues after NKT cell-targeted adoptive immunotherapy. Clin Immunol 2011;138(3):255–65.
[96] Exley MA, Friedlander P, Alatrakchi N, Vriend L, Yue S, Sasada T, et al. Adoptive transfer of invariant NKT cells as immunotherapy for advanced melanoma: a phase I clinical trial. Clin Cancer Res 2017;23(14):3510–9.
[97] Xu X, Huang W, Heczey A, Liu D, Guo L, Wood M, et al. NKT cells coexpressing a GD2-specific chimeric antigen receptor and IL15 show enhanced in vivo persistence and antitumor activity against neuroblastoma. Clin Cancer Res 2019;25(23):7126–38.
[98] Yamada D, Iyoda T, Vizcardo R, Shimizu K, Sato Y, Endo TA, et al. Efficient regeneration of human Vα24+ invariant natural killer T cells and their anti-tumor activity in vivo. Stem Cells 2016;34(12):2852–60.
[99] Smith DJ, Liu S, Ji S, Li B, McLaughlin J, Cheng D, et al. Genetic engineering of hematopoietic stem cells to generate invariant natural killer T cells. Proc Natl Acad Sci 2015;112(5):1523–8.
[100] Zhu Y, Smith DJ, Zhou Y, Li Y-R, Yu J, Lee D, et al. Development of hematopoietic stem cell-engineered invariant natural killer T cell therapy for cancer. Cell Stem Cell 2019;25(4):542–57. e9.
[101] Lan F, Zeng D, Higuchi M, Higgins JP, Strober S. Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. Biol Blood Marrow Transplant 2003;9(6):355–63.
[102] Schneidawind D, Pierini A, Alvarez M, Pan Y, Baker J, Buechele C, et al. CD4+ invariant natural killer T cells protect from murine GVHD lethality through expansion of donor CD4+ CD25+ FoxP3+ regulatory T cells. Blood 2014;124(22):3320–8.
[103] Schneidawind D, Baker J, Pierini A, Buechele C, Luong RH, Meyer EH, et al. Third-party CD4+ invariant natural killer T cells protect from murine GVHD lethality. Blood 2015;125(22):3491–500.
[104] Lowsky R, Takahashi T, Liu YP, Dejbakhsh-Jones S, Grumet FC, Shizuru JA, et al. Protective conditioning for acute graft-versus-host disease. N Engl J Med 2005;353(13):1321–31.
[105] Kohrt HE, Turnbull BB, Heydari K, Shizuru JA, Laport GG, Miklos DB, et al. TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors. Blood 2009;114(5):1099–109.
[106] Chaidos A, Patterson S, Szydlo R, Chaudhry MS, Dazzi F, Kanfer E, et al. Graft invariant natural killer T-cell dose predicts risk of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. Blood 2012;119(21):5030–6.
[107] Patterson S, Chaidos A, Neville DC, Poggi A, Butters TD, Roberts IA, et al. Human invariant NKT cells display alloreactivity instructed by invariant TCR-CD1d interaction and killer Ig receptors. J Immunol 2008;181(5):3268–76.
[108] Pillai AB, George TI, Dutt S, Strober S. Host natural killer T cells induce an interleukin-4–dependent expansion of donor CD4+ CD25+ Foxp3+ T regulatory cells that protects against graft-versus-host disease. Blood 2009;113 (18):4458–67.

[109] Hashimoto D, Asakura S, Miyake S, Yamamura T, Van Kaer L, Liu C, et al. Stimulation of host NKT cells by synthetic glycolipid regulates acute graft-versus-host disease by inducing Th2 polarization of donor T cells. J Immunol 2005;174(1):551–6.
[110] Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. Front Immunol 2019;10:128.
[111] Heczey A, Liu D, Tian G, Courtney AN, Wei J, Marinova E, et al. Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. Blood 2014;124(18):2824–33.
[112] Tian G, Courtney AN, Jena B, Heczey A, Liu D, Marinova E, et al. CD62L+ NKT cells have prolonged persistence and antitumor activity in vivo. J Clin Invest 2016;126(6):2341–55.
[113] Heczey A, Courtney AN, Montalbano A, Robinson S, Liu K, Li M, et al. Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis. Nat Med 2020;26(11):1686–90.
[114] Rotolo A, Caputo VS, Holubova M, Baxan N, Dubois O, Chaudhry MS, et al. Enhanced anti-lymphoma activity of CAR19-iNKT cells underpinned by dual CD19 and CD1d targeting. Cancer Cell 2018;34(4):596–610. e11.
[115] Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med 2017;9(374), eaaj2013.
[116] Iriguchi S, Kaneko S. Toward the development of true "off-the-shelf" synthetic T-cell immunotherapy. Cancer Sci 2019;110(1):16–22.
[117] Themeli M, Kloss CC, Ciriello G, Fedorov VD, Perna F, Gonen M, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. Nat Biotechnol 2013;31(10):928–33.
[118] Montel-Hagen A, Seet CS, Li S, Chick B, Zhu Y, Chang P, et al. Organoid-induced differentiation of conventional T cells from human pluripotent stem cells. Cell Stem Cell 2019;24(3):376–89. e8.
[119] Nagano S, Maeda T, Ichise H, Kashima S, Ohtaka M, Nakanishi M, et al. High frequency production of T cell-derived iPSC clones capable of generating potent cytotoxic T cells. Mol Ther Methods Clin Dev 2020;16:126–35.
[120] Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. Cell Stem Cell 2018;23(2):181–92. e5.
[121] Zhu H, Kaufman DS. An improved method to produce clinical-scale natural killer cells from human pluripotent stem cells. In: Vitro differentiation of T-Cells. Springer; 2019. p. 107–19.
[122] Bernareggi D, Pouyanfard S, Kaufman DS. Development of innate immune cells from human pluripotent stem cells. Exp Hematol 2019;71:13–23.
[123] Zhu H, Blum RH, Bjordahl R, Gaidarova S, Rogers P, Lee TT, et al. Pluripotent stem cell–derived NK cells with high-affinity noncleavable CD16a mediate improved antitumor activity. Blood 2020;135(6):399–410.
[124] Kitayama S, Zhang R, Liu T-Y, Ueda N, Iriguchi S, Yasui Y, et al. Cellular adjuvant properties, direct cytotoxicity of re-differentiated Vα24 invariant NKT-like cells from human induced pluripotent stem cells. Stem Cell Rep 2016;6(2):213–27.
[125] Matsuda JL, Gapin L, Sidobre S, Kieper WC, Tan JT, Ceredig R, et al. Homeostasis of V α 14 i NKT cells. Nat Immunol 2002;3(10):966–74.
[126] Baev DV, Peng X-H, Song L, Barnhart JR, Crooks GM, Weinberg KI, et al. Distinct homeostatic requirements of CD4+ and CD4-subsets of Vα24-invariant natural killer T cells in humans. Blood 2004;104(13):4150–6.
[127] Durgan K, Ali M, Warner P, Latchman YE. Targeting NKT cells and PD-L1 pathway results in augmented anti-tumor responses in a melanoma model. Cancer Immunol Immunother 2011;60(4):547–58.
[128] Parekh VV, Lalani S, Kim S, Halder R, Azuma M, Yagita H, et al. PD-1/PD-L blockade prevents anergy induction and enhances the anti-tumor activities of glycolipid-activated invariant NKT cells. J Immunol 2009;182(5):2816–26.
[129] Chang W-S, Kim J-Y, Kim Y-J, Kim Y-S, Lee J-M, Azuma M, et al. Cutting edge: programmed death-1/programmed death ligand 1 interaction regulates the induction and maintenance of invariant NKT cell anergy. J Immunol 2008;181(10):6707–10.
[130] Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, et al. Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. Mol Ther 2018;26(7):1855–66.
[131] Nicol AJ, Tazbirkova A, Nieda M. Comparison of clinical and immunological effects of intravenous and intradermal administration of α-galactosylceramide (KRN7000)-pulsed dendritic cells. Clin Cancer Res 2011;17(15):5140–51.