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REVIEW

CAR Cells beyond Classical CAR T Cells: Functional Properties and Prospects of Application

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Abstract—Chimeric antigen receptors (CARs) are genetically engineered receptors that recognize antigens and activate signaling cascades in a cell. Signal recognition and transmission are mediated by the CAR domains derived from different proteins. T cells carrying CARs against tumor-associated antigens have been used in the development of the CAR T cell therapy, a new approach to fighting malignant neoplasms. Despite its high efficacy in the treatment of oncohematological diseases, CAR T cell therapy has a number of disadvantages that could be avoided by using other types of leukocytes as effector cells. CARs can be expressed in a wide range of cells of adaptive and innate immunity with the emergence or improvement of cytotoxic properties. This review discusses the features of CAR function in different types of immune cells, with a particular focus on the results of preclinical and clinical efficacy studies and the safety of potential CAR cell products.

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Keywords: chimeric antigen receptor, immunotherapy, cell therapy, CAR, CAR T cell, CAR NK cell

INTRODUCTION

A chimeric receptor consisting of the variable immunoglobulin domain and constant regions of the T cell receptor (TCR) was first created in 1987 [1]. It recognized the bacterial antigen phosphorylcholine and was expressed in EL4 lymphoblastic T cells. The first T lymphocytes with a chimeric antigen receptor (CAR) were obtained in 1993 [2]. This receptor recognized 2,4,6-trinitrophenol and was one of the first generation of CARs. It consisted of the extracellular antigen-recognizing single-chain variable fragment (scFv) linked by a transmembrane region to the intracellular CD3ζ signaling domain (a fragment of the endogenous TCR) [3]. CAR T cells expressing the first-generation receptors were poorly effective against malignant cells because, despite of their strong cytotoxic properties, they were easily exhausted [4]. In 2002, second-generation CARs were obtained. They contained the CD28 costimulatory domain between the signaling and transmembrane domains [5]. Second-generation CAR T cells targeting the CD19 antigen were effective in recognizing and eliminating B cell tumors in a mouse model [6]. Since then, CAR T lymphocytes targeting various tumor-associated antigens have been actively developed.

CAR T therapy has been particularly effective in the treatment of hematological oncological diseases. To date, the Food and Drug Administration (FDA) has approved six CAR-T therapies for the treatment of B-cell neoplasms [7]. So far, CAR-T products that have

Abbreviations: CAR, chimeric antigen receptor; CAR M, macrophage expressing CAR; CAR T, T cell expressing CAR; CBCR, chimeric B cell receptor; CIK, cytokine-induced killers; CRS, cytokine release syndrome; DC, dendritic cell; DN T cell, double-negative T cell; GD2, disialoganglioside GD2; GVHD, graft-versus-host disease; iNKT cell, invariant NKT cell; MAIT cell, mucosal-associated invariant T cell; MHC, major histocompatibility complex; NK, natural killer cell; NKT cells, natural killer T cell; NSCAR, non-signaling CAR; TCR, T cell receptor; Treg, regulatory T cell.

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successfully passed clinical trials and have been approved by FDA are based on the second-generation CARs containing CD28 or 4-1BB signaling domains as costimulatory domains. Other variants of CAR T cells containing receptors with different immunomodulatory domains are being researched and developed. For example, third-generation CARs containing two costimulatory domains have been developed, although their clinical use has been limited due to strong severe side effects. CAR T cells expressing fourth-generation CARs also secrete cytokines that increase the persistence of these cells in the tumor microenvironment [8].

Despite significant success in the treatment of oncohematological diseases, CAR T cell therapy has a number of drawbacks. For example, it becomes ineffective when malignant cells lose the tumor antigens targeted by the CARs [8]. CAR T cells are often unable to infiltrate solid tumors and recognize the antigen. In addition, the cytotoxic function of CAR T cells is significantly influenced by the immunosuppressive tumor microenvironment. Currently, progress in the treatment of solid tumors with CAR T cells has reached its limits [9]. Finally, the secretion of pro-inflammatory cytokines by activated CAR T cells can lead to severe side effects, such as cytokine release syndrome (CRS) and neurotoxicity [10], which are due to the rapid activation and proliferation of T cells producing pro-inflammatory cytokines and typically develop within one week of CAR T cell administration. It has been suggested that excessive release of pro-inflammatory cytokines increases capillary permeability in the brain, leading to severe neurological symptoms and even death, although the molecular mechanisms of neurotoxicity in the case of CAR T cell therapy are not fully understood [10]. The adverse effects of this therapy are often due to the poor regulation of the activity of CAR T cells, which can sometimes be activated even in the absence of an antigenic stimulus [11].

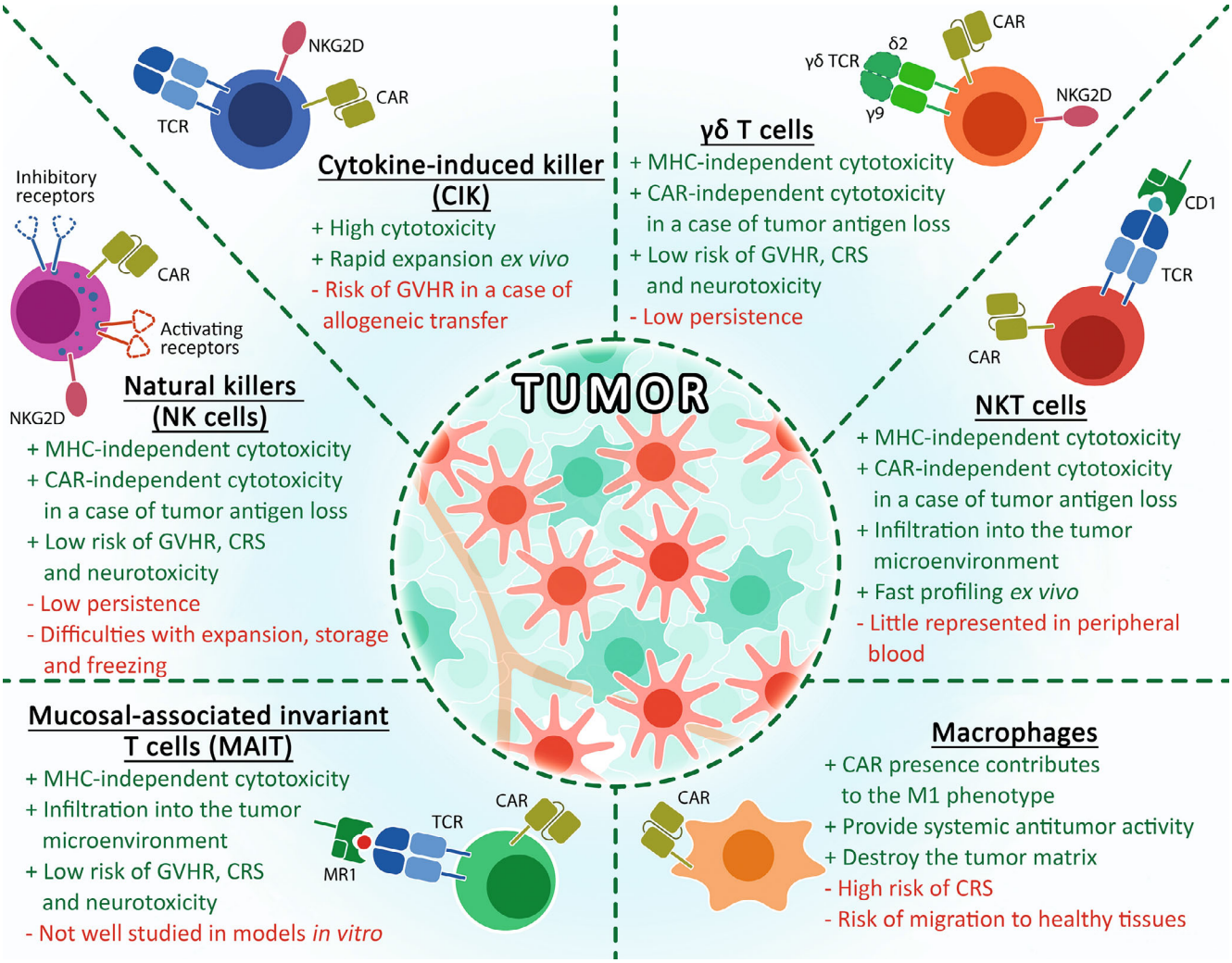

Fig. 1. Advantages and disadvantages of different types of immune cells in the development of CAR-bearing cell products. Nomenclature: TCR, T cell receptor; CAR, chimeric antigen receptor; NKG2D, activating receptor; MR1, non-canonical major histocompatibility complex close to class I; CD1, non-canonical major histocompatibility complex close to class I; CRS, cytokine release syndrome; MHC, major histocompatibility complex; GVHD, graft-versus-host disease.

To overcome these drawbacks, new strategies for the CAR T cell generation are being actively being developed [12, 13], including the use of different (non-T cells) leukocytes as CAR-bearing cells (Fig. 1). CAR-expressing cells have been derived from γδ T cells, regulatory T cells (Tregs), mucosal-associated invariant T cells (MAIT cells), double-negative T cells (DN T cells), natural killer (NK) cells, natural killer T cells (NKT cells), cytokine-induced killers (CIKs), macrophages, dendritic cells (DCs), and even B lymphocytes.

Preclinical studies have shown that many alternative immune CAR cells with a high antitumor activity have fewer or none of the typical drawbacks of CAR T cells. However, such cells are poorly studied. Only a few cell products have entered phase I/II clinical trials; some have preclinical data, while others are still under development. This review discusses the features of CAR function in different types of immune cells and the results of available preclinical and clinical studies of CAR-bearing immune cells.

CARs IN DIFFERENT TYPES OF IMMUNE CELLS

Below we discuss the properties of different immune cells that could be used in the development of novel CAR-mediated immunotherapies, focusing on the results of preclinical and, if available, clinical studies of CAR expression and efficacy in these cells.

Natural killer (NK) cells are innate immune cells of the lymphoid lineage. NK cells account approximately 10% of peripheral blood lymphocytes [14]. Unlike T lymphocytes, they recognize malignant and infected cells with a variety of non-polymorphic activating and inhibitory receptors. The directionality of the NK cell response in each case is the result of a balance of various signals. When the signals from the activating receptors outweigh those from the inhibitory receptors, an NK cell causes a lysis of the target cell. NK cells express a variety of activating and inhibiting receptors on their surface, the most important among them are receptors that recognize the major histocompatibility complex MHC I and MHC I-like molecules. Cells that express these molecules are recognized as healthy by NK cells [15]. It is important to note that red blood cells do not carry the ligands (either activating or inhibiting) for the NK cell receptors on their surface and therefore are not recognized as targets by these cells. In addition to MHC, NK cells can recognize other cell surface ligands, in particular, the stress markers MICA, MICB, and UL16BP1, due to the presence of specific receptors, including the NKG2D receptor, which is also present on the surface of γδ T cells [16]. In addition, the expression of FcγRIIIa allows NK cells to recognize and kill cells opsonized by antibodies [17].

The cytotoxic function of NK cells is manifested by the formation of an immunological synapse between the NK cell and the target cell and the subsequent secretion of granzyme-containing lytic granules. NK cells can also kill target cells by inducing programmed cell death through FasL and TRAIL. Finally, activated NK cells release a number of pro-inflammatory cytokines, in particular, interferon γ (IFNγ) and tumor necrosis factor (TNF) [14].

The cytotoxic properties of NK cells and the lack of need for the antigen presentation in the MHC context required for the recognition of target cells (unlike in T cells), have significantly contributed to the development of anti-cancer therapies based on genetically modified NK cells, including those expressing CARs. In most studies, NK cells have been transduced with the CAR constructs originally developed for the CAR T cells therapy. In addition to the CD28 and 4-1BB costimulatory domains, some of the CARs expressed in NK cells contained the 2B4 costimulatory domain (Fig. 2). The 2B4 receptor is one of the activating receptors of the SLAM (signaling lymphocytic activation molecule) family on NK cells. It is important to note that CAR constructs originally designed for expression in T cells can also function in NK cells due to the presence of common signaling pathways that control cell activation in both cell types. In particular, the signaling from some activating receptors in NK cells involves the CD3ζ signaling domain which is intended for use in T cells [18]. NK cells transduced with the second-generation 2B4-containing CAR targeting CD5 had a higher cytotoxic activity against malignant cells and showed more rapid proliferation and enhanced cytokine production compared to NK cells expressing CARs with the CD28 domain [19]. In addition to the CD3ζ domain, CARs developed for the expression in NK cells can contain the DAP10 and DAP12 domains, which are involved in the signaling from a number of activating NK cell receptors. It has been shown that the antitumor activity observed for the constructs with the CD3ζ domain was higher than that of those with the DAP10 domain, but lower than the activity of CARs with the DAP12 domain [20, 21]. Cifaldi et al. [22] proposed the use of DNAM-1 as a part of a CAR adapted to NK cells. DNAM-1 recognizes the poliovirus receptor (PVR) and nectin-2, which are expressed on the virus-infected cells and many malignant cells. Receptors containing the 2B4 and CD3ζ domains in addition to DNAM-1 cause further activation of NK cells.

It should be emphasized that CAR-expressing NK cells can potentially exhibit cytotoxic activity against malignant cells in a CAR-independent manner due to their own activating receptors, as well as their ability to recognize cells opsonized by antibodies [20, 23]. The presence of a CAR-independent antitumor activity enhances the efficacy of the CAR NK cell therapy.

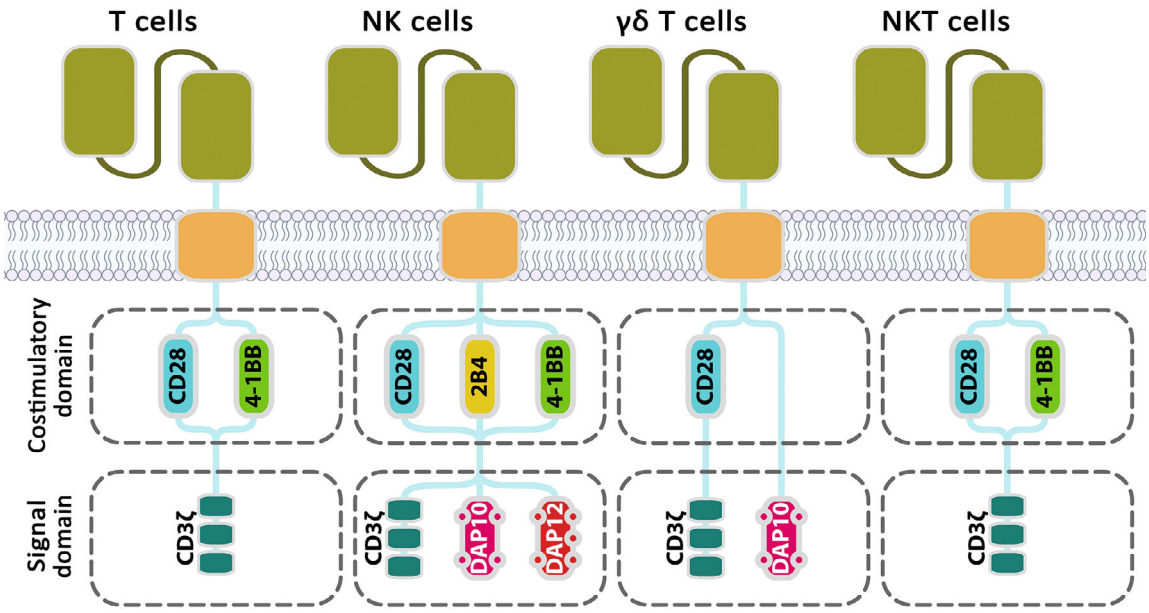

Fig. 2. Structure of CARs used in the development of products based on different cell types. Nomenclature: NK cells, natural killer cells; NKT cells, natural killer T cells.

In addition, the diversity of activating receptors allows CAR NK cells to recognize and destroy tumor cells with an altered phenotype that have survived a long-term treatment [24]. NK cells treated with exogenous IL-12 and IL-18 acquire a phenotype similar to that of memory cells. The efficacy of such cells has already been demonstrated in phase I trials in patients with relapsed or resistant acute myeloid leukemia. Additional expression of a CAR directed against nucleophosmin 1 (NPM1) in these cells increased the efficacy of the therapy and reduced the incidence of side effects [25]. An increased sensitivity of NK cells to reactive oxygen species (compared to T and B cells) can be reduced by expression of peroxiredoxin 1, which would promote the persistence of CAR NK cells in the acidified environment of solid tumors [26].

Preclinical and clinical studies have shown that CAR NK cells lack many of the disadvantages of CAR T cells. Since NK cells do not carry variable TCRs, their adoptive transfer does not lead to the development of the graft-versus-host disease (GVHD) [27, 28]. This opens up the possibility for the producing off-the-shelf allogeneic cell preparations suitable for many patients at the same time. In addition, CAR NK cell transfer carries almost no risk of CRS or neurotoxicity [29, 30]. Finally, CAR NK cells have been shown in preclinical studies to be effective not only against oncohematological diseases, but also in the treatment of solid tumors [31]. Currently, many developed CAR NK cell preparations, including those against solid tumors, have moved from preclinical studies to clinical trials.

Several dozen clinical trials have been registered to investigate the use of CAR NK cells directed against various tumor antigens, both as a monotherapy and in combination with other therapeutic approaches. For example, in one trial (NCT04847466), the participants treated with CAR NK cells against PD-L1 also received the immunostimulant N-803, which induces proliferation and activation of NK and $CD8^+$ T cells, and pembrolizumab (monoclonal antibody against PD-1). NK cells sequentially transfected with the chemokine receptor CXCR4 and a CAR directed against B cell maturation antigen (BCMA) efficiently destroyed multiple myeloma cells [32]. Currently, all clinical trials of the CAR NK cell therapy are in phase I/II. Several registered trials are testing CAR NK cells against oncohematological diseases, such as multiple myeloma (target, BCMA; NCT05008536), B cell lymphomas (target, CD19; NCT05379647) and acute lymphoblastic leukemia (target, CD19; NCT05563545). Clinical trials investigating the efficacy and safety of CAR NK cells in the treatment of various solid tumors are ongoing: ovarian cancer (target, claudin 6; NCT05410717); colon cancer (target, NKGD2L; NCT05213195); pancreatic cancer (target, ROBO1; NCT03941457); prostate cancer (target, PSMA; NCT03692663); and others (table). The successful completion of phase I in several clinical trials of CAR NK cells has demonstrated the high safety of this type of therapy and the almost complete absence of side effects [33].

Despite many advantages, CAR NK cell therapy has some limitations. CAR NK cells are characterized by a low persistence after adoptive transfer: the lifespan of CAR T cells in a patient's body can be up to 10 years [34], while the lifespan of CAR NK cells does not exceed several weeks [35]. CAR NK cells are

Some registered CAR NK cell clinical trials

Clinical trial	Started in	Phase	Disease	Target
NCT02892695	2016	I/II	lymphomas and leukemias	CD19
NCT03940833	2019	I/II	multiple myeloma	BCMA
NCT02742727	2016	I/II	lymphomas and leukemias	CD7
NCT02944162	2016	I/II	acute myeloid leukemia	CD33
NCT02839954	2016	I/II	solid tumors	MUCL
NCT03383978	2017	I	glioblastoma	HER2
NCT03415100	2018	I	metastatic solid tumors	NKG2DL
NCT03941457	2019	I/II	pancreatic cancer	ROBO1
NCT03692663	2018	I	prostate cancer	PSMA
NCT05410717	2022	I/II	ovarian cancer	claudin 6 (CLDN6)
NCT05194709	2021	I	developed solid tumors	5T4
NCT05507593	2022	I	non-small cell lung cancer	DLL3

difficult to expand *ex vivo* and do not tolerate freezing and storage well [36]. However, there is hope that these technical difficulties will be soon be overcome. In particular, it has been shown that the persistence of CAR NK cells can be doubled by knocking out the *CISH* gene, which encodes the CIS protein, a negative regulator of the IL-15 signaling pathway. The absence of CIS in CAR NK cells activates IL-15 secretion and, since CAR NK cells carry IL-15 receptors on their surface, causes autocrine activation of the IL-15 signaling pathway. Activation of IL-15-mediated signaling, in turn, promotes the expansion of CAR NK cells *ex vivo*, increases their persistence and enhances the antitumor properties of these cells [37]. Incorporation of IL-15 into a CAR construct can also improve the cell metabolic status of the cells and activate the effector functions of CAR NK cells that are attenuated by the interaction with metabolically active tumors [38].

Another factor that can reduce the efficacy of CAR NK therapy is the capture of tumor antigens by the therapeutic CAR NK cells via the trogocytosis mechanism. After capturing the tumor antigen, CAR NK cells become the target of the therapy and are destroyed by other NK cells via the fratricide pathway, while the amount of tumor antigen on the targeted malignant cells decreases. The risk of fratricide among CAR NK cells can be reduced by co-expression of activating CARs directed against the tumor antigen and inhibitory CARs that recognize antigens specific for NK cells [39].

γδ T cells. Approximately 3.7% of T cells circulating in the bloodstream have TCRs formed by the γ and δ chains on their surface [40, 41]. Such “non-classical” (as opposed to “classical” αβ T cells) γδ T cells differ significantly from αβ T cells, which carry TCRs formed by the α and β chains and constitute a predominant population of circulating T cells. Recognition of antigens by αβ T cells is only possible when the antigen is presented in the context of the MHC, whereas γδ T cells do not require the involvement of classical MHC molecules in the antigen recognition, greatly expanding the possibilities for their transfer between different organisms and significantly reducing the likelihood of complications such as GVHD. Relatively safe adoptive transfer of γδ T cells is also possible because the repertoire of γ and δ chains in a population is much less diverse than the repertoire of α and β chains, so that γδ T cells tend to recognize molecular signatures common to different individuals, indicating the development of an infectious process or the emergence of malignant cells. For this reason, they are often thought to be similar to the cells of the innate immunity. γδ T cells also resemble innate immune cells in the expression of Toll-like receptors and receptors similar to the activating receptors of NK cells, in particular, NKG2D [42]. However, γδ T cells are also capable of forming immunological memory and are therefore classified as components of the adaptive immunity [43]. γδ T cells can differentiate into Th-like cells and produce a wide range of cytokines [44].

Most circulating γδ T cells are Vγ9Vδ2 T cells. Their TCRs consist of the Vγ9 and Vδ2 segments of the γ and δ chains, respectively. Since Vγ9Vδ2 T cells recognize

phosphoantigens, synthetic analogs such as zoledronic acid are used to expand Vγ9Vδ2 T cells *ex vivo* [45]. γδ T cells, which have the Vδ1 segment in the TCRs, localize mainly to the mucosa [23].

The role of γδ T cells in providing antitumor protection was first demonstrated in mice lacking these cells. In these mice, chemical mutagens caused early development of squamous cell carcinoma of the skin [46]. Later, an important role of γδ T cells in antitumor immunity has been demonstrated in various tumor models. In particular, the extent of malignant tumor infiltration by γδ T cells has been shown to correlate with a favorable prognosis in many types of cancer, such as melanoma [47] and gastric cancer [48]. It is believed that the activity of γδ T cells against malignant cells is ensured by their cytotoxic properties, as well as the production of IFNγ and TNF [33]. The cytotoxicity of γδ T cells is provided by the action of perforin and granzymes; the cells are also capable of antibody-dependent cellular cytotoxicity. Antitumor activity is inherent to both Vγ9Vδ2 and Vδ1 T cells [45, 48, 49].

γδ T cells have both the antitumor and pro-tumor properties. The pro-tumor effect of γδ T cells is usually due to the production of interleukin-17 (IL-17) and some other cytokines. For example, in pancreatic cancer, γδ T cells suppress the activity of αβ T cells by secreting IL-10 and IL-17 and promoting PD-L1 expression in tumor cells [50].

The strong intrinsic antitumor activity of γδ T cells, together with a highly safe adoptive transfer, makes these cells a promising tool in the immunotherapy of oncological diseases. Since the transfer of unmodified γδ T cells to a patient after their *ex vivo* expansion appeared to be safe but ineffective [45], the enhancement of the antitumor activity of γδ T cells via CAR expression has been actively investigated. It was shown that CD19-directed CAR γδ T cells effectively recognized and destroyed malignant cells, including those that had lost CD19. The cytotoxic properties of CAR γδ T cells were enhanced by their treatment with zoledronic acid [51, 52]. The ability of CAR γδ T lymphocytes to kill malignant blood cells that have lost antigen is due to the high non-specific cytotoxicity of γδ T cells towards leukemia cells, which is further enhanced by zoledronic acid during *ex vivo* expansion of CAR γδ T cells. Such retention of cytotoxic properties against malignant blood cells that have lost the antigen is an important advantage of CAR γδ T cells [52].

CAR γδ T cells can also be effective against solid tumors. It has been shown that CAR γδ T cells directed against the neuroblastoma antigen (GD2) not only destroy malignant cells, but also present antigens to and activate αβ T cells *in vitro*. It is possible that CAR γδ T cells also present antigens and activate αβ T cells *in vivo*, but further experiments are needed to clarify this issue [23].

Most CARs that have been successfully expressed in γδ T cells are the second-generation receptors and contain CD28 and CD3ζ as costimulatory and signaling domains, respectively [23] (Fig. 2). In [53], the first-generation GD-recognizing CAR with a single intracellular domain DAP10 was introduced into γδ T cells. It should be noted that γδ T cells express of costimulatory molecules, such as CD28, CD27, and 4-1BB, which contribute to the activation of these cells during signaling by CARs [54].

Some studies have used γδ T cells to create a combinatorial antigen recognition system acting as logical operators. For example, Fisher et al. [53] obtained γδ CAR T cells expressing GD2-recognizing DAP10-CAR and γδ TCR that bound to phosphoantigens expressed by malignant (but not normal) cells. These γδ CAR T cells were activated only by interaction with the target cells exposing both antigens recognized by the CAR and TCR, thus significantly reducing the likelihood of killing off-target cells.

Fleischer et al. [55] obtained γδ T cells expressing non-signaling CAR (NSCAR), which lack intracellular activation domains and are therefore unable to trigger the cytotoxic response upon antigen recognition. NSCAR-expressing γδ T cells can recognize the tumor antigens CD19 and CD5 and, through this interaction, approach malignant cells. However, direct antitumor activity requires endogenous MHC-independent cytotoxicity of γδ T cells. In acute lymphoblastic leukemia, γδ T cells expressing NSCARs were found to be more cytotoxic against B and T cells than γδ T cells expressing CARs against the same antigens (CD19 and CD5). Expression of NSCARs instead of CARs in αβ T cells did not significantly increase their cytotoxic properties, suggesting that the molecular mechanisms underlying the enhanced antitumor activity of γδ T cells expressing NSCARs are fundamentally different from those of αβ T cells [55].

Several ongoing clinical trials are focusing on the use of γδ CAR T cells in the immunotherapy of different types of cancer, such as acute myeloid leukemia (targets, CD33 and CD123; NCT03885076, NCT04796441), relapsed or resistant $CD7^+$ T cell leukemia (target, CD7; NCT04702841), various B cell oncohematological diseases (targets, CD20 and CD19; NCT04735471, NCT02656147, NCT04911478) and relapsed or resistant solid tumors of various origins (target, NKG2DL, NCT04107142, NCT05302037). To date, all these clinical trials are in phase I, so it is too early to assess the clinical efficacy of γδ CAR T cells. It is believed that the γδ CAR T cell-based therapy will have an advantage over conventional CAR T cell therapy by reducing the risk of developing side effects, such as CRS and neurotoxicity [49]. Studies on the function of γδ CAR T cells *in vivo* have shown that these cells only persist in the body for a short time [52], which may be a significant limitation for their clinical application.

NKT cells are similar to γδ T cells and combine the properties of adaptive and innate immune cells. NKT cells represent less than 1% of T cells in the blood [56] and express αβ TCRs, as well as molecules characteristic of NK cells, such as CD16 (FcγR) and CD56 [57]. Unlike T lymphocytes, NKT cells recognize antigens only in the context of CD1d, a non-classical MHC molecule close to MHC class I. NKT cells are usually classified into invariant NKT (iNKT) cells, or type I NKT cells, and type II NKT cells. iNKT cells are characterized by a restricted TCR repertoire and recognize α-galactosylceramide (α-GalCer), whereas type II NKT cells are characterized by a broader TCR repertoire and do not recognize α-GalCer [58].

The number of NKT cells in the blood is often reduced in patients with various malignancies, particularly oncohematological ones, and the functions of these cells (e.g., IFNγ production) are also impaired [60]. An increased number of NKT cells in the peripheral blood, as well as NKT cell infiltration into the tumor tend to correlate with a favorable prognosis for patients [61, 62]. Only iNKT cells have a pronounced antitumor effect, whereas type II NKT cells can even suppress the immune response against malignant cells. The cytotoxicity of iNKT cells, which is mediated by granzymes or FasL, is promoted by the recognition of the lipid antigen associated with CD1d [63]. In addition to direct lysis of tumor cells, iNKT cells can modulate the activity of other immune cells (in particular, DCs, NK cells, B and T lymphocytes) through the secretion of pro- and anti-inflammatory cytokines, such as IFNγ, TNF, IL-4, IL-10, and IL-13 [64, 65]. Cytokines released by iNKT cells can also affect the tumor microenvironment and thus indirectly influence the immune response against tumors [64]. NKT cells with a transient CAR expression produced less IL-6 than transduced $CD8^+$ CAR T lymphocytes, while maintaining their cytotoxic activity. For this reason, it has been suggested that the risk of CRS is lower with CAR NKT therapy than with CAR-T therapy, but the issue needs further clarification [66].

Some properties of NKT cells make them particularly attractive as effectors for CAR therapy. Their intrinsic antitumor activity (not only the cytotoxic properties, but also the ability to rearrange the tumor environment) can complement the CAR-mediated effect against malignant cells. Since NKT cells recognize antigens in the context of non-polymorphic CD1d, their allogeneic adoptive transfer does not cause GVHD, allowing the generation of allogeneic CAR iNKT cells from the cells of healthy donors [23]. In addition, NKT cells are able to proliferate rapidly *ex vivo* and are relatively easy to obtain in the quantities required to produce a clinical product [56].

In the first work on the generation of CAR iNKT cells, the expressed CAR was directed against the neuroblastoma antigen GD2 and contained the 4-1BB and CD3ζ signaling domains (Fig. 2). 4-1BB was shown to polarize CAR iNKT cells towards the Th1 phenotype, enabling these cells to efficiently destroy neuroblastoma cells *in vivo* and persist in the body for a long time [67]. CD62L expression has been shown to be a marker of increased persistence and anti-tumor activity of NKT cells, including those expressing CARs. Administration of anti-CD19 CAR NKT cells expressing CD62L to mice with the B cell lymphoma resulted in a significant disease regression [68]. The study of the efficacy of CAR NKT cells recognizing chondroitin sulfate proteoglycan 4 (CSPG4; melanoma cell antigen) showed that CAR NKT cells destroyed melanoma cells *in vitro* no less efficiently than CAR T cells [69]. The generation of NKT cells expressing the third-generation CARs directed against glypican-3 (hepatocellular carcinoma cell antigen) were obtained and containing CD28 and 4-1BB costimulatory domains has recently been announced [70].

Advances in the preclinical application of CAR NKT cells have led to the initiation of clinical trials for the treatment of neuroblastoma (target, GD2; NCT03294954, NCT02439788), relapsed or resistant B cell lymphoma (target, CD19; NCT03774654) and other B cell neoplasms (target, CD19; NCT05487651). The small number of CAR NKT clinical trials is probably due to the insufficient preclinical research. Another major limitation for the production of clinical products from CAR NKT cells is the low number of NKT cells in the peripheral blood.

Regulatory T cells (Tregs) are a specialized subset of $CD4^+$ T lymphocytes with the immunosuppressive function. Tregs account for 5 to 10% of $CD4^+$ T cells in the circulation [71]. The inhibitory influence of Tregs on the effector T cells or antigen-presenting cells can be direct or indirect. In the first case, Tregs act on target cells by secreting anti-inflammatory cytokines (IL-35, IL-10, or TGF-β) or by releasing granzymes from the lytic granules, resulting in the target cell death. Indirect mechanisms include targeting of other cells exposed to Tregs [72].

The ability of Tregs to inhibit effector immune cells makes them potentially useful for therapeutic applications and in various conditions associated with an exaggerated immune response, such as transplantation. However, the first studies of the therapeutic properties of polyclonal Tregs showed that their adoptive transfer induces non-specific tolerance, reduces the body's resistance to infection and increases the risk of developing malignant neoplasms [73]. CAR expression allows Tregs to selectively attack target tissues while maintaining the original inhibitory activity of these cells and avoiding induction of unwanted immunological tolerance. The first-generated CAR Tregs were intended for the therapy of colitis in a mouse model [74];

soon after, the development of the first human CAR Tregs was reported [75].

In most studies of CAR Tregs, these cells expressed second-generation CARs with the CD28 or 4-1BB as costimulatory domains. Systematic analysis of the activity of second-generation CARs directed against the human leukocyte antigen HLA-A2 with different costimulatory domains in Tregs showed that CARs with CD28 had the most pronounced activity. The suppressive properties of these cells were further increased by the expression of IL-10 [76]. CAR Tregs with the CD28 domain showed better persistence than CAR Tregs with the 4-1BB domain (unlike classic CAR T) [77, 78]. Tregs expressing third-generation CARs with both CD28 and 4-1BB costimulatory domains are currently being studied [79-81].

In the decade since the first CAR Treg cells were generated, several CAR Tregs with different specificities have been obtained. These cells are characterized by a high efficacy, stability, and increased persistence in mouse models [82]. CAR Tregs are also characterized by a reduced requirement for IL-2 compared to CAR T [83]. CAR Treg prevent or reduce the symptoms of GVHD, disorders associated with the excessive activity of the immune system, hemophilia, and other diseases in mouse models [73]. Currently, only one CAR Treg study has entered clinical trial (NCT04817774), which aims to assess the efficacy and safety of CAR Treg in kidney transplantation. In this research, CARs recognize HLA-A2 in the recipient organ, so that CAR Tregs are attracted to the antigen and suppress the immune response.

Mucosal-associated T cells. Mucosal-associated invariant T cells (MAIT) recognize metabolites of vitamins B2 and B9 of bacterial origin in a complex with the non-classical MHC molecule MR1 [84]. MAIT cells account for 1 to 8% of peripheral blood T cells [85] and are localized to mucous membranes and lymphoid tissues. MAIT cell TCRs consist of non-polymorphic α and β chains; most MAIT cells also express CD8. They also carry granzyme-containing lytic granules and have cytotoxic properties [86]. When activated, MAIT cells secrete the pro-inflammatory cytokines IFNγ, TNF, and IL-17 [87].

MAIT cells are involved in protection against mostly bacterial infections and in the pathogenesis of many non-communicable diseases, such as autoimmune disorders, inflammatory bowel disease, celiac disease, and cancer [84]. The level of MAIT cells in the blood decreases in colorectal cancer. MAIT cells migrate into the tumors, where they exert their antitumor cytotoxic effect by releasing granzymes [88]. A reduction in the number of MAIT cells is also observed in patients with multiple myeloma [89]. In some cases, actively migrating MAIT cells become immunosuppressed by the tumor microenvironment and lose the ability to secrete IFNγ [90].

The fact that MAIT cells actively infiltrate tumor microenvironment and have the cytotoxic properties suggests that they can be used in the development of novel anti-tumor immunotherapeutic approaches. Since MAIT cells only recognize antigens associated with the non-classical MHC class I molecule MR1, the likelihood of GVHD development when using CAR MAIT cells would be lower than in the case of cytotoxic CAR T cells [23]. Dogan et al. [86] expressed CARs against CD19 or HER2 antigens in primary human MAIT cells and showed that the resulting cells were cytotoxic effects against B cell lymphoma and breast cancer cells, respectively. The activated CAR MAIT cells were not inferior to $CD8^+$ CAR T lymphocytes in their cytotoxicity against target cells, but produced significantly lower levels of IFNγ and other pro-inflammatory cytokines. This latter observation suggests that the likelihood of CRS and neurotoxicity following injection of CAR MAIT cells into the patient's body may be lower than with CAR T cells [87]. Further evaluation of the safety and antitumor properties of CAR MAIT cells should be performed in *in vivo* in laboratory animals and in clinical trials.

Double negative (DN) T cells. DN T cells are characterized by CD3 expression in the absence of CD4 and CD8 expression. DN T cells also express CD25 [91], a molecule that is highly expressed by Tregs. TCRs of DN T cells can contain both αβ and γδ chains. DN T cells make up 3 to 5% of T cells in the blood [92]. They exert a strong suppressive effect on several groups of immune cells ($CD4^+$ and $CD8^+$ T cells, B cells, and NK cells) both *in vitro* and *in vivo*. Thus, DN T cells play an important role in the prevention of GVHD and the maintenance of tolerance to allografts and xenografts [93-95]. Thus, DN T cells can be considered as a non-canonical subset of Tregs.

Despite their immunosuppressive effect on many immune cells, DN T cells possess their own MHC-independent cytotoxic properties. DN T cells can induce malignant cell death via FasL [96], TRAIL, and other cytotoxicity-associated surface proteins [97]. They also express perforin and granzymes and secrete TNF and IFNγ [97]. In addition to MHC-independent cytotoxicity, DN T cells have other properties, such as the possibility of easy expansion *ex vivo* [98], lack of extra-tumor cytotoxic activity, and reduced risk of rejection after allogeneic transfer [99], which allow their use in immunotherapy of malignant diseases.

The possibility of CAR expression in DN T cells has been poorly investigated. In 2022, Vasic et al. [99] reported the generation of DN T cells with the CD19-directed CAR and compared their antitumor properties with traditional CAR T cells directed against the same antigen. The authors found that the CAR DN T cells were not inferior to conventional CAR T cells in their *in vitro* and *in vivo* anti-tumor properties, but did

not cause GVHD [99]. However, these results require independent confirmation by other investigators.

Cytokine-induced killer (CIK) cells are a heterogeneous group of $CD8^+$ T cells that are phenotypically and functionally close to NK cells [100, 101]. CIKs are derived from $CD3^+$ T lymphocytes that begin to express CD56 during expansion. Initially, CIKs were derived from the lymphokine-activated killer cells (LAKs, lymphocytes with the ability to lyse tumor cells after incubation with IL-2 [102]) during the optimization of the expansion protocol. LAKs had already been obtained in the 1980s [103], but despite their strong cytotoxic properties against tumor cells, the difficulties in expanding these cells *ex vivo* have limited their use. The timed addition of IFNγ, IL-2, and anti-CD3 monoclonal antibody (which had the mitogenic effect) to LAKs led to the development of CIKs [104]. Currently, these cells can be produced relatively cheaply from peripheral or umbilical cord blood T cells.

The cytotoxic effect of CIKs requires the involvement of the NKG2D receptor and is manifested by the release of the contents of granzyme-containing lytic granules. NKG2D recognizes stress-induced molecules, such as UL16BP and MICA/MICB, on the surface of target cells [105]. As TCR signaling is also possible, so the use of CIKs has for a long time been limited to autologous therapy due to the fear of acute GVHD. However, it has been recently been shown that the use of donor-based CIKs after hematopoietic stem cells allotransplantation is relatively safe, which may be due to the short persistence of terminally differentiated $CD3^+CD56^+$ cells and the reduced expression of some homing receptors [106].

Due to their strong cytotoxic properties, rapid *ex vivo* expansion, tumor cell recognition via NKG2D, and safety of allogeneic adoptive transfer, CIKs are considered as promising effectors for CAR cell therapy [107]. Several preclinical studies have demonstrated the efficacy of CAR CIKs against hematological and solid tumors. For example, CIKs expressing a CAR directed against CD19 and containing CD28/4-1BB and CD3ζ domains efficiently destroyed B cell acute lymphoblastic leukemia cells [108, 109]. CIKs bearing CARs to relevant tumor antigens effectively destroyed colon cancer cells, acute myeloid leukemia cells, soft tissue sarcoma cells, and cells of other tumor [107]. Expression of CARs in CIKs leads to increased production of IFNγ and TNF compared to CIKs that do not express CARs [110, 111].

As of 2022, there is only one clinical trial investigating the efficacy and safety of CD19-directed CAR CIKs in acute lymphoblastic leukemia (NCT03389035). Further studies are therefore needed to provide information on the therapeutic efficacy of CAR CIKs.

Macrophages as specialized phagocytic cells that heavily infiltrate the stroma of solid tumors and are an important component of the tumor microenvironment. Following recognition of foreign agents by innate immune receptors (mainly Toll-like and NOD-like receptors), macrophages become activated and start to produce pro-inflammatory cytokines (TNF, IL-1β, IL-6, IL-12, and IL-23) that affect many cells in the tumor microenvironment and in particular promote the antitumor activity of T cells and NK cells. Macrophages with the pro-inflammatory properties have the so-called M1 phenotype, whereas macrophages with M2 phenotype inhibit inflammation and the development of antitumor T cell immunity and promote tumor growth by producing IL-4, IL-5, and IL-13 cytokines. The major physiological role of M2 macrophages is wound healing [112]. Macrophages in the tumor microenvironment are known as tumor-associated macrophages (TAMs). The TAMs with M2 phenotype promote tumor growth and metastasis and has the immunosuppressive properties [66]. Therefore, polarization of TAMs towards the M1 phenotype, which contributes to the destruction of neoplasia, is an important component of the anti-tumor immunotherapy using these cells.

The phagocytic capacity of macrophages can be enhanced by the expression of CARs bearing intracellular domains that trigger phagocytosis-activating signaling. In the one of the first studies focusing on CAR macrophages (CAR Ms), primary human monocytes were transduced with a CAR directed against carcinoembryonic antigen (CEA) and bearing CD64 (FcγRI) as a signaling domain. The resulting CAR Ms showed antitumor activity *in vitro* and *in vivo*, although the molecular mechanism of this activity remained unclear [113]. Further studies showed that expression of CARs containing the phagocytic receptor Megf10 or FcγR as signaling domains promoted the phagocytic activity of macrophages [114, 115]. Phagocytosis was also activated by the expression of CARs with the CD3ζ signaling domain, which is homologous to FcγRI.

Macrophages expressing CARs directed against CD19 with Megf10 or FcγR domains efficiently destroyed CD19-positive tumor cells. Phagocytosis was only observed for a small fraction of malignant cells, while the majority were destroyed by trogocytosis [116]. Zhang et al. [116] derived macrophages expressing second-generation anti-CD19 CAR and possessing CD3ζ signaling and 4-1BB costimulatory domains from the induced pluripotent stem cells (iPSCs). These macrophages phagocytized tumor cells in culture and produced pro-inflammatory cytokines, but had little effect on the tumor growth *in vivo* [117].

It has been shown that the process of CAR M generation itself induces their polarization into pro-inflammatory cells. In particular, it was shown that transduction with a CAR-encoding adenoviral vector and subsequent CAR expression polarized macrophages towards the M1 phenotype, which was maintained

in the tumor microenvironment [118]. In addition, CAR Ms are capable of cross-presenting tumor antigens after phagocytosis. Macrophages expressing CARs directed against HER2 (breast cancer antigen) phagocytized tumor cells and presented the processed antigens. CAR Ms have been shown to activate systemic antitumor immunity [119]. A single administration of CAR Ms in a mouse model of ovarian cancer significantly prolonged animal survival and slowed (but did not completely suppress) tumor development [115]. Nevertheless, the ability of CAR Ms to transform the tumor microenvironment into a pro-inflammatory one makes them an additional tool in the CAR T cell therapy or other immunotherapies.

Macrophages can produce matrix metalloproteinases that cause significant changes in the tumor extracellular matrix and affect the architecture of the neoplasm, which can be exploited in the CAR therapy. Expression of CARs directed against HER2 and containing CD147 as a transmembrane/intracellular domain in macrophages stimulated the secretion of matrix metalloproteinases by these cells. Using a mouse model of breast cancer, it was shown that administration of these CAR Ms led to the tumor matrix rearrangement, which promoted T cell infiltration into the tumor. Levels of CRS-associated pro-inflammatory cytokines (IFNγ, TNF, and IL-6) in the blood of mice with breast cancer were reduced, while the levels of IL-12 and IFNγ in the tumor were increased [120]. It is reasonable to assume that CAR M therapy is associated with a reduced risk of CRS.

At present, there is not enough information to draw conclusions about the efficacy and safety of CAR M therapy in humans. The fact that macrophages are one of the main cell types of that trigger the CRS requires a detailed review of the safety of CAR M therapy. In addition, peripheral blood monocytes are heterogeneous, and there is a possibility that CAR Ms derived from these cells may migrate to healthy tissues more often than to the tumor when used systemically [23], which may lead to significant side effects in different organ systems. To date, only one HER2-overexpressing CAR M against solid tumors (NCT04660929) has entered the clinical trial.

Dendritic cells. As “professional” antigen-presenting cells, DCs are involved in the maturation of naive T cells and the reactivation of memory T cells. During antigen presentation to T cells, DCs produce cytokines that modulate T cell activity. When present in the tumor microenvironment, DCs can both induce immune tolerance and contribute to the development of the antitumor immune response [121, 122]. In addition, DCs are able to cross-present tumor antigens to the cytotoxic $CD8^+$ T cells, thereby promoting the antitumor response [123].

The potential of CAR-expressing DCs in controlling of the antitumor activity of CAR T cells has been poorly investigated. Suh et al. [123] investigated the possibility of using CAR-expressing DCs (CAR DCs) to attract to the tumor microenvironment and to activate CAR T cells through the cytokine secretion. Expression of CARs against CD33 on DCs promoted the homing of these cells to the bone marrow in mice with acute myeloid leukemia. Mice receiving CAR DCs and CAR T cells targeting CD33 had significantly elevated levels of IL-12, IFNγ, and TNF, and the overall survival of these animals was longer than that of mice receiving CAR T cells alone. Thus, co-administration of CAR DCs and CAR T cells significantly increased the efficiency of malignant cell destruction [124]. The safety of clinical application of CAR DCs should be investigated, as IL-12 secreted in high concentrations may have a systemic toxic effect [125]. To date, there are no registered clinical trials focusing on CAR DCs.

B cells. In addition to T cells and innate immune cells, B cells are also of interest for CAR technology. Because of their ability to differentiate into long-lived antibody-secreting plasma cells, B lymphocytes could become a safe and controlled source of therapeutic monoclonal antibodies. By expressing CARs against tumor antigens in these cells, targeted antibody delivery can avoid the side effects of systemic antibody administration.

Despite these prospects, the studies on the CAR-expressing B cells and their therapeutic use are currently limited. The possibility of lentiviral transduction of B cells and subsequent expression of CARs in them was described in 2018, when a patient experienced a relapse of B cell lymphoma caused by a single B cell clone accidentally transduced with a CAR [126]. Soon after, Pesch et al. [126] demonstrated that genes encoding chimeric B cell receptors (CBCRs) could be inserted into B cells using the CRISPR/Cas9 genome editing technology. The CBCR used in this study contained the transmembrane region CD28 and the BCR signaling domain CD79β instead of the classical CD3ζ. Introducing of the gene encoding this CBCR into primary mouse B cells resulted in the abundant presence of CBCR on their surface, allowing the cells to recognize antigens without the involvement of their own B cell receptors [127]. The antitumor activity and the therapeutic efficacy of CAR-expressing B cells, as well as their safety in humans, are currently unknown.

ONE TARGET – MANY TYPES OF CAR CELLS

Early studies focused on the antitumor effects of CAR-expressing leukocytes of different types have used a small set of tumor-associated antigens, including CD19, GD2 and others as CAR targets. At present, CAR cells that effectively recognize tumor antigens have been derived from different types of white blood cells.

The generation of CAR cells that recognize the same tumor antigen from different cell sources could provide scope for selecting the optimal therapy for each patient, as the effector properties of different groups of white blood cells are different.

Four out of the six FDA-approved CAR T cell therapies target the CD19 antigen and are designed to treat B cell neoplasias. Despite proven high efficacy, the anti-CD19 CAR T cell therapy is associated with serious side effects, primarily CRS and neurotoxicity, in a significant proportion of patients. For example, the use of axicabtagene ciloleucel (CAR T cells with the CD28 costimulatory domain and CD3ζ signaling domain) causes CRS in 93% of patients and neurotoxicity in 67% [128, 129]. To date, CAR cells that efficiently recognize and destroy CD19-positive malignant cells have been derived from γδ T cells [52], MAIT cells [87], NKT cells [68], NK cells [29], DN T cells [100], CIKs [108] and macrophages [114, 115]. Tregs expressing CD19 have been engineered to suppress the antibody production and prevent GVHD [130].

Preclinical studies and studies in small groups of patients, have shown that anti-CD19 CAR cells, which are different from the cytotoxic T cells, have a strong antitumor effect with fewer side effects than the CAR T cell therapy. However, many of the cell products mentioned above have not yet reached full-scale clinical trials, while those that have are still in the early stages of clinical testing, so it is too early to draw conclusions about their safety and efficacy.

Two other FDA-approved CAR T cell therapies, idecabtagene vicleucel and ciltacabtagene autoleucel, which target the BCMA antigen, have been developed for the treatment of multiple myeloma. As with the anti-CD19 CAR T therapy, the high efficacy of these drugs is associated with a significant risk of CRS or neurotoxicity: 76 and 42% for idecabtagene vicleucel and 92 and 20% for ciltacabtagene autoleucel, respectively [131, 132]. It has been reported that CAR NK cells targeting BCMA have been obtained [133], but have not yet entered clinical trials.

There are also CAR cells targeting the same tumor-associated antigen, derived from different types of white blood cells, intended for the therapy of solid tumors. For example, GD2-recognizing CAR cells have been derived from γδ T cells and NKT cells. Currently, GD2-recognizing CAR NKT cells are in phase I clinical trials, so the safety and efficacy of both cell products remain to be investigated.

CONCLUSION

Over the past 20 years, the CAR T cell approach has been marked by a recognized success in the treatment of hematologic cancers, and six CAR T cell therapies have been approved for clinical use by the FDA. Despite the drawbacks of "traditional" CAR T cell therapy, the possibility of generating CAR cells from other types of white blood cells has long been overlooked. The results of preclinical studies of the efficacy and safety of CAR cells in cell culture and mouse models suggest a wide range of applications for CAR cells in the treatment of human cancer, including solid tumors. Data on the expression of CARs against different targets and in different leukocyte subsets are summarized in Table 1 in the article by Qin et al. [33]. Currently, existing cellular products are only in phase I/II of clinical trials, while the vast majority of therapeutic drugs based on CAR-expressing immune cells have not yet progressed beyond the preclinical studies.

Although alternative CAR cell products have significant advantages over traditional CAR T cells (e.g., reduced risk of CRS and neurotoxicity), the latter are still superior in a number of parameters. Since the signaling pathways underlying the activity of CARs are different in different groups of adaptive and innate immune cells, the choice of costimulatory and signaling domains is crucial for the development of CARs intended for the expression in different types of immune cells. Inclusion of a domain that is not sufficiently functional in a particular cell type can lead to a reduction in the CAR activity and therapeutic efficacy. For example, in macrophages, the Megf10 signaling domain in the CAR construct is more efficient than the CD3ζ domain, which has shown optimal activity in T cells. Therefore, each new CAR-expressing cell may require "customization" of the receptors and identification of domains that provide the required level of signaling from the activated receptor. In the case of traditional CAR T cells, the choice of domains for CARs has been much better studied. In addition, some immune cells are difficult to expand *ex vivo*, and obtaining sufficient numbers of these cells can be expensive and time-consuming, significantly limiting the ability to produce clinically relevant products. However, in some cases, it is possible to find alternative sources of white blood cells that can at least partially overcome the problem of cell expansion. For example, CAR NK cells may be derived from cell lines and iPSCs [20]; CAR Ms may be derived from monocytes and iPSCs [116]. However, this method of generating CAR cells may be associated with a potential risk of tumor formation due to the high oncogenic potential of iPSCs, and therefore its safety needs to be further investigated.

Despite significant limitations associated with the development of CAR-mediated therapies based on different types of white blood cells, these cells have good prospects in the immunotherapy of solid tumors. The use of CAR T cells in the treatment of solid tumors has been largely ineffective due to the insufficient infiltration of CAR T cells into the tumor, suppressive effects

of the tumor microenvironment, and heterogeneity and frequent loss of tumor antigens. Preclinical studies have shown that many alternative CAR-expressing immune cells successfully destroy solid tumors. Some cells, in particular CAR-Ms, can transform tumor microenvironment into an inflammatory environment that promotes tumor elimination. The issue of infiltration of CAR-expressing immune cells into solid tumors has also not been resolved. Efficient tumor infiltration of CAR cells has only been demonstrated for NK cells and macrophages [117]. Nevertheless, it can be assumed that CAR leukocytes will become effective agents for the treatment of solid tumors when used in combination with other therapeutic approaches.

In conclusion, CAR cell therapy based on different types of immune cells is moving towards its clinical application. However, it may be years before alternative CAR cells become effective, safe, and routinely used tools in the treatment of cancer and other diseases.

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REFERENCES

1. Kuwana, Y., Asakura, Y., Utsunomiya, N., Nakanishi, M., Arata, Y., Itoh, S., Nagase, F., and Kurosawa, Y. (1987) Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions, *Biochem. Biophys. Res. Commun.*, **149**, 960-968, doi: 10.1016/0006-291X(87)90502-X.
2. Eshhar, Z., Waks, T., Gross, G., and Schindler, D. G. (1993) Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ or ζ subunits of the immunoglobulin and T-cell receptors, *Proc. Natl. Acad. Sci. USA*, **90**, 720-724, doi: 10.1073/pnas.90.2.720.
3. Sadelain, M., Brentjens, R., and Riviere, I. (2013) The basic principles of chimeric antigen receptor design, *Cancer Discov.*, **3**, 388-398, doi: 10.1158/2159-8290.CD-12-0548.
4. Gomes-Silva, D., Mukherjee, M., Srinivasan, M., Krenciute, G., Dakhova, O., Zheng, Y., Cabral, J. M. S., Rooney, C. M., Orange, J. S., Brenner, M. K., and Mamonkin, M. (2017) Tonic 4-1BB costimulation in chimeric antigen receptors impedes T cell survival and is vector-dependent, *Cell Rep.*, **21**, 17-26, doi: 10.1016/j.celrep.2017.09.015.
5. Maher, J., Brentjens, R. J., Gunset, G., Rivière, I., and Sadelain, M. (2002) Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor, *Nat. Biotechnol.*, **20**, 70-75, doi: 10.1038/nbt0102-70.
6. Brentjens, R. J., Latouche, J. B., Santos, E., Marti, F., Gong, M. C., Lyddane, C., King, P. D., Larson, S., Weiss, M., Riviere, I., and Sadelain, M. (2003) Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15, *Nat. Med.*, **9**, 279-286, doi: 10.1038/nm827.
7. List of FDA approved cell and gene therapies, URL: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products.
8. Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., and Rosenberg, S. A. (2010) Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2, *Mol. Ther.*, **18**, 843-851, doi: 10.1038/mt.2010.24.
9. D'Aloia, M. M., Zizzari, I. G., Sacchetti, B., Pierelli, L., and Alimandi, M. (2018) CAR-T cells: the long and winding road to solid tumors, *Cell Death Dis.*, **9**, 282, doi: 10.1038/s41419-018-0278-6.
10. Larson, R. C., and Maus, M. V. (2021) Recent advances and discoveries in the mechanisms and functions of CAR T cells, *Nat. Rev. Cancer*, **21**, 145-161, doi: 10.1038/s41568-020-00323-z.
11. Long, A. H., Haso, W. M., Shern, J. F., Wanhainen, K. M., Murgai, M., Ingaramo, M., Smith, J. P., Walker, A. J., Kohler, M. E., Venkateshwara, V. R., Kaplan, R. N., Patterson, G. H., Fry, T. J., Orentas, R. J., and Mackall, C. L. (2015) 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors, *Nat. Med.*, **21**, 581-590, doi: 10.1038/nm.3838.
12. Hong, M., Clubb, J. D., and Chen, Y. Y. (2020) Engineering CAR-T cells for next-generation cancer therapy, *Cancer Cell*, **38**, 473-488, doi: 10.1016/j.ccell.2020.07.005.
13. Rafiq, S., Hackett, C. S., and Brentjens, R. J. (2020) Engineering strategies to overcome the current roadblocks in CAR T cell therapy, *Nat. Rev. Clin. Oncol.*, **17**, 147-167, doi: 10.1038/s41571-019-0297-y.
14. Meza Guzman, L. G., Keating, N., and Nicholson, S. E. (2020) Natural killer cells: tumor surveillance and signaling, *Cancers (Basel)*, **12**, 952, doi: 10.3390/cancers12040952.
15. Kumar, S. (2018) Natural killer cell cytotoxicity and its regulation by inhibitory receptors, *Immunology*, **154**, 383-393, doi: 10.1111/imm.12921.
16. Fauriat, C., Long, E. O., Ljunggren, H. G., and Bryceson, Y. T. (2010) Regulation of human NK-cell cytokine and chemokine production by target cell recognition, *Blood*, **115**, 2167-2176, doi: 10.1182/blood-2009-08-238469.

17. Lo Nigro, C., Macagno, M., Sangiolo, D., Bertolaccini, L., Aglietta, M., and Merlano, M. C. (2019) NK-mediated antibody-dependent cell-mediated cytotoxicity in solid tumors: biological evidence and clinical perspectives, *Ann. Transl. Med.*, **7**, 105, doi: 10.21037/atm.2019.01.42.
18. Tyshchuk, E. V., Mikhailova, V. A., Selkov, S. A., and Sokolov, D. I. (2021) Natural killer cells: origin, phenotype, function, *Medical Immunology (Russia)*, **23**, 1207-1228, doi: 10.15789/1563-0625-NKC-2330.
19. Xu, Y., Liu, Q., Zhong, M., Wang, Z., Chen, Z., Zhang, Y., Xing, H., Tian, Z., Tang, K., Liao, X., Rao, Q., Wang, M., and Wang, J. (2019) 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell malignancies, *J. Hematol. Oncol.*, **12**, 49, doi: 10.1186/s13045-019-0732-7.
20. Xie, G., Dong, H., Liang, Y., Ham, J. D., Rizwan, R., and Chen, J. (2020) CAR-NK cells: a promising cellular immunotherapy for cancer, *EBioMed.*, **59**, 102975, doi: 10.1016/j.ebiom.2020.102975.
21. Töpfer, K., Cartellieri, M., Michen, S., Wiedemuth, R., Muller, N., Lindemann, D., Bachmann, M., Fussel, M., Schackert, G., and Temme, A. (2015) DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy, *J. Immunol.*, **194**, 3201-3212, doi: 10.4049/jimmunol.1400330.
22. Cifaldi, L., Melaiu, O., Giovannoni, R., Benvenuto, M., Focaccetti, C., Nardozi, D., Barillari, G., and Bei, R. (2023) DNAM-1 chimeric receptor-engineered NK cells: a new frontier for CAR-NK cell-based immunotherapy, *Front. Immunol.*, **14**, 1197053, doi: 10.3389/fimmu.2023.1197053.
23. Oei, V. Y. S., Siernicka, M., Graczyk-Jarzynka, A., Hoel, H. J., Yang, W., Palacios, D., Almasbak, H., Bajor, M., Clement, D., Brandt, L., Onfelt, B., Goodridge, J., Winiarska, M., Zagozdzon, R., Olweus, J., Kyte, J. A., and Malmberg, K. J. (2018) Intrinsic functional potential of NK-cell subsets constrains retargeting driven by chimeric antigen receptors, *Cancer Immunol. Res.*, **6**, 467-480, doi: 10.1158/2326-6066.CIR-17-0207.
24. Xia, J., Minamino, S., and Kuwabara, K. (2020) CAR-expressing NK cells for cancer therapy: a new hope, *Biosci. Trends*, **14**, 354-359, doi: 10.5582/bst.2020.03308.
25. Dong, H., Ham, J. D., Hu, G., Xie, G., Vergara, J., Liang, Y., Ali, A., Tarannum, M., Donner, H., Baginska, J., Abdulhamid, Y., Dinh, K., Soiffer, R. J., Ritz, J., Glimcher, L. H., Chen, J., and Romee, R. (2022) Memory-like NK cells armed with a neoepitope-specific CAR exhibit potent activity against NPM1 mutated acute myeloid leukemia, *Proc. Natl. Acad. Sci. USA*, **119**, e2122379119, doi: 10.1073/pnas.2122379119.
26. Klopotowska, M., Bajor, M., Graczyk-Jarzynka, A., Kraft, A., Pilch, Z., Zhylko, A., Firczuk, M., Baranowska, I., Lazniewski, M., Plewczynski, D., Goral, A., Soroczynska, K., Domagala, J., Marhelava, K., Slusarczyk, A., Retecki, K., Ramji, K., Krawczyk, M., Temples, M. N., Sharma, B., Lachota, M., Netskar, H., Malmberg, K.-J., Zagozdzon, R., and Winiarska, M. (2022) PRDX-1 supports the survival and antitumor activity of primary and CAR-modified NK cells under oxidative stress, *Cancer Immunol. Res.*, **10**, 228-244, doi: 10.1158/2326-6066.CIR-20-1023.
27. Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M. F., and Velardi, A. (2002) Effectiveness of donor natural killer cell aloreactivity in mismatched hematopoietic transplants, *Science*, **295**, 2097-2100, doi: 10.1126/science.1068440.
28. Miller, J. S., Soignier, Y., Panoskaltsis-Mortari, A., McNearney, S. A., Yun, G. H., Fautsch, S. K., McKenna, D., Le, C., Defor, T. E., Burns, L. J., Orchard, P. J., Blazar, B. R., Wagner, J. E., Slungaard, A., Weisdorf, D. J., Okazaki, I. J., and McGlave, P. B. (2005) Successful adoptive transfer and *in vivo* expansion of human haploidentical NK cells in patients with cancer, *Blood*, **105**, 3051-3057, doi: 10.1182/blood-2004-07-2974.
29. Liu, E., Marin, D., Banerjee, P., Macapinlac, H. A., Thompson, P., Basar, R., Nassif Kerbauy, L., Overman, B., Thall, P., Kaplan, M., Nandivada, V., Kaur, I., Nunez Cortes, A., Cao, K., Daher, M., Hosing, C., Cohen, E. N., Kebriaei, P., Mehta, R., Neelapu, S., Nieto, Y., Wang, M., Wierda, W., Keating, M., Champlin, R., Shpall, E., and Rezvani, K. (2020) Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors, *N. Engl. J. Med.*, **382**, 545-553, doi: 10.1056/NEJMoa1910607.
30. Xiao, L., Cen, D., Gan, H., Sun, Y., Huang, N., Xiong, H., Jin, Q., Su, L., Liu, X., Wang, K., Yan, G., Dong, T., Wu, S., Zhou, P., Zhang, J., Liang, W., Ren, J., Teng, Y., Chen, C., and Xu, X. H. (2019) Adoptive transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients, *Mol. Ther.*, **27**, 1114-1125, doi: 10.1016/j.ymthe.2019.03.011.
31. Wrona, E., Borowiec, M., and Potemski, P. (2021) CAR-NK cells in the treatment of solid tumors, *Int. J. Mol. Sci.*, **22**, 5899, doi: 10.3390/ijms22115899.
32. Ng, Y. Y., Du, Z., Zhang, X., Chng, W. J., and Wang, S. (2022) CXCR4 and anti-BCMA CAR co-modified natural killer cells suppress multiple myeloma progression in a xenograft mouse model, *Cancer Gene Ther.*, **29**, 475-483, doi: 10.1038/s41417-021-00365-x.
33. Qin, V. M., D'Souza, C., Neeson, P. J., and Zhu, J. J. (2021) Chimeric antigen receptor beyond CAR-T cells, *Cancers (Basel)*, **13**, 404, doi: 10.3390/cancers13030404.
34. Melenhorst, J. J., Chen, G. M., Wang, M., Porter, D. L., Chen, C., Collins, M. A., Gao, P., Bandyopadhyay, S., Sun, H., Zhao, Z., Lundh, S., Pruteanu-Malinici, I., Nobles, C. L., Maji, S., Frey, N. V., Gill, S. I., Loren, A. W., Tian, L., Kulikovskaya, I., Gupta, M., Ambrose, D. E., Davis, M. M., Fraietta, J. A., Brogdon, J. L., Young, R. M., Chew, A., Levine, B. L., Siegel, D. L., Alanio, C.,

Wherry, E. J., Bushman, F. D., Lacey, S. F., Tan, K., and June, C. H. (2022) Decade-long leukaemia remissions with persistence of CD4$^+$ CAR T cells, *Nature*, **602**, 503-509, doi: 10.1038/s41586-021-04390-6.

35. Sabbah, M., Jondreville, L., Lacan, C., Norol, F., Vieillard, V., Roos-Weil, D., and Nguyen, S. (2022) CAR-NK cells: a chimeric hope or a promising therapy? *Cancers (Basel)*, **14**, 3839, doi: 10.3390/cancers14153839.
36. Ebrahimiyan, H., Tamimi, A., Shokoohian, B., Minaei, N., Memarnejadian, A., Hossein-Khannazer, N., Hassan, M., and Vosough, M. (2022) Novel insights in CAR-NK cells beyond CAR-T cell technology; promising advantages, *Int. Immunopharmacol.*, **106**, 108587, doi: 10.1016/j.intimp.2022.108587.
37. Daher, M., Basar, R., Gokdemir, E., Baran, N., Uprety, N., Nunez Cortes, A. K., Mendt, M., Kerbauy, L. N., Banerjee, P. P., Shanley, M., Imahashi, N., Li, L., Lim, F. L. W. I., Fathi, M., Rezvan, A., Mohanty, V., Shen, Y., Shaim, H., Lu, J., Ozcan, G., Ensley, E., Kaplan, M., Nandivada, V., Bdiwi, M., Acharya, S., Xi, Y., Wan, X., Mak, D., Liu, E., Jiang, X. R., Ang, S., Muniz-Feliciano, L., Li, Y., Wang, J., Kordasti, S., Petrov, N., Varadarajan, N., Marin, D., Brunetti, L., Skinner, R. J., Lyu, S., Silva, L., Turk, R., Schubert, M. S., Rettig, G. R., McNeill, M. S., Kurgan, G., Behlke, M. A., Li, H., Fowlkes, N. W., Chen, K., Konopleva, M., Champlin, R. E., Shpall, E. J., and Rezvani, K. (2021) Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells, *Blood*, **137**, 624-636, doi: 10.1182/blood.2020007748.
38. Pulè, M. A., Straathof, K. C., Dotti, G., Heslop, H. E., Rooney, C. M., and Brenner, M. K. (2005) A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells, *Mol. Ther.*, **12**, 933-941, doi: 10.1016/j.ymthe.2005.04.016.
39. Li, Y., Basar, R., Wang, G., Liu, E., Moyes, J. S., Li, L., Kerbauy, L. N., Uprety, N., Fathi, M., Rezvan, A., Banerjee, P. P., Muniz-Feliciano, L., Laskowski, T. J., Ensley, E., Daher, M., Shanley, M., Mendt, M., Acharya, S., Liu, B., Biederstadt, A., Rafei, H., Guo, X., Garcia, L. M., Lin, P., Ang, S., Marin, D., Chen, K., Bover, L., Champlin, R. E., Varadarajan, N., Shpall, E. J., and Rezvani, K. (2022) KIR-based inhibitory CARs overcome CAR-NK cell trogocytosis-mediated fratricide and tumor escape, *Nat. Med.*, **28**, 2133-2144, doi: 10.1038/s41591-022-02003-x.
40. Li, L., Mohanty, V., Dou, J., Huang, Y., Banerjee, P. P., Miao, Q., Lohr, J. G., Vijaykumar, T., Frede, J., Knoechel, B., Muniz-Feliciano, L., Laskowski, T. J., Liang, S., Moyes, J. S., Nandivada, V., Basar, R., Kaplan, M., Daher, M., Liu, E., Li, Y., Acharya, S., Lin, P., Shanley, M., Rafei, H., Marin, D., Mielke, S., Champlin, R. E., Shpall, E. J., Chen, K., and Rezvani, K. (2023) Loss of metabolic fitness drives tumor resistance after CAR-NK cell therapy and can be overcome by cytokine engineering, *Sci. Adv.*, **9**, eadd6997, doi: 10.1126/sciadv.add6997.
41. Papadopoulou, M., Sanchez Sanchez, G., and Vermijlen, D. (2020) Innate and adaptive γδ T cells: how, when, and why, *Immunol. Rev.*, **298**, 99-116, doi: 10.1111/imr.12926.
42. Simões, A. E., Di Lorenzo, B., and Silva-Santos, B. (2018) Molecular determinants of Target cell recognition by human γδ T cells, *Front. Immunol.*, **9**, 929, doi: 10.3389/fimmu.2018.00929.
43. Chien, Y. H., Meyer, C., and Bonneville, M. (2014) γδ T cells: first line of defense and beyond, *Annu. Rev. Immunol.*, **32**, 121-155, doi: 10.1146/annurev-immunol-032713-120216.
44. Deng, J., and Yin, H. (2022) Gamma delta (γδ) T cells in cancer immunotherapy; where it comes from, where it will go? *Eur. J. Pharmacol.*, **919**, 174803, doi: 10.1016/j.ejphar.2022.174803.
45. Sebestyen, Z., Prinz, I., Dechanet-Merville, J., Silva-Santos, B., and Kuball, J. (2020) Translating gammadelta (γδ) T cells and their receptors into cancer cell therapies, *Nat. Rev. Drug Discov.*, **19**, 169-184, doi: 10.1038/s41573-019-0038-z.
46. Girardi, M., Oppenheim, D. E., Steele, C. R., Lewis, J. M., Glusac, E., Filler, R., Hobby, P., Sutton, B., Tigelaar, R. E., and Hayday, A. C. (2001) Regulation of cutaneous malignancy by gammadelta T cells, *Science*, **294**, 605-609, doi: 10.1126/science.1063916.
47. Donia, M., Ellebaek, E., Andersen, M. H., Straten, P. T., and Svane, I. M. (2012) Analysis of Vdelta1 T cells in clinical grade melanoma-infiltrating lymphocytes, *Oncoimmunology*, **1**, 1297-1304, doi: 10.4161/onci.21659.
48. Wang, J., Lin, C., Li, H., Li, R., Wu, Y., Liu, H., Zhang, H., He, H., Zhang, W., and Xu, J. (2017) Tumor-infiltrating γδT cells predict prognosis and adjuvant chemotherapeutic benefit in patients with gastric cancer, *Oncoimmunology*, **6**, e1353858, doi: 10.1080/2162402X.2017.1353858.
49. Silva-Santos, B., Mensurado, S., and Coffelt, S. B. (2019) γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer, *Nat. Rev. Cancer*, **19**, 392-404, doi: 10.1038/s41568-019-0153-5.
50. Gunderson, A. J., Kaneda, M. M., Tsujikawa, T., Nguyen, A. V., Affara, N. I., Ruffell, B., Gorjestani, S., Liudahl, S. M., Truitt, M., Olson, P., Kim, G., Hanahan, D., Tempero, M. A., Sheppard, B., Irving, B., Chang, B. Y., Varner, J. A., and Coussens, L. M. (2016) Bruton tyrosine kinase-dependent immune cell cross-talk drives pancreas cancer, *Cancer Discov.*, **6**, 270-285, doi: 10.1158/2159-8290.CD-15-0827.
51. Deniger, D. C., Switzer, K., Mi, T., Maiti, S., Hurton, L., Singh, H., Huls, H., Olivares, S., Lee, D. A., Champlin, R. E., and Cooper, L. J. (2013) Bispecific T-cells expressing polyclonal repertoire of endogenous γδ T-cell receptors and introduced CD19-specific chimeric antigen receptor, *Mol. Ther.*, **21**, 638-647, doi: 10.1038/mt.2012.267.

52. Rozenbaum, M., Meir, A., Aharony, Y., Itzhaki, O., Schachter, J., Bank, I., Jacoby, E., and Besser, M. J. (2020) Gamma-delta CAR-T cells show CAR-directed and independent activity against leukemia, *Front. Immunol.*, **11**, 1347, doi: 10.3389/fimmu.2020.01347.
53. Fisher, J., Abramowski, P., Wisidagamage Don, N. D., Flutter, B., Capsomidis, A., Cheung, G. W., Gustafsson, K., and Anderson, J. (2017) Avoidance of on-target off-tumor activation using a co-stimulation-only chimeric antigen receptor, *Mol. Ther.*, **25**, 1234-1247, doi: 10.1016/j.ymthe.2017.03.002.
54. Morandi, F., Yazdanifar, M., Cocco, C., Bertaina, A., and Airoldi, I. (2020) Engineering the bridge between innate and adaptive immunity for cancer immunotherapy: focus on γδ T and NK cells, *Cells*, **9**, 1757, doi: 10.3390/cells9081757.
55. Fleischer, L. C., Becker, S. A., Ryan, R. E., Fedanov, A., Doering, C. B., and Spencer, H. T. (2020) Non-signaling chimeric antigen receptors enhance antigen-directed killing by γδ T cells in contrast to αβ T cells, *Mol. Ther. Oncolytics*, **18**, 149-160, doi: 10.1016/j.omto.2020.06.003.
56. Ali, A. K., Tarannum, M., and Romee, R. (2021) Is adoptive cellular therapy with non-T-cell immune effectors the future? *Cancer J.*, **27**, 168-175, doi: 10.1097/PPO.0000000000000517.
57. Vivier, E., and Anfossi, N. (2004) Inhibitory NK-cell receptors on T cells: witness of the past, actors of the future, *Nat. Rev. Immunol.*, **4**, 190-198, doi: 10.1038/nri1306.
58. Kriegsmann, K., Kriegsmann, M., von Bergwelt-Baildon, M., Cremer, M., and Witzens-Harig, M. (2018) NKT cells – new players in CAR cell immunotherapy? *Eur. J. Haematol.*, **101**, 750-757, doi: 10.1111/ejh.13170.
59. Yoneda, K., Morii, T., Nieda, M., Tsukaguchi, N., Amano, I., Tanaka, H., Yagi, H., Narita, N., and Kimura, H. (2005) The peripheral blood Valpha24$^+$ NKT cell numbers decrease in patients with haematopoietic malignancy, *Leuk. Res.*, **29**, 147-152, doi: 10.1016/j.leukres.2004.06.005.
60. Tahir, S. M., Cheng, O., Shaulov, A., Koezuka, Y., Bubley, G. J., Wilson, S. B., Balk, S. P., and Exley, M. A. (2001) Loss of IFN-gamma production by invariant NK T cells in advanced cancer, *J. Immunol.*, **167**, 4046-4050, doi: 10.4049/jimmunol.167.7.4046.
61. Tachibana, T., Onodera, H., Tsuruyama, T., Mori, A., Nagayama, S., Hiai, H., and Imamura, M. (2005) Increased intratumor Valpha24-positive natural killer T cells: a prognostic factor for primary colorectal carcinomas, *Clin. Cancer Res.*, **11**, 7322-7327, doi: 10.1158/1078-0432.CCR-05-0877.
62. Schneiders, F. L., de Bruin, R. C., van den Eertwegh, A. J., Scheper, R. J., Leemans, C. R., Brakenhoff, R. H., Langendijk, J. A., Verheul, H. M., de Gruijl, T. D., Molling, J. W., and van der Vliet, H. J. (2012) Circulating invariant natural killer T-cell numbers predict outcome in head and neck squamous cell carcinoma: updated analysis with 10-year follow-up, *J. Clin. Oncol.*, **30**, 565-567, doi: 10.1200/jco.2011.39.3975.
63. Bassiri, H., Das, R., Guan, P., Barrett, D. M., Brennan, P. J., Banerjee, P. P., Wiener, S. J., Orange, J. S., Brenner, M. B., Grupp, S. A., and Nichols, K. E. (2014) iNKT cell cytotoxic responses control T-lymphoma growth *in vitro* and *in vivo*, *Cancer Immunol. Res.*, **2**, 59-69, doi: 10.1158/2326-6066.CIR-13-0104.
64. Nair, S., and Dhodapkar, M. V. (2017) Natural killer T cells in cancer immunotherapy, *Front. Immunol.*, **8**, 1178, doi: 10.3389/fimmu.2017.01178.
65. McEwen-Smith, R. M., Salio, M., and Cerundolo, V. (2015) The regulatory role of invariant NKT cells in tumor immunity, *Cancer Immunol. Res.*, **3**, 425-435, doi: 10.1158/2326-6066.CIR-15-0062.
66. Cortés-Selva, D., Dasgupta, B., Singh, S., and Grewal, I. S. (2021) Innate and innate-like cells: the future of chimeric antigen receptor (CAR) cell therapy, *Trends Pharmacol. Sci.*, **42**, 45-59, doi: 10.1016/j.tips.2020.11.004.
67. Heczey, A., Liu, D., Tian, G., Courtney, A. N., Wei, J., Marinova, E., Gao, X., Guo, L., Yvon, E., Hicks, J., Liu, H., Dotti, G., and Metelitsa, L. S. (2014) Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy, *Blood*, **124**, 2824-2833, doi: 10.1182/blood-2013-11-541235.
68. Tian, G., Courtney, A. N., Jena, B., Heczey, A., Liu, D., Marinova, E., Guo, L., Xu, X., Torikai, H., Mo, Q., Dotti, G., Cooper, L. J., and Metelitsa, L. S. (2016) CD62L$^+$ NKT cells have prolonged persistence and antitumor activity *in vivo*, *J. Clin. Invest.*, **126**, 2341-2355, doi: 10.1172/JCI83476.
69. Simon, B., Wiesinger, M., Marz, J., Wistuba-Hamprecht, K., Weide, B., Schuler-Thurner, B., Schuler, G., Dorrie, J., and Uslu, U. (2018) The generation of CAR-transfected natural killer T cells for the immunotherapy of melanoma, *Int. J. Mol. Sci.*, **19**, 2365, doi: 10.3390/ijms19082365.
70. Shaik, R. S., Rathi, P., Courtney, A., Schneller, N., Guo, L., Barragan, G., Zhang, C., Xu, X., Sumazin, P., Metelitsa, L., and Heczey, A. (2022) Glypican-3-specific CAR-NKT cells overexpressing BATF3 mediate potent antitumor activity against hepatocellular carcinoma, *J. Clin. Oncol.*, **40**, e14521, doi: 10.1200/jco.2022.40.16_suppl.e14521.
71. Itoh, M., Takahashi, T., Sakaguchi, N., Kuniyasu, Y., Shimizu, J., Otsuka, F., and Sakaguchi, S. (1999) Thymus and autoimmunity: production of CD25$^+$CD4$^+$ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance, *J. Immunol.*, **162**, 5317-5326, doi: 10.4049/jimmunol.162.9.5317.
72. Romano, M., Fanelli, G., Albany, C. J., Giganti, G., and Lombardi, G. (2019) Past, present, and future of reg-

ulatory T cell therapy in transplantation and autoimmunity, *Front. Immunol.*, **10**, 43, doi: 10.3389/fimmu.2019.00043.

73. Arjomandnejad, M., Kopec, A. L., and Keeler, A. M. (2022) CAR-T regulatory (CAR-Treg) cells: engineering and applications, *Biomedicines*, **10**, 287, doi: 10.3390/biomedicines10020287.
74. Elinav, E., Waks, T., and Eshhar, Z. (2008) Redirection of regulatory T cells with predetermined specificity for the treatment of experimental colitis in mice, *Gastroenterology*, **134**, 2014-2024, doi: 10.1053/j.gastro.2008.02.060.
75. Hombach, A. A., Kofler, D., Rappl, G., and Abken, H. (2009) Redirecting human $CD4^+CD25^+$ regulatory T cells from the peripheral blood with pre-defined target specificity, *Gene Ther.*, **16**, 1088-1096, doi: 10.1038/gt.2009.75.
76. Dawson, N. A. J., Rosado-Sánchez, I., Novakovsky, G. E., Fung, V. C. W., Huang, Q., McIver, E., Sun, G., Gillies, J., Speck, M., Orban, P. C., Mojibian, M., and Levings, M. K. (2020) Functional effects of chimeric antigen receptor co-receptor signaling domains in human regulatory T cells, *Sci. Transl. Med.*, **12**, eaaz3866, doi: 10.1126/scitranslmed.aaz3866.
77. Mohseni, Y. R., Saleem, A., Tung, S. L., Dudreuilh, C., Lang, C., Peng, Q., Volpe, A., Adigbli, G., Cross, A., Hester, J., Farzaneh, F., Scotta, C., Lechler, R. I., Issa, F., Fruhwirth, G. O., and Lombardi, G. (2021) Chimeric antigen receptor-modified human regulatory T cells that constitutively express IL-10 maintain their phenotype and are potently suppressive, *Eur. J. Immunol.*, **51**, 2522-2530, doi: 10.1002/eji.202048934.
78. Boroughs, A. C., Larson, R. C., Choi, B. D., Bouffard, A. A., Riley, L. S., Schiferle, E., Kulkarni, A. S., Cetrulo, C. L., Ting, D., Blazar, B. R., Demehri, S., and Maus, M. V. (2019) Chimeric antigen receptor costimulation domains modulate human regulatory T cell function, *JCI Insight*, **5**, e126194, doi: 10.1172/jci.insight.126194.
79. Fu, R. Y., Chen, A. C., Lyle, M. J., Chen, C. Y., Liu, C. L., and Miao, C. H. (2020) $CD4^+$ T cells engineered with FVIII-CAR and murine Foxp3 suppress anti-factor VIII immune responses in hemophilia a mice, *Cell Immunol.*, **358**, 104216, doi: 10.1016/j.cellimm.2020.104216.
80. Arjomandnejad, M., Sylvia, K., Blackwood, M., Nixon, T., Tang, Q., Muhuri, M., Gruntman, A. M., Gao, G., Flotte, T. R., and Keeler, A. M. (2021) Modulating immune responses to AAV by expanded polyclonal T-regs and capsid specific chimeric antigen receptor T-regulatory cells, *Mol. Ther. Methods Clin. Dev.*, **23**, 490-506, doi: 10.1016/j.omtm.2021.10.010.
81. Rana, J., and Biswas, M. (2020) Regulatory T cell therapy: current and future design perspectives, *Cell Immunol.*, **356**, 104193, doi: 10.1016/j.cellimm.2020.104193.
82. MacDonald, K. G., Hoeppli, R. E., Huang, Q., Gillies, J., Luciani, D. S., Orban, P. C., Broady, R., and Levings, M. K. (2016) Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor, *J. Clin. Invest.*, **126**, 1413-1424, doi: 10.1172/JCI82771.
83. D'Souza, C., Chen, Z., and Corbett, A. J. (2018) Revealing the protective and pathogenic potential of MAIT cells, *Mol. Immunol.*, **103**, 46-54, doi: 10.1016/j.molimm.2018.08.022.
84. Howson, L. J., Salio, M., and Cerundolo, V. (2015) MR1-restricted mucosal-associated invariant T cells and their activation during infectious diseases, *Front. Immunol.*, **6**, 303, doi: 10.3389/fimmu.2015.00303.
85. Le Bourhis, L., Dusseaux, M., Bohineust, A., Bessoles, S., Martin, E., Premel, V., Core, M., Sleurs, D., Serriari, N. E., Treiner, E., Hivroz, C., Sansonetti, P., Gougeon, M. L., Soudais, C., and Lantz, O. (2013) MAIT cells detect and efficiently lyse bacterially-infected epithelial cells, *PLoS Pathog.*, **9**, e1003681, doi: 10.1371/journal.ppat.1003681.
86. Dogan, M., Karhan, E., Kozhaya, L., Placek, L., Chen, X., Yigit, M., and Unutmaz, D. (2022) Engineering human mucosal associated invariant T (MAIT) cells with chimeric antigen receptors for cancer immunotherapy, *J. Immunol.*, **209**, 1523-1531, doi: 10.1101/2022.07.28.501764.
87. Won, E. J., Ju, J. K., Cho, Y. N., Jin, H. M., Park, K. J., Kim, T. J., Kwon, Y.-S., Kee, H. J., Kim, J. C., Kee, S.-J., and Park, Y.-W. (2016) Clinical relevance of circulating mucosal-associated invariant T cell levels and their anti-cancer activity in patients with mucosal-associated cancer, *Oncotarget*, **7**, 76274-76290, doi: 10.18632/oncotarget.11187.
88. Gherardin, N. A., Loh, L., Admojo, L., Davenport, A. J., Richardson, K., Rogers, A., Darcy, P. K., Jenkins, M. R., Prince, H. M., Harrison, S. J., Quach, H., Fairlie, D. P., Kedzierska, K., McCluskey, J., Uldrich, A. P., Neeson, P. J., Ritchie, D. S., and Godfrey, D. I. (2018) Enumeration, functional responses and cytotoxic capacity of MAIT cells in newly diagnosed and relapsed multiple myeloma, *Sci. Rep.*, **8**, 4159, doi: 10.1038/s41598-018-22130-1.
89. Reantragoon, R., Boonpattanaporn, N., Corbett, A. J., and McCluskey, J. (2016) Mucosal-associated invariant T cells in clinical diseases, *As. Pac. J. Allergy Immunol.*, **34**, 3-10.
90. Zhang, Z.-X., Yang, L., Young, K. J., Dutemple, B., and Zhang, L. I. (2000) Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression, *Nat. Med.*, **6**, 782-789, doi: 10.1038/77513.
91. Wu, Z., Zheng, Y., Sheng, J., Han, Y., Yang, Y., Pan, H., and Yao, J. (2022) $CD3^+CD4^-CD8^-$ (Double-Negative) T cells in inflammation, immune disorders and cancer, *Front. Immunol.*, **13**, 816005, doi: 10.3389/fimmu.2022.816005.
92. Zhang, Z.-X., Yang, L., Young, K. J., and Zhang, L. (2001) Suppression of alloimmune responses *in vitro* and

in vivo by CD3 CD8 CD4 regulatory T cells, *Transplant. Proc.*, **33**, 84-85, doi: 10.1016/s0041-1345(00)01915-1.

93. Young, K. J., DuTemple, B., Phillips, M. J., and Zhang, L. (2003) Inhibition of graft-versus-host disease by double-negative regulatory T cells, *J. Immunol.*, **171**, 134-141, doi: 10.4049/jimmunol.171.1.134.
94. Chen, W., Ford, M. S., Young, K. J., Cybulsky, M. I., and Zhang, L. (2003) Role of double-negative regulatory T cells in long-term cardiac xenograft survival, *J. Immunol.*, **170**, 1846-1853, doi: 10.4049/jimmunol.170.4.1846.
95. Chen, J., Hu, P., Wu, G., and Zhou, H. (2019) Antipancreatic cancer effect of DNT cells and the underlying mechanism, *Pancreatology*, **19**, 105-113, doi: 10.1016/j.pan.2018.12.006.
96. Chen, X., Wang, D., and Zhu, X. (2022) Application of double-negative T cells in haematological malignancies: recent progress and future directions, *Biomark. Res.*, **10**, 11, doi: 10.1186/s40364-022-00360-w.
97. Merims, S., Li, X., Joe, B., Dokouhaki, P., Han, M., Childs, R. W., Wang, Z. Y., Gupta, V., Minden, M. D., and Zhang, L. (2011) Anti-leukemia effect of *ex vivo* expanded DNT cells from AML patients: a potential novel autologous T-cell adoptive immunotherapy, *Leukemia*, **25**, 1415-1422, doi: 10.1038/leu.2011.99.
98. Lee, J. B., Kang, H., Fang, L., D'Souza, C., Adeyi, O., and Zhang, L. (2019) Developing allogeneic double-negative T cells as a novel off-the-shelf adoptive cellular therapy for cancer, *Clin. Cancer Res.*, **25**, 2241-2253, doi: 10.1158/1078-0432.CCR-18-2291.
99. Vasic, D., Lee, J. B., Leung, Y., Khatri, I., Na, Y., Abate-Daga, D., and Zhang, L. (2022) Allogeneic double-negative CAR-T cells inhibit tumor growth without off-tumor toxicities, *Sci. Immunol.*, **7**, eabl3642, doi: 10.1126/sciimmunol.abl3642.
100. Gao, X., Mi, Y., Guo, N., Xu, H., Xu, L., Gou, X., and Jin, W. (2017) Cytokine-induced killer cells as pharmacological tools for cancer immunotherapy, *Front. Immunol.*, **8**, 774, doi: 10.3389/fimmu.2017.00774.
101. Grimm, E. A., Mazumder, A., Zhang, H. Z., and Rosenberg, S. A. (1982) Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes, *J. Exp. Med.*, **155**, 1823-1841, doi: 10.1084/jem.155.6.1823.
102. Rosenberg, S. A., Spiess, P., and Lafreniere, R. (1986) A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes, *Science*, **233**, 1318-1321, doi: 10.1126/science.3489291.
103. Cappuzzello, E., Sommaggio, R., Zanovello, P., and Rosato, A. (2017) Cytokines for the induction of antitumor effectors: the paradigm of cytokine-induced killer (CIK) cells, *Cytokine Growth Factor Rev.*, **36**, 99-105, doi: 10.1016/j.cytogfr.2017.06.003.
104. Diefenbach, A., Jamieson, A. M., Liu, S. D., Shastri, N., and Raulet, D. H. (2000) Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages, *Nat. Immunol.*, **1**, 119-126, doi: 10.1038/77793.
105. Merker, M., Wagner, J., Kreyenberg, H., Heim, C., Moser, L. M., Wels, W. S., Bonig, H., Ivics, Z., Ullrich, E., Klingebiel, T., Bader, P., and Rettinger, E. (2020) ERBB2-CAR-engineered cytokine-induced killer cells exhibit both CAR-mediated and innate immunity against high-risk rhabdomyosarcoma, *Front. Immunol.*, **11**, 581468, doi: 10.3389/fimmu.2020.581468.
106. Rotolo, R., Leuci, V., Donini, C., Cykowska, A., Gammaitoni, L., Medico, G., Valabrega, G., Aglietta, M., and Sangiolo, D. (2019) CAR-based strategies beyond T lymphocytes: integrative opportunities for cancer adoptive immunotherapy, *Int. J. Mol. Sci.*, **20**, 2839, doi: 10.3390/ijms20112839.
107. Marin, V., Dander, E., Biagi, E., Introna, M., Fazio, G., Biondi, A., and D'Amico, G. (2006) Characterization of *in vitro* migratory properties of anti-CD19 chimeric receptor-redirected CIK cells for their potential use in B-ALL immunotherapy, *Exp. Hematol.*, **34**, 1219-1229, doi: 10.1016/j.exphem.2006.05.004.
108. Marin, V., Kakuda, H., Dander, E., Imai, C., Campana, D., Biondi, A., and D'Amico, G. (2007) Enhancement of the anti-leukemic activity of cytokine induced killer cells with an anti-CD19 chimeric receptor delivering a 4-1BB-zeta activating signal, *Exp. Hematol.*, **35**, 1388-1397, doi: 10.1016/j.exphem.2007.05.018.
109. Marin, V., Pizzitola, I., Agostoni, V., Attianese, G. M., Finney, H., Lawson, A., Pule, M., Rousseau, R., Biondi, A., and Biagi, E. (2010) Cytokine-induced killer cells for cell therapy of acute myeloid leukemia: improvement of their immune activity by expression of CD33-specific chimeric receptors, *Haematologica*, **95**, 2144-2152, doi: 10.3324/haematol.2010.026310.
110. Magnani, C. F., Mezzanotte, C., Cappuzzello, C., Bardini, M., Tettamanti, S., Fazio, G., Cooper, L. J. N., Dastoli, G., Cazzaniga, G., Biondi, A., and Biagi, E. (2018) Preclinical efficacy and safety of CD19CAR cytokine-induced killer cells transfected with sleeping beauty transposon for the treatment of acute lymphoblastic leukemia, *Hum. Gene Ther.*, **29**, 602-613, doi: 10.1089/hum.2017.207.
111. Jarosz-Biej, M., Kaminska, N., Matuszczak, S., Cichon, T., Pamula-Pilat, J., Czapla, J., Smolarczyk, R., Skwarzynska, D., Kulik, K., and Szala, S. (2018) M1-like macrophages change tumor blood vessels and microenvironment in murine melanoma, *PLoS One*, **13**, e0191012, doi: 10.1371/journal.pone.0191012.
112. Biglari, A., Southgate, T. D., Fairbairn, L. J., and Gilham, D. E. (2006) Human monocytes expressing a CEA-specific chimeric CD64 receptor specifically target CEA-expressing tumour cells *in vitro* and *in vivo*, *Gene Ther.*, **13**, 602-610, doi: 10.1038/sj.gt.3302706.
113. Morrissey, M. A., Williamson, A. P., Steinbach, A. M., Roberts, E. W., Kern, N., Headley, M. B., and Vale, R. D.

(2018) Chimeric antigen receptors that trigger phagocytosis, *Elife*, **7**, e36688, doi: 10.7554/eLife.36688.

114. Klichinsky, M., Ruella, M., Shestova, O., Lu, X. M., Best, A., Zeeman, M., Schmierer, M., Gabrusiewicz, K., Anderson, N. R., Petty, N. E., Cummins, K. D., Shen, F., Shan, X., Veliz, K., Blouch, K., Yashiro-Ohtani, Y., Kenderian, S. S., Kim, M. Y., O'Connor, R. S., Wallace, S. R., Kozlowski, M. S., Marchione, D. M., Shestov, M., Garcia, B. A., June, C. H., and Gill, S. (2020) Human chimeric antigen receptor macrophages for cancer immunotherapy, *Nat. Biotechnol.*, **38**, 947-953, doi: 10.1038/s41587-020-0462-y.
115. Cannac, M., Nikolic, J., and Benaroch, P. (2022) Cancer immunotherapies based on genetically engineered macrophages, *Cancer Immunol. Res.*, **10**, 1156-1166, doi: 10.1158/2326-6066.CIR-22-0030.
116. Zhang, L., Tian, L., Dai, X., Yu, H., Wang, J., Lei, A., Zhu, M., Xu, J., Zhao, W., Zhu, Y., Sun, Z., Zhang, H., Hu, Y., Wang, Y., Xu, Y., Church, G. M., Huang, H., Weng, Q., and Zhang, J. (2020) Pluripotent stem cell-derived CAR-macrophage cells with antigen-dependent anti-cancer cell functions, *J. Hematol. Oncol.*, **13**, 153, doi: 10.1186/s13045-020-00983-2.
117. Hagemann, T., Lawrence, T., McNeish, I., Charles, K. A., Kulbe, H., Thompson, R. G., Robinson, S. C., and Balkwill, F. R. (2008) "Re-educating" tumor-associated macrophages by targeting NF-kappaB, *J. Exp. Med.*, **205**, 1261-1268, doi: 10.1084/jem.20080108.
118. Niu, Z., Chen, G., Chang, W., Sun, P., Luo, Z., Zhang, H., Zhi, L., Guo, C., Chen, H., Yin, M., and Zhu, W. (2021) Chimeric antigen receptor-modified macrophages trigger systemic anti-tumour immunity, *J. Pathol.*, **253**, 247-257, doi: 10.1002/path.5585.
119. Zhang, W., Liu, L., Su, H., Liu, Q., Shen, J., Dai, H., Zheng, W., Lu, Y., Zhang, W., Bei, Y., and Shen, P. (2019) Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix, *Br. J. Cancer*, **121**, 837-845, doi: 10.1038/s41416-019-0578-3.
120. Williford, J. M., Ishihara, J., Ishihara, A., Mansurov, A., Hosseinchi, P., Marchell, T. M., Potin, L., Swartz, M. A., and Hubbell, J. A. (2019) Recruitment of CD103$^+$ dendritic cells via tumor-targeted chemokine delivery enhances efficacy of checkpoint inhibitor immunotherapy, *Sci. Adv.*, **5**, eaay1357, doi: 10.1126/sciadv.aay1357.
121. Maier, B., Leader, A. M., Chen, S. T., Tung, N., Chang, C., LeBerichel, J., Chudnovskiy, A., Maskey, S., Walker, L., Finnigan, J. P., Kirkling, M. E., Reizis, B., Ghosh, S., D'Amore, N. R., Bhardwaj, N., Rothlin, C. V., Wolf, A., Flores, R., Marron, T., Rahman, A. H., Kenigsberg, E., Brown, B. D., and Merad, M. (2020) A conserved dendritic-cell regulatory program limits antitumour immunity, *Nature*, **580**, 257-262, doi: 10.1038/s41586-020-2134-y.
122. Hildner, K., Edelson, B. T., Purtha, W. E., Diamond, M., Matsushita, H., Kohyama, M., Calderon, B., Schraml, B. U., Unanue, E. R., Diamond, M. S., Schreiber, R. D., Murphy, T. L., and Murphy, K. M. (2008) Batf3 deficiency reveals a critical role for CD8α$^+$ dendritic cells in cytotoxic T cell immunity, *Science*, **322**, 1097-1100, doi: 10.1126/science.1164206.
123. Suh, H. C., Pohl, K. A., Termini, C., Kan, J., Timmerman, J. M., Slamon, D. J., and Chute, J. P. (2018) Bioengineered autologous dendritic cells enhance car t cell cytotoxicity by providing cytokine stimulation and intratumoral dendritic cells, *Blood*, **132**, 3693, doi: 10.1182/blood-2018-99-115296.
124. Zhang, L., Morgan, R. A., Beane, J. D., Zheng, Z., Dudley, M. E., Kassim, S. H., Nahvi, A. V., Ngo, L. T., Sherry, R. M., Phan, G. Q., Hughes, M. S., Kammula, U. S., Feldman, S. A., Toomey, M. A., Kerkar, S. P., Restifo, N. P., Yang, J. C., and Rosenberg, S. A. (2015) Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma, *Clin. Cancer Res.*, **21**, 2278-2288, doi: 10.1158/1078-0432.CCR-14-2085.
125. Ruella, M., Xu, J., Barrett, D. M., Fraietta, J. A., Reich, T. J., Ambrose, D. E., Klichinsky, M., Shestova, O., Patel, P. R., Kulikovskaya, I., Nazimuddin, F., Bhoj, V. G., Orlando, E. J., Fry, T. J., Bitter, H., Maude, S. L., Levine, B. L., Nobles, C. L., Bushman, F. D., Young, R. M., Scholler, J., Gill, S. I., June, C. H., Grupp, S. A., Lacey, S. F., and Melenhorst, J. J. (2018) Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell, *Nat. Med.*, **24**, 1499-1503, doi: 10.1038/s41591-018-0201-9.
126. Pesch, T., Bonati, L., Kelton, W., Parola, C., Ehling, R. A., Csepregi, L., Kitamura, D., and Reddy, S. T. (2019) Molecular design, optimization, and genomic integration of chimeric B cell receptors in murine B cells, *Front. Immunol.*, **10**, 2630, doi: 10.3389/fimmu.2019.02630.
127. Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., Braunschweig, I., Oluwole, O. O., Siddiqi, T., Lin, Y., Timmerman, J. M., Stiff, P. J., Friedberg, J. W., Flinn, I. W., Goy, A., Hill, B. T., Smith, M. R., Deol, A., Farooq, U., McSweeney, P., Munoz, J., Avivi, I., Castro, J. E., Westin, J. R., Chavez, J. C., Ghobadi, A., Komanduri, K. V., Levy, R., Jacobsen, E. D., Witzig, T. E., Reagan, P., Bot, A., Rossi, J., Navale, L., Jiang, Y., Aycock, J., Elias, M., Chang, D., Wiezorek, J., and Go, W. Y. (2017) Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma, *N. Engl. J. Med.*, **377**, 2531-2544, doi: 10.1056/NEJMoa1707447.
128. Schuster, S. J., Svoboda, J., Chong, E. A., Nasta, S. D., Mato, A. R., Anak, O., Brogdon, J. L., Pruteanu-Malinici, I., Bhoj, V., Landsburg, D., Wasik, M., Levine, B. L., Lacey, S. F., Melenhorst, J. J., Porter, D. L., and June, C. H. (2017) Chimeric antigen receptor T cells in refractory B-cell lymphomas, *N. Engl. J. Med.*, **377**, 2545-2554, doi: 10.1056/NEJMoa1708566.
129. Imura, Y., Ando, M., Kondo, T., Ito, M., and Yoshimura, A. (2020) CD19-targeted CAR regulatory T cells sup-

press B cell pathology without GvHD, *JCI Insight*, **5**, e136185, doi: 10.1172/jci.insight.136185.

130. Raje, N., Berdeja, J., Lin, Y., Siegel, D., Jagannath, S., Madduri, D., Liedtke, M., Rosenblatt, J., Maus, M. V., Turka, A., Lam, L. P., Morgan, R. A., Friedman, K., Massaro, M., Wang, J., Russotti, G., Yang, Z., Campbell, T., Hege, K., Petrocca, F., Quigley, M. T., Munshi, N., and Kochenderfer, J. N. (2019) Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma, *N. Engl. J. Med.*, **380**, 1726-1737, doi: 10.1056/NEJMoa1817226.
131. Berdeja, J. G., Madduri, D., Usmani, S. Z., Jakubowiak, A., Agha, M., Cohen, A. D., Stewart, A. K., Hari, P., Htut, M., Lesokhin, A., Deol, A., Munshi, N. C., O'Donnell, E., Avigan, D., Singh, I., Zudaire, E., Yeh, T. M., Allred, A. J., Olyslager, Y., Banerjee, A., Jackson, C. C., Goldberg, J. D., Schecter, J. M., Deraed, W., Zhuang, S. H., Infante, J., Geng, D., Wu, X., Carrasco-Alfonso, M. J., Akram, M., Hossain, F., Rizvi, S., Fan, F., Lin, Y., Martin, T., and Jagannath, S. (2021) Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study, *Lancet*, **398**, 314-324, doi: 10.1016/S0140-6736(21)00933-8.
132. Wang, X., Jasinski, D. L., Medina, J. L., Spencer, D. M., Foster, A. E., and Bayle, J. H. (2020) Inducible MyD88/CD40 synergizes with IL-15 to enhance antitumor efficacy of CAR-NK cells, *Blood Adv.*, **4**, 1950-1964, doi: 10.1182/bloodadvances.2020001510.
133. Shin, M. H., Oh, E., Kim, Y., Nam, D. H., Jeon, S. Y., Yu, J. H., and Minn, D. (2023) Recent advances in CAR-based solid tumor immunotherapy, *Cells*, **12**, 1606, doi: 10.3390/cells12121606.