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Allogeneic gamma delta T cells as adoptive cellular therapy for hematologic malignancies

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Abstract

Cancer immunotherapy, especially T-cell driven targeting, has significantly evolved and improved over the past decade, paving the way to treat previously refractory cancers. Hematologic malignancies, given their direct tumor accessibility and less immunosuppressive microenvironment compared to solid tumors, are better suited to be targeted by cellular immunotherapies. Gamma delta (γδ) T cells, with their unique attributes spanning the entirety of the immune system, make a tantalizing therapeutic platform for cancer immunotherapy. Their inherent anti-tumor properties, ability to act like antigen-presenting cells, and the advantage of having no major histocompatibility complex (MHC) restrictions, allow for greater flexibility in their utility to target tumors, compared to their αβ T cell counterpart. Their MHC-independent anti-tumor activity, coupled with their ability to be easily expanded from peripheral blood, enhance their potential to be used as an allogeneic product. In this review, the potential of utilizing γδ T cells to target hematologic malignancies is described, with a specific focus on their applicability as an allogeneic adoptive cellular therapy product.

Keywords

Gamma delta T cells, allogeneic, immunotherapy, leukemia, chimeric antigen receptor

Introduction

Gamma delta (γδ) T cells, a unique population of lymphocytes that mature in the thymus, account for 1–10% of circulating human T cells in the peripheral blood and up to 20% of intraepithelial T cells in the intestinal mucosa [1–5]. This T-cell subset has the distinctive ability to interact and display qualities of both the innate and adaptive immune systems. The robust properties of γδ T cells, allow it to polarize its immune response between anti- or pro-inflammatory, anti- or pro-tumorigenic, as well as between regulatory

and effector functions in the immune system depending on the situation [5, 6]. A sought-after attribute of γδ T cells is their inherent cytotoxic properties against malignant and infected cells. A study involving the molecular profiling of ~5,000 tumors showed that infiltrating γδ T cells were the strongest favorable leukocyte predictor of survival [7]. The major contributor towards cytotoxicity is the γδ T-cell receptor (TCR), which can identify antigens independent of major histocompatibility complex (MHC) presentation, in stark contrast to αβ T cells, which respond predominantly to antigens bound and restricted to MHC molecules. This singular property amplifies the potential of developing γδ T cells into an allogeneic product, given the minimal risk of graft-versus-host disease (GvHD). γδ T cells also express multiple activating natural killer (NK) cell surface receptors such as NK group 2D (NKG2D), NK protein 30 (NKp30) and NKp44 as well as the Fas ligand (FasL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) leading to the release of lysing mediators such as perforin and granzymes. Additionally, activating DNAX accessory molecule-1 (DNAM-1) receptors, leukocyte function-associated antigen-1 (LFA-1) and costimulatory receptor CD27, all lead to T-cell activation and enhanced cytotoxicity [8–12]. Tumor cell death can also occur by antibody-dependent cellular cytotoxicity (ADCC) in a CD16-dependent manner, binding to the Fc region of immunoglobulin G (IgG) deposited on tumor cells. Finally, γδ T cells can also act like antigen-presenting cells (APCs), thereby playing an important role in the adaptive immune system. Thus, γδ T cells facilitate direct-targeted cell death, aiding in tumor and pathogen clearance while releasing immune-modulatory cytokines such as interferon-γ (IFN-γ), interleukin-17 (IL-17) and TNF-α. The multimodal approach γδ T cells that utilize in directing their natural cytotoxicity, make them an attractive tool for development into an anti-cancer cellular immunotherapeutic. Hematologic malignancies, which include leukemias, lymphomas and myeloma, are more suited to be targeted by cellular therapies, given the direct access to tumor cells through blood vasculature and lymphatics. Additionally, these cancers typically have a less immunosuppressive tumor microenvironment compared to solid tumors, further enhancing potential therapeutic effects. Here, we will explore the use of allogeneic T cells in targeting hematologic malignancies by examining the properties that make allogeneic γδ T cells an attractive immunotherapeutic candidate and reviewing all reported preclinical and clinical studies investigating the use of γδ T cells against blood cancers.

Different types of γδ T cells: Vδ1 *vs.* Vδ2

Classically, T cells can be divided into two broad categories based on the structure of their TCR, alpha beta (αβ) T cells, the majority subset and γδ T cells, the minority subset. In γδ T cells, TCR loci encode for the gamma chain [TCR gamma locus (*TRG*)] and the delta chain [TCR delta locus (*TRD*)]. The TCRs expressed in γδ T cells can rearrange depending on the expression of recombination-activating genes (*RAGs*) [5, 13]. The heterodimer of γδ *V*(*D*)*J* gene segments is restricted by the γ gene TRG locus only having 12 variables (6 of which are functional) and the δ gene TRD locus only having eight functional variable genes [6]. In γδ T cells, the γ chains most frequently used are Vγ1, Vγ7, Vγ4, Vγ5, Vγ6, and Vγ9 while frequently used δ chains are Vδ1, Vδ2, Vδ3 and Vδ5. This is in comparison to the more dominant αβ T cells, which have 52 variable β and 10 variable α loci [6, 14, 15]. *Vγ* and *Vδ* genes tend to delineate a particular organ/location in the body in abundance. Vδ1 is specifically found in the thymus, skin, lungs and intestines [16] with Vγ5 present in the skin and Vγ7 in the intestine, Vγ6 in the reproductive mucosa and Vγ1/Vγ4 in secondary lymphoid organs [15]. Vδ2 is found primarily in peripheral blood alongside Vγ9 and Vδ3 is mainly found in the liver [15, 16]. Both Vδ1 and Vδ2 are associated with Vγ9-recognizing phosphoantigens (pAgs), such as non-peptide prenyl-pyrophosphate metabolites, which in turn are associated with stress-related antigens and selective expansion of specific γδ TCR clonotypes [17–19]. As stated before, the γδ TCR recognizes antigens in an MHC-independent manner, and thus unlike its αβ counterpart does not require antigen presentation by APCs.

In peripheral blood, Vδ1 T cells are typically a minority population compared to the more dominate Vγ9Vδ2 [5, 18]. However, Vδ1 T cells paired with Vγ8 and Vγ9 chains, enriched in tissues, have targeted a variety of host and microbial antigens [5, 18–21]. Some studies have also shown Vδ1 T cells, through

the γδ TCR, recognizing class 1b MHC-like proteins such as CD1 proteins similar to other unconventional T cells such as NKT or mucosal-associated invariant (MAIT) cells [22, 23]. Current studies have also indicated great benefits of Vδ1 T cells following allogeneic hematopoietic stem cell transplantation (allo-HSCT) and cytomegalovirus (CMV)-infections in patients with leukemia [3]. Some data also imply a balancing ratio between Vδ1 and Vδ2 T cells in tumor cells necessitates γδ T cell to either pro- or anti-tumorigenic responses [24, 25]. Despite multiple benefits indicated by Vδ1 T cells in targeting malignancies and post-transplantation survival, the prior inability to expand this small subset of T cells had hindered its clinical therapeutic benefits as an adoptive cellular product [3]. Notably, two recent publications have challenged that narrative by successfully expanding Vδ1 T cells with an anti-Vδ1 antibody [26, 27]. This new expansion method has now opened avenues for allogenic Vδ1 T cell therapy in clinical settings.

Vγ9Vδ2 T cells, the majority population in peripheral blood, have been directly implicated in both anti-viral and anti-tumor immunity. Their TCR is specifically reactive to pAgs, such as isopentenyl pyrophosphate (IPP), which are upregulated in certain stressed, infected and tumor cells. Butyrophilins (BTNs) have also emerged as an essential tool in γδ T-cell activation. BTNs are a large family of proteins and members of the extended B7 family of costimulatory molecules [28, 29]. BTN3A1 and BTN2A1 have been identified as crucial molecules indispensable for activation of Vγ9 T cells by pAgs. Vγ9Vδ2 TCR recognizes the BTN3A1/BTN2A1 complex in the membrane presenting IPP leading to activation [29–33]. IPP expression can also be artificially induced via inhibition of farnesyl pyrophosphate synthetase (FPPS) in the mevalonate pathway by amino-bisphosphonates. This unique property of the γδ TCR has been exploited by several groups, including ours, to isolate and expand Vγ9Vδ2 T cells from peripheral blood mononuclear cells (PBMCs) using bisphosphonates such as zoledronic acid [34–38]. This ability to easily activate and expand Vγ9Vδ2 T cells from peripheral blood makes them an attractive candidate to develop into a cellular immunotherapeutic product.

Non-TCR mediated cytotoxic mechanisms in γδ T cells

Along with the γδ TCR, expression of the NKG2D receptor plays a significant role in the cytotoxic ability of γδ T cells. The NKG2D receptor recognizes markers of cellular stress, which include the unique long 16 binding proteins (ULBPs) 1–6, and the MHC class I chain-related protein A and B (MICA/B) ligands. The NKG2D receptor-ligand interaction results in increased granzyme and perforin expression leading to target cell killing [39–45]. Along with NKG2D receptors, γδ T cells also express other activating NK cell receptors such as NKp30 and NKp44 to augment anti-tumor activity and cell signaling [46, 47]. The DNAM-1 receptor can trigger cytotoxicity upon interaction with its ligands CD112 (nectin) and CD155 (PVR), which are commonly expressed on hematologic malignancies [48–50]. Additionally, upregulation of FasL and TRAIL through TCR activation can also lead to enhanced tumor killing by interaction with Fas and TRAIL-R1/R2 respectively expressed on target cells. Other activating receptors include LFA-1 and the costimulatory receptor CD27 [8–12]. Cytokines such as IL-2, IL-15, IL-12, IL-18, IL-21 and IL-36γ also aid in γδ T-cell mediated cytotoxicity against malignant cells [51–53]. Finally, γδ T cells can mediate ADCC through the upregulation of CD16. γδ T cells can trigger cytotoxicity by recognizing the Fc regions of specific monoclonal antibodies (mAbs) bound to target cells, resulting in expression of CD107a, IFN-γ and TNF-α [54–56]. Apart from its innate-like direct cytotoxic mechanisms, γδ T cells also participate in the adaptive immune system by functioning as APCs, analogous to dendritic cells [5, 57, 58]. Vγ9Vδ2 T cells can process a wide range of microbial and tumor antigens for presentation to $CD4^+$ and $CD8^+$ T cells, and can also induce dendritic cell maturation through TNF-α production [57–59]. Thus, in addition to their TCR-dependent cytotoxicity, γδ T cells can employ several different killing mechanisms to target malignant cells (Figure 1).

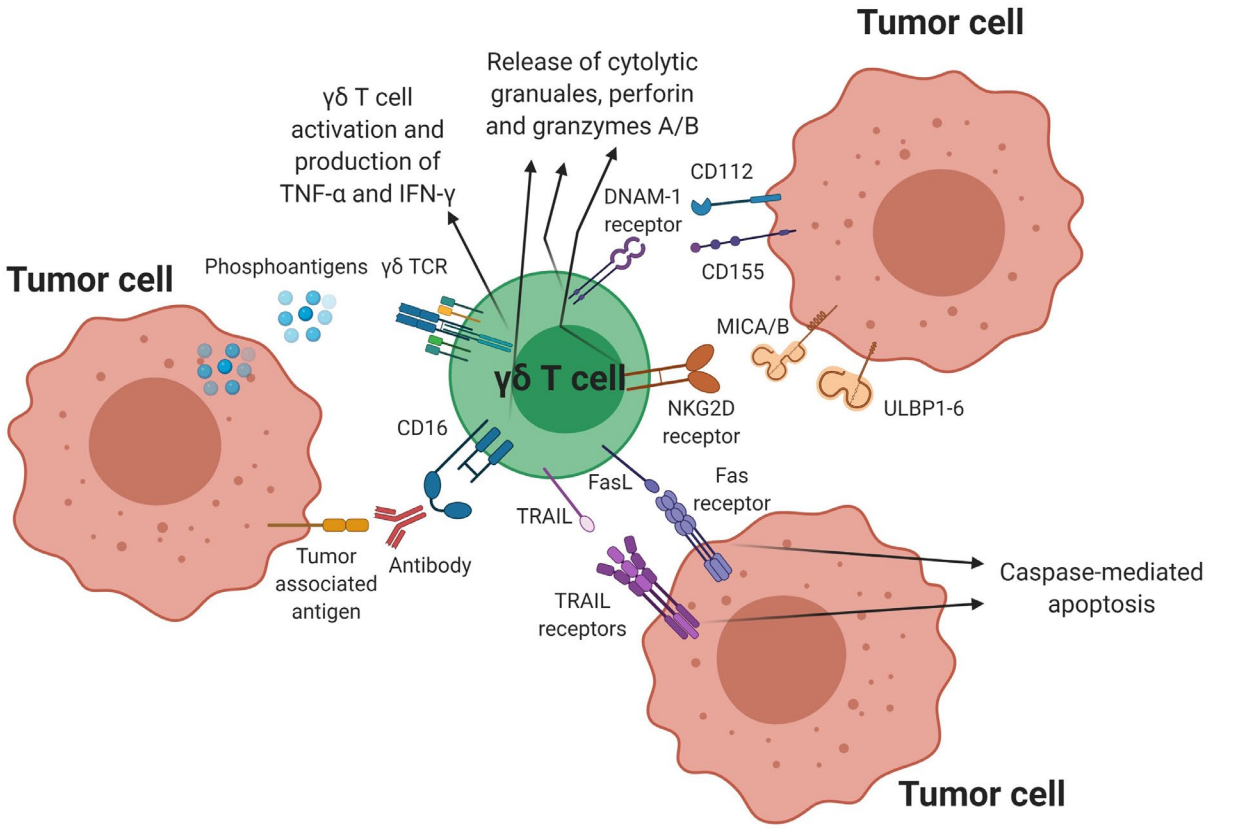

Figure 1. γδ T cell-mediated cytotoxicity against tumor cells. γδ T cells have several direct cytotoxic mechanisms against tumor cells as shown above. Binding of the pAg to the γδ TCR triggers activation resulting in target cell lysis and also stimulates the release of TNF-α and IFN-γ, which enhances the anti-tumor activity of other immune cells. Additional cytotoxic mechanisms include ADCC through CD16 expression, NKG2D and DNAM-1 receptor-ligand interactions as well as the activation of the TRAIL-TRAIL receptor and FasL-Fas receptor pathway

Ex vivo expansion of γδ T cells from peripheral blood

A critical factor in the manufacturing of a cellular therapy product is the ability to expand the product to reach desirable cell numbers in a robust efficient manner. The ability to expand Vγ9Vδ2 T cells from peripheral blood by taking advantage of the unique properties of the γδ TCR coupled with its non-MHC target recognition makes it an ideal candidate to develop into an allogeneic cellular therapy product. The γδ TCR is specifically reactive to pAgs, such as IPP, which are upregulated in infected and tumor cells. As mentioned before, it is now known that BTN3A1 and BTN2A1 are essential for the presentation of pAgs to the γδ TCR [29–33]. Expression of IPP can be artificially induced via inhibition of FPPS in the mevalonate pathway by amino-bisphosphonates such as zoledronic acid, pamidronate and risedronate. Several groups, including ours, have utilized this strategy to isolate and expand Vγ9Vδ2 T cells from PBMCs [39–43]. We have further characterized the variability in γδ T cell expansion among different donors, and have shown that IL-21 can be used to improve expansion in donors with poor *ex vivo* γδ T cell expansion [60]. Furthermore, we successfully depleted αβ T cells on day 6 of the expansion, providing a better environment for the γδ T cells to expand, while confirming that the αβ T cell population remains below clinically acceptable standards for T cell-depleted allogeneic stem cell products [60, 61]. Two recent studies have shown successful expansion of Vδ1 T cells from peripheral blood [26, 27]. These studies performed by Adicet Bio, Inc. (Boston, USA) utilize a proprietary agonist anti-Vδ1 mAb that selectively activates and expands Vδ1 T cells from healthy donor derived PBMCs. Similar to our studies, an αβ T cell depletion step is utilized before the final product formulation [26].

Role of γδ T cells in the setting of allo-HSCT for hematologic malignancies

Allo-HSCT can be an effective treatment option for patients with high-risk leukemia and other hematologic malignancies, which are refractory to conventional treatments. The success of an allogeneic transplant depends on several different factors, such as disease status prior to hematopoietic stem cell transplantation (HSCT), type of hematologic malignancy, and donor characteristics such as human leukocyte antigen (HLA) match status, age, and stem cell source. GvHD remains the most significant toxicity in patients undergoing allo-HSCT, and the pathogenesis of GvHD is primarily driven by donor αβ T cells. While several measures are taken to reduce potential GvHD, the graft-versus-leukemia (GvL) effect seen in the setting of allo-HSCT is known to be beneficial to patients. Given that γδ T cells identify antigens in an MHC-independent manner, they can provide therapeutic GvL effects without the risk of GvHD; hence there is a growing interest in the role γδ T cells play in the success of allo-HSCT [46]. Indeed, high γδ T cell immune reconstitution after allo-HSCT of αβ T cell and $CD19^+$ depleted grafts has been shown to result in overall higher survival rates and decreased rate of acute GvHD [62–64]. In a large cohort of patients with leukemia undergoing allo-HSCT that received a T-cell depleted bone marrow graft from partially mismatched HLA donors, patients in which γδ T cells accounted for greater than 10% of circulating lymphocytes had superior disease-free 30 months after treatment [64]. There was no significant difference in acute and chronic GvHD, suggesting a superior GvL effect without GvHD. Two subsequent long term follow-up studies for this population, at 42 months and then 8 years, confirmed there was a significantly better disease-free survival (DFS) and overall survival for patients with higher proportion of γδ T cells [65, 66]. A more recent pediatric study of 102 patients from St Jude Children's Research Hospital analyzing the γδ T cell reconstitution after allo-HSCT, showed that a significantly better event-free survival and overall survival was seen in patients with increased γδ T cells at a median follow-up of 2.7 years [67]. Additionally, the patients with higher γδ T cells had a lower incidence of bacterial and viral infections, emphasizing the anti-microbial properties of γδ T cells [68]. Based on the superior GvL effects of γδ T cells without causing GvHD, combined with their ability to fight infections, several clinical studies are now exploring the utility of the adoptive transfer of allogenic donor-derived γδ T cells in the post-transplant setting, as we discuss in a later section in this article [46].

Adoptive cellular therapies for hematologic malignancies

Hematologic malignancies, which include leukemias, lymphomas and myelomas, have become an attractive target for cellular therapies over the past decade, especially with the advent of chimeric antigen receptor (CAR) based T-cell therapies. In this innovative therapy, T cells are genetically modified to express a receptor, called a CAR, which can identify target tumor antigens with the specificity of an mAb, thereby enabling the T cell to directly kill its tumor target [69]. CAR T-cell therapy has been very successful in hematologic malignancies, especially B-cell malignancies and more recently multiple myeloma (MM), compared to solid tumors [70–72]. Hematologic malignancies are more suited to be targeted by cellular therapies, given the direct accessibility to tumor cells through the blood vasculature and lymphatics. Additionally, blood cancers typically have a less immunosuppressive tumor microenvironment compared to solid tumors, further enhancing potential therapeutic effects [73].

However, most current cellular therapies use autologous patient-derived αβ T cells. T cells are first collected from the patient through a process called leukapheresis and then genetically modified using a viral vector encoding the CAR. Cells are then expanded to the desired numbers and finally given back to the patient after lympho-depleting chemotherapy [69]. While there has been significant progress in the manufacturing process over the past few years, the production and administration of an autologous cellular therapy product are still very complex and time-consuming, taking at minimum between 2–4 weeks from collection to infusion. Although this strategy has been successful in B-cell malignancies, the delay in delivering the therapeutic product may not be feasible in more aggressive cancers such as acute myeloid leukemia (AML) and T-cell malignancies [74, 75]. Furthermore, we are now learning that poor T-cell fitness is

a major factor in the failure of these therapies, especially when cells are collected from patients heavily pre-treated with chemotherapy [70].

To overcome this challenge, there has been a concerted effort to develop "off-the-shelf allogeneic cellular therapies using healthy donors as the effector cell source. However, given the severe risk of GvHD using αβ T cells from non-HLA-matched donors, certain genetic modifications are necessary to make allogeneic αβ T cells a safe and feasible therapeutic. The most common approach has been to knock down the expression of the αβ TCR by gene editing of the TCR alpha constant (*TRAC*) and/or TCR beta constant (*TRBC*) locus. The different gene editing tools that have used in this setting include Zinc finger nucleases (ZFN) [76–78], transcription activator-like effector nucleases (TALEN) [79–81], and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing [82, 83]. While gene editing of αβ T cells is an exciting approach, the translatability to a clinical product can be challenging and expensive. γδ T cells, given their non-MHC dependence for antigen recognition, provide an excellent alternative as the effector cell source, and do not require any further genome editing to be developed into an allogeneic product. Furthermore, given their inherent anti-tumor properties, they form a promising candidate to move forward as an "off-the-shelf cellular therapeutic.

Preclinical studies of γδ T cells against hematologic malignancies

In this section, we will review the different approaches that have been tested in the preclinical setting to enhance the cytotoxic effect of γδ T cells against hematologic malignancies. These include the use of γδ T cells in combination with amino-bisphosphonates, checkpoint inhibitors, chemotherapeutic drugs, mAbs and bispecific T-cell engagers (BiTEs) as well as the use of γδ T cells genetically modified to express CARs.

Use of amino-bisphosphonates and anti-BNT3A1 antibodies

As stated before, most studies have used amino-bisphosphonates to selectively expand γδ T cells *ex vivo*. Several studies have shown that γδ T cells are cytotoxic towards AML blasts. Gertner-Dardenne et al. [48] showed that Vγ9Vδ2 T cells efficiently killed autologous AML blasts via the perforin/granzyme pathway utilizing both TCR and DNAM-1 dependent mechanisms. Vγ9Vδ2 T cells also killed AML blast in a xenograft mouse model improving survival. More recently, Benyamine et al. [84] showed that anti-BTN3A1 antibodies have the ability to mimic pAg stimulation, which in turn selectively activates Vγ9Vδ2 T cells. Incubation of AML blasts with anti-BNT3A1 triggered BTN3A1 on the blasts, resulting in enhanced Vγ9Vδ2 T cell-mediated killing, while also sensitizing resistant blasts to Vγ9Vδ2 T cell lysis. They further validated their results in an AML xenograft model demonstrating that the agonistic anti-CD277/BTN3A1 antibody mAb 20.1, enhanced the therapeutic efficacy of adoptively transferred Vγ9Vδ2 T cells [84]. Vγ9Vδ2 T cells have also been shown to be effective against lymphoma cell line Daudi and MM cell lines RPMI8226 and U266 [85, 86]. Interestingly, the Vδ1 T cell subset appears to play a more important role in targeting chronic lymphocytic leukemia (CLL) blasts, with increased number having been reported in patients with CLL [87]. Siegers et al. [88] developed an expansion protocol using the mitogen concanavalin A (Con A) that selectively expanded Vδ1 cells over the Vδ2 subset when combined with IL-2 and IL-4. They subsequently showed that these were more cytotoxic against the CLL cell line MEC1 compared to Vδ2 cells [88]. A subsequent Vδ1 T cell expansion protocol developed by Almeida et al. [89], resulting in a cellular product called Delta One T (DOT) cells, showed impressive efficacy in CLL xenograft models. The DOT cells have also been tested in an AML xenograft model and were shown to have impressive efficacy [90].

Combination with chemotherapy

Several studies have taken advantage of the NKG2D receptor-ligand axis as a means to effectively target hematologic malignancies using γδ T cells. Expression of the NKG2D ligands ULBP1–6 and MICA/B can be upregulated in leukemia and myeloma cells by pre-treatment with chemotherapeutic agents. Different classes of chemotherapeutics have been. The proteasome inhibitor, bortezomib, has been tested in MM, AML and T-cell acute lymphoblastic leukemia (T-ALL) [61, 91, 92]. Niu et al. [92] showed treatment of MM cells with low-dose bortezomib resulted in enhanced killing by γδ T cells and NK cells, through increased

NKG2D and DNAM-1 ligand expression. Story et al. [61] showed that bortezomib increased the ULBP2/5/6 expression in both AML and T-ALL cell lines, enhancing γδ T cell-mediated killing. Importantly, both studies showed that bortezomib had minimal inhibitory effects on γδ T cell proliferation and function. The other class of chemotherapeutics that have been tested in this setting are epigenetic drugs. These include histone deacetylase inhibitors such as valproic acid and romidepsin as well as demethylating agents such as azacitidine and decitabine [93–95]. de Weerdt et al. [96] showed that treatment of CLL patient-derived Vγ9Vδ2 T cells with the tyrosine kinase inhibitor irutinib restored its functional phenotype and improved cytotoxicity against CLL cells.

ADCC with mAbs and other antibody-based constructs

γδ T cells mediate ADCC through expression of the Fc-γ receptor III CD16. Multiple studies have evaluated the combination of γδ T cells with anti-CD20 mAbs such as rituximab, ofatumumab and obinutuzumab (GA101) to target B-cell malignancies. Tokuyama et al. [56] demonstrated the rituximab enhanced the ADCC effect of γδ T cells against CLL and follicular lymphoma cells. Braza et al. [97] showed that the highest ADCC effect against follicular lymphoma cells was seen when using obinutuzumab, compared to rituximab and ofatumumab. Similarly, Gertner-Dardenne et al. [98] found that alemtuzumab, an anti-CD52 antibody, also increased γδ T-cell dependent ADCC against lymphoma cell lines. Another new category of antibody-based drugs is BiTEs which consist of two single chain variable fragments (scFvs) binding domains, one typically specific to CD3 present on T cells, and another to a tumor associated antigen on cancerous cells [99–101]. Concurrent binding of a BiTE combination results in forming a lytic immune synapse between the cytotoxic T cell and the cancerous target cell. A recent preclinical study by Chen et al. [102] showed that combining the CD19-directed BiTE blinatumomab with γδ T-cells improved overall survival in a murine B-ALL model. Previously, Seidel et al. [54] had tested both CD19-CD3 and CD19-CD16 using the CD19 antibody 4G7SDIE as its backbone, and showed that the dual antibody constructs could induce cytotoxic reactions from γδ T cells. Schiller et al. [103] then created a single chain triple antibody (CD19-CD19-CD16) called SPM-1 and showed it had higher NK and γδ T-cell mediated killing when compared to 4G7SDIE. More excitingly, a new γδ T-cell specific BiTE against AML has now been created, TRGV9/CD123, which binds the Vγ9 chain of the Vγ9Vδ2 T cell, thereby enabling it to selectively target CD123-expressing AML cells [104, 105].

CAR-expressing γδ T cells

CAR T-cell therapy has revolutionized cancer immunotherapy over the past decade, with great success seen in hematologic malignancies. However, given the complexity and cost involved in manufacturing autologous CAR T cells, there is a growing need to develop “off-the-shelf allogeneic CAR T-cell therapeutics. Given the lack of an MHC-dependent TCR, γδ T cells have not been implicated in GvHD pathogenesis, and are ideal candidates to be developed into allogeneic CAR T-cell therapeutics. While several companies are now developing CAR γδ T cells, only limited preclinical data has been published so far. A few reported studies have demonstrated effective preclinical CAR-γδ T cell cytotoxicity against hematologic malignancies. Deniger et al. [106] used a sleeping beauty transposase system to develop CD19 CAR γδ T cells, which showed efficacy against CD19 positive tumor cell lines *in vitro* and reduced leukemia burden in xenograft models. Similarly, Rozenbaum et al. [107] recently showed the CD19-directed CAR γδ T cells generated from lentiviral transduction were effective in both *in vitro* and *in vivo* studies. Multiple doses of γδ T cells were given to attain the desired effect given their short lifespan. Additionally, CD19 negative leukemia cells when primed with zoledronate were also killed. In an interesting study by Fleischer et al. [108], non-signaling CARs (NSCARs) were expressed in γδ T cells, to prevent fratricide while targeting T-cell leukemia. The NSCARs lacked signaling/activation domains, but retained the ability to interact with the tumor cell with antigen-specificity, thereby acting as an anchor. This then allowed the γδ T cell to use its inherent MHC-independent mechanism to lyse the tumors cells. They demonstrated that both CD5 and CD19-NSCAR modified γδ T cells had a significant increase in killing against T-ALL and B-ALL cell lines respectively. Importantly, as hypothesized, no increase in cytotoxicity was seen in the NSCAR approach when using αβ

T cells [108]. Finally, a recent study by Nishimoto et al. [26] (Adicet Bio, Inc., Boston, USA) demonstrated successful large-scale manufacturing of anti-CD20 CAR γδ T cells utilizing healthy donor derived Vδ1 T cells. As mentioned before, Vδ1 T cells were expanded utilizing an agonist anti-Vδ1 mAb. The anti-CD20 CAR Vδ1 T cells exhibited effectively *in vitro* tumor cell killing and pro-inflammatory cytokine production, as well as *in vivo* tumor growth inhibition of B-cell lymphoma xenografts in immunodeficient mice. Interestingly, the CAR Vδ1 T cells exhibited a naïve-like T-cell memory phenotype and only a single dose of γδ T cells was used in mouse studies [26]. Based on these findings, a phase 1 clinical trial has been initiated in patients with CD20-positive B-cell malignancies (NCT04735471).

Clinical studies using γδ T cells against hematologic malignancies

Clinical trials evaluating the use of γδ T cells to target hematologic malignancies fall into three separate categories: (a) *in vivo* stimulation of autologous γδ T cells, (b) adoptive transfer of *ex vivo* expanded autologous γδ T cells, and (c) adoptive transfer of *ex vivo* expanded allogeneic γδ T cells. Most studies using allogeneic γδ T cells having been the post-HSCT setting where cells are derived from the allo-HSCT donor; however, newer trials are now beginning to evaluate the use of allogeneic γδ T cells as a standalone cellular therapeutic. The results from all past completed trials utilizing γδ T cells to target hematologic malignancies are reviewed in Table 1.

Table 1. Clinical studies utilizing γδ T cells against hematologic malignancies with published results

Reference	Year	Disease	*N*	Intervention	Response
In vivo stimulation of γδ T cells					
Wilhelm et al. [109]	2003	NHL, CLL, MM	19	Pamidronate and IL-2	3/19 had objective response
Laurent et al. [110]	2009	Follicular lymphoma	45	Rituximab + BrHPP + IL-2	75% of first 12 patients had response
Kunzmann et al. [111]	2012	AML	8	Zoledronate and IL-2	2/8 had partial remission
Bertaina et al. [112]	2017	ALL and AML	43	Zoledronate post allo-HSCT	Improved DFS, higher circulating γδ T cells
Merli et al. [113]	2020	ALL, AML and MPAL	46	Zoledronate x 3 post allo-HSCT	Improved DFS, lower TRM, reduced GvHD
Adoptive transfer of γδ T cells					
Abe et al. [116]	2009	MM	6	Four infusions of *ex vivo* expanded autologous Vγ9γδ2 T cells	4/6 had stable disease, no toxicity
Wilhelm et al. [117]	2014	T-NHL, AML, MM, plasma cell leukemia	4	Haploidentical γδ T cells, followed by zoledronate + IL-2	3/4 had complete response

N: total number of patients; NHL: non-Hodgkin lymphoma; BrHPP: bromohydrin pyrophosphate; ALL: acute lymphoblastic leukemia; MPAL: mixed phenotype acute leukemia; TRM: transplant-related mortality; T-NHL: T-cell NHL

In one of the first completed trials, Wilhelm et al. [109] tested the effects of pamidronate and IL-2 on Vγ9Vδ2 T cell activation and anti-tumor activity in 19 patients with NHL, CLL or MM. Objective responses were seen in only three patients, and corresponded to the Vγ9Vδ2 T cell proliferation *in vitro* [109]. Laurent et al. [110] studied the combination of rituximab with IPH1101 (BrHPP, a Vγ9Vδ2 T cell agonist) along with IL-2 in 45 patients with follicular lymphoma. Only data for the first 12 patients were reported in an abstract with 75% showing a response; however, the final outcomes were never published. In another study, Kunzmann et al. [111] studied the use of zoledronate and IL-2 in patients with different advance malignancies, including eight patients with refractory AML. Two of the eight AML patients had a partial remission. In a pediatric study of patients undergoing allo-HSCT for acute leukemia, patients who received a zoledronate infusion post-transplant had improved DFS and a higher number of circulating γδ T cells [112]. A subsequent trial showed that three or more infusions of zoledronate lowered TRM, improved DFS and was associated with a reduced incidence of GvHD [113].

The adoptive transfer of γδ T cells has more commonly been tested in solid tumor malignancies. Published data from a recent study using allogeneic Vγ9Vδ2 T cells in 132 advanced stage liver and lung cancer patients showed that allogeneic Vγ9Vδ2 T cells produced no significant adverse effects (e.g., immune

rejection, cytokine storm, or GVHD effects) [114]. A follow-up case report was published on one of these patients, who is a 30-year-old male with stage 4 cholangiocarcinoma, post liver transplantation, with recurrent mediastinal lymph node metastasis [115]. This patient successfully received 8 infusions of allogenic Vγ9Vδ2 T cells (4×10^8 cells total) and had a significant reduction in the size of his lymph nodes without any significant adverse effects, thereby confirming its potential as a therapeutic. Several current ongoing trials are now evaluating this approach in hematologic malignancies. All the current ongoing γδ T cell clinical trials targeting hematologic malignancies, including two CAR-based γδ T cell studies are listed in Table 2. Results of only two studies using adoptively transferred γδ T cells in hematologic malignancies have been published. Abe et al. [116] treated six MM patients with *ex vivo* expanded autologous Vγ9γδ2 T cells. Cells were expanded from PBMCs using zoledronate and IL-2, and each patient received four infusions of cells at 2-week intervals. The infusions were safely tolerated and disease remained stable in 4/6 patients [116]. In a pilot study, Wilhelm et al. [117] reported the successful transfer of haploidentical γδ T cells in four patients with refractory hematologic malignancies (one T-NHL, one AML, one secondary plasma cell leukemia, and one MM), followed by *in vivo* stimulation with zoledronate and IL-2. Three out the four patients achieved complete remission, with one patient having a sustained response for 8 months [117].

Table 2. Active adoptive γδ T-cell immunotherapy clinical trials against hematologic malignancies

ClinicalTrials.gov identifier	Sponsor	Disease	Intervention	Phase	Status
NCT04696705	Institute of Hematology & Blood Diseases Hospital	NHL and PTCL	*Ex vivo* expanded allogeneic γδ T cells	Early Phase I	Recruiting
NCT05015426	H. Lee Moffiff Cancer Center and Research Institute	AML	*Ex vivo* expanded donor γδ T cells post allo-HSCT	Phase I/Ib	Recruiting
NCT04764513	Chinese PLA General Hospital	AML, ALL, MDS, lymphoma	*Ex vivo* expanded donor γδ T cells post allo-HSCT	Phase I/II	Recruiting
NCT03533816	University of Kanas Medical Center	AML, CML, ALL, MDS	EAGD T-cell infusion following haplo-HSCT	Phase I	Recruiting
NCT05001451	GammaDelta Therapeutics Limited	AML	GDX012 infusion–allogeneic Vδ1$^+$ γδ T cells	Phase I	Recruiting
NCT04008381	Wuhan Union Hospital	AML	*Ex vivo* expanded allogeneic γδ T cells from suitable donor	Phase I	Recruiting
NCT03790072	TC Biopharm	AML	*Ex vivo* expanded allogeneic γδ T cells from suitable donor (OmnImmune®)	Phase I	Completed
NCT04702841	PersonGen BioTherapeutics	CD7$^+$ T-cell malignancies	CAR–γδ T cells	Early Phase I	Recruiting
NCT04735471	Adicet Bio. Inc.	B-cell malignancies	Anti-CD20-CAR-T	Phase 1	Recruiting

PTCL: peripheral T-cell lymphoma; MDS: myelodysplastic syndrome; CML: chronic myeloid leukemia; EAGD: *ex vivo* expanded/activated γδ; haplo: haploidentical

Conclusions

As we have discussed here, γδ T cells provide an optimal platform for development into an "off-the-shelf" allogeneic cellular therapeutic. There continues to be a growing interest in the use of these unique multi-functional immune cells as reflected in the increasing number of companies developing γδ T-cell based immunotherapies (Table 3). While there has been remarkable success in utilizing CAR T-cell therapies for hematologic malignancies, the overall efficacy has somewhat been limited primarily to B-cell malignancies. In order to extend this therapy to other disease types, newer approaches need to be considered. Additionally, we now have a better understanding of the mechanisms of treatment failure, and a poor quality cellular product remains one of the major concerns. A shift to an allogeneic platform may be necessary to overcome these manufacturing challenges and increase the availability of the unique immunotherapy to the general population. γδ T cell therapies can be utilized in number of different approaches, with adjuncts such as zoledronic acid, in combination with chemotherapies to upregulate NKG2D ligand expression, enhancing ADCC when used with mAbs or BiTEs, on by genetic modification to

express a CAR. Thus, allogeneic γδ T cells have great potential to be an effective cellular therapy option for hematologic malignancies. Continued efforts are needed to enhance and maximize the benefits of this unique cellular therapeutic.

Table 3. Current companies developing γδ T cell-based immunotherapies

Company	γδ T cell type	Allogeneic *vs.* autologous	Targeting strategy/engineering
GammaCell Biotechnologies	Vδ2	Autologous/allogeneic	Unmodified
Hebei Senlang Biotechnology	Vδ2	Autologous	CAR/αβTCR
Incysus Therapeutics	Vδ2	Autologous	Engineered for chemo-resistance
Adicet Bio, Inc.	Vδ1	Allogeneic	CAR
Beijing Doing Biomedical	Vδ2	Allogeneic	Unmodified/CAR
Cytomed Therapeutics	Vδ2	Allogeneic	CAR
GammaDelta Therapeutics	Vδ1	Allogeneic	CAR
Immatics	Vδ2	Allogeneic	αβTCR
PhosphoGam Inc.	Vδ2	Allogeneic	Unmodified
TC BioPharm	Vδ1/Vδ2	Autologous/allogeneic	Unmodified/CAR
Imcheck Therapeutics	Vδ2	Autologous (*in vivo*)	Vδ2 activation with BTN3A
Lava Therapeutics	Vδ2	Autologous (*in vivo*)	Activated Vδ2 with BiTE
PersonGen BioTherapeutics	γδ T	Allogeneic	TAA3-UCAR, CD7 UCAR
Expression Therapeutics	Vδ2	Allogeneic	Unmodified/CAR

UCAR: universal CAR

Note. Adapted from "Cancer immunotherapy with γδ T cells: many paths ahead of us", by Kabelitz D, Serrano R, Kouakanou L, Peters C, Kalyan S. Cell Mol Immunol. 2020;17:925–39 (https://www.nature.com/articles/s41423-020-0504-x). CC-BY.

Abbreviations

ADCC: antibody-dependent cellular cytotoxicity

ALL: acute lymphoblastic leukemia

allo-HSCT: allogeneic hematopoietic stem cell transplantation

AML: acute myeloid leukemia

APCs: antigen-presenting cells

BiTEs: bispecific T-cell engagers

BTNs: Butyrophilins

CAR: chimeric antigen receptor

CLL: chronic lymphocytic leukemia

DFS: disease-free survival

DNAM-1: DNAX accessory molecule-1

GvHD: graft-versus-host disease

GvL: graft-versus-leukemia

HLA: human leukocyte antigen

HSCT: hematopoietic stem cell transplantation

IFN-γ: interferon-γ

IL-17: interleukin-17

IPP: isopentenyl pyrophosphate

mAbs: monoclonal antibodies

MHC: major histocompatibility complex

MM: multiple myeloma

NHL: non-Hodgkin lymphoma

NK: natural killer

NKG2D: natural killer group 2D

NKp30: natural killer protein 30

NSCARs: non-signaling chimeric antigen receptors

pAgs: phosphoantigens

PBMCs: peripheral blood mononuclear cells

T-ALL: T-cell acute lymphoblastic leukemia

TCR: T-cell receptor

TNF: tumor necrosis factor

TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

TRG: T-cell rearranging gamma locus

ULBP: unique long 16-binding proteins

Declarations

Author contributions

NJ and SSR both wrote the first draft, edited, read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

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References

1. Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. Nat Rev Immunol. 2015;15:683–91.
2. Coffelt SB, Kabelitz D, Silva-Santos B, Kuball J, Born W, Bank I. Editorial: γδ T cells in cancer. Front Immunol. 2020;11:602411.
3. Nussbaumer O, Koslowski M. The emerging role of γδ T cells in cancer immunotherapy. Immuno-Oncology Technology. 2019;1:3–10.

4. Kabelitz D, Serrano R, Kouakanou L, Peters C, Kalyan S. Cancer immunotherapy with γδ T cells: many paths ahead of us. Cell Mol Immunol. 2020;17:925–39.
5. Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. Nat Rev Immunol. 2013;13:88–100.
6. Sullivan LC, Shaw EM, Stankovic S, Snell GI, Brooks AG, Westall GP. The complex existence of γδ T cells following transplantation: the good, the bad and the simply confusing. Clin Transl Immunology. 2019;8:e1078.
7. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med. 2015;21:938–45.
8. Wrobel P, Shojaei H, Schittek B, Gieseler F, Wollenberg B, Kalthoff H, et al. Lysis of a broad range of epithelial tumour cells by human gamma delta T cells: involvement of NKG2D ligands and T-cell receptor-versus NKG2D-dependent recognition. Scand J Immunol. 2007;66:320–8.
9. Todaro M, D'Asaro M, Caccamo N, Iovino F, Francipane MG, Meraviglia S, et al. Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. J Immunol. 2009;182:7287–96.
10. Spada FM, Grant EP, Peters PJ, Sugita M, Melián A, Leslie DS, et al. Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. J Exp Med. 2000;191:937–48.
11. Couzi L, Pitard V, Sicard X, Garrigue I, Hawchar O, Merville P, et al. Antibody-dependent anti-cytomegalovirus activity of human γδ T cells expressing CD16 (FcγRIIIa). Blood. 2012;119:1418–27.
12. Farrington LA, Callaway PC, Vance HM, Baskevitch K, Lutz E, Warrier L, et al. Opsonized antigen activates Vδ2$^+$ T cells via CD16/FCγRIIIa in individuals with chronic malaria exposure. PLoS Pathog. 2020;16:e1008997.
13. Davey MS, Willcox CR, Hunter S, Kasatskaya SA, Remmerswaal EBM, Salim M, et al. The human Vδ2$^+$ T-cell compartment comprises distinct innate-like Vγ9$^+$ and adaptive Vγ9$^-$ subsets. Nat Commun. 2018;9:1760.
14. Legut M, Cole DK, Sewell AK. The promise of γδ T cells and the γδ T cell receptor for cancer immunotherapy. Cell Mol Immunol. 2015;12:656–68.
15. Wo J, Zhang F, Li Z, Sun C, Zhang W, Sun G. The role of gamma-delta T cells in diseases of the central nervous system. Front Immunol. 2020;11:580304.
16. Lawand M, Déchanet-Merville J, Dieu-Nosjean MC. Key features of gamma-delta T-cell subsets in human diseases and their immunotherapeutic implications. Front Immunol. 2017;8:761.
17. Girardi M. Immunosurveillance and immunoregulation by gammadelta T cells. J Invest Dermatol. 2006;126:25–31.
18. Davey MS, Willcox CR, Baker AT, Hunter S, Willcox BE. Recasting human Vδ1 lymphocytes in an adaptive role. Trends Immunol. 2018;39:446–59.
19. Hviid L, Smith-Togobo C, Willcox BE. Human Vδ1$^+$ T cells in the immune response to *Plasmodium falciparum* infection. Front Immunol. 2019;10:259.
20. Pang DJ, Neves JF, Sumaria N, Pennington DJ. Understanding the complexity of γδ T-cell subsets in mouse and human. Immunology. 2012;136:283–90.
21. Siegers GM, Lamb LS Jr. Cytotoxic and regulatory properties of circulating Vδ1$^+$ γδ T cells: a new player on the cell therapy field? Mol Ther. 2014;22:1416–22.
22. Di Lorenzo B, Ravens S, Silva-Santos B. High-throughput analysis of the human thymic Vδ1$^+$ T cell receptor repertoire. Sci Data. 2019;6:115.
23. Li Y, Li G, Zhang J, Wu X, Chen X. The dual roles of human γδ T cells: anti-tumor or tumor-promoting. Front Immunol. 2021;11:619954.
24. Zhao Y, Niu C, Cui J. Gamma-delta (γδ) T cells: friend or foe in cancer development? J Transl Med. 2018;16:3.

25. Lee HW, Chung YS, Kim TJ. Heterogeneity of human γδ T cells and their role in cancer immunity. Immune Netw. 2020;20:e5.
26. Nishimoto KP, Barca T, Azameera A, Makkouk A, Romero JM, Bai L, et al. Allogeneic CD20-targeted γδ T cells exhibit innate and adaptive antitumor activities in preclinical B-cell lymphoma models. Clin Transl Immunology. 2022;11:e1373.
27. Makkouk A, Yang X, Barca T, Lucas A, Turkoz M, Nishimoto K, et al. Allogeneic Vδ1 gamma delta T cells engineered with glypican-3 (GPC3)-specific CAR expressing soluble IL-15 have enhanced antitumor efficacy against hepatocellular carcinoma in preclinical models. J Clin Oncol. 2021;39:e14511.
28. Rhodes DA, Reith W, Trowsdale J. Regulation of immunity by butyrophilins. Annu Rev Immunol. 2016;34:151-72.
29. Harly C, Guillaume Y, Nedellec S, Peigné CM, Mönkkönen H, Mönkkönen J, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. Blood. 2012;120:2269–79.
30. Sandstrom A, Peigné CM, Léger A, Crooks JE, Konczak F, Gesnel MC, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells. Immunity. 2014;40:490–500.
31. Sebestyen Z, Scheper W, Vyborova A, Gu S, Rychnavska Z, Schiffler M, et al. RhoB mediates phosphoantigen recognition by Vγ9Vδ2 T cell receptor. Cell Rep. 2016;15:1973–85.
32. Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, et al. Butyrophilin 2A1 is essential for phosphoantigen reactivity by gammadelta T cells. Science. 2020;367:eaay5516.
33. Karunakaran MM, Willcox CR, Salim M, Paletta D, Fichtner AS, Noll A, et al. Butyrophilin-2A1 directly binds germline-encoded regions of the Vgamma9Vdelta2 TCR and is essential for phosphoantigen sensing. Immunity. 2020;52:487–98.e6.
34. Sutton KS, Dasgupta A, McCarty D, Doering CB, Spencer HT. Bioengineering and serum free expansion of blood-derived gammadelta T cells. Cytotherapy. 2016;18:881–92.
35. Kondo M, Izumi T, Fujieda N, Kondo A, Morishita T, Matsushita H, et al. Expansion of human peripheral blood gammadelta T cells using zoledronate. J Vis Exp. 2011;55:3182.
36. Li H, Pauza CD. Rapamycin increases the yield and effector function of human gammadelta T cells stimulated *in vitro*. Cancer Immunol Immunother. 2011;60:361–70.
37. Lamb LS Jr, Bowersock J, Dasgupta A, Gillespie GY, Su Y, Johnson A, et al. Engineered drug resistant gammadelta T cells kill glioblastoma cell lines during a chemotherapy challenge: a strategy for combining chemo- and immunotherapy. PLoS One. 2013;8:e51805.
38. Di Carlo E, Bocca P, Emionite L, Cilli M, Cipollone G, Morandi F, et al. Mechanisms of the antitumor activity of human Vgamma9Vdelta2 T cells in combination with zoledronic acid in a preclinical model of neuroblastoma. Mol Ther. 2013;21:1034–43.
39. Hudspeth K, Fogli M, Correia DV, Mikulak J, Roberto A, Della Bella S, et al. Engagement of NKp30 on Vδ1 T cells induces the production of CCL3, CCL4, and CCL5 and suppresses HIV-1 replication. Blood. 2012;119:4013–6.
40. Correia DV, Fogli M, Hudspeth K, da Silva MG, Mavilio D, Silva-Santos B. Differentiation of human peripheral blood Vδ1$^+$ T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. Blood. 2011;118:992–1001.
41. Nausch N, Cerwenka A. NKG2D ligands in tumor immunity. Oncogene. 2008;27:5944–58.
42. Shafi S, Vantourout P, Wallace G, Antoun A, Vaughan R, Stanford M, et al. An NKG2D-mediated human lymphoid stress surveillance response with high interindividual variation. Sci Transl Med. 2011;3:113ra124.

43. Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T. Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. Proc Natl Acad Sci U S A. 1999;96:6879–84.
44. Bodman-Smith MD, Anand A, Durand V, Youinou PY, Lydyard PM. Decreased expression of FcgammaRIII (CD16) by gammadelta T cells in patients with rheumatoid arthritis. Immunology. 2000;99:498–503.
45. Busche A, Goldmann T, Naumann U, Steinle A, Brandau S. Natural killer cell-mediated rejection of experimental human lung cancer by genetic overexpression of major histocompatibility complex class I chain-related gene A. Hum Gene Ther. 2006;17:135–46.
46. Handgretinger R, Schilbach K. The potential role of γδ T cells after allogeneic HCT for leukemia. Blood. 2018;131:1063–72.
47. Shojaei H, Oberg HH, Juricke M, Marischen L, Kunz M, Mundhenke C, et al. Toll-like receptors 3 and 7 agonists enhance tumor cell lysis by human gammadelta T cells. Cancer Res. 2009;69:8710–7.
48. Gertner-Dardenne J, Castellano R, Mamessier E, Garbit S, Kochbati E, Etienne A, et al. Human Vγ9Vδ2 T cells specifically recognize and kill acute myeloid leukemic blasts. J Immunol. 2012;188:4701–8.
49. Knight A, Mackinnon S, Lowdell MW. Human Vdelta1 gamma-delta T cells exert potent specific cytotoxicity against primary multiple myeloma cells. Cytotherapy. 2012;14:1110–8.
50. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75:555–62.
51. Silva-Santos B, Mensurado S, Coffelt SB. γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer. Nat Rev Cancer. 2019;19:392–404.
52. Ribot JC, Ribeiro ST, Correia DV, Sousa AE, Silva-Santos B. Human γδ thymocytes are functionally immature and differentiate into cytotoxic type 1 effector T cells upon IL-2/IL-15 signaling. J Immunol. 2014;192:2237–43.
53. Park JH, Lee HK. Function of γδ T cells in tumor immunology and their application to cancer therapy. Exp Mol Med. 2021;53:318–27.
54. Seidel UJ, Vogt F, Grosse-Hovest L, Jung G, Handgretinger R, Lang P. γδ T cell-mediated antibody-dependent cellular cytotoxicity with CD19 antibodies assessed by an impedance-based label-free real-time cytotoxicity assay. Front Immunol. 2014;5:618.
55. Chen Z, Freedman MS. $CD16^+$ gammadelta T cells mediate antibody dependent cellular cytotoxicity: potential mechanism in the pathogenesis of multiple sclerosis. Clin Immunol. 2008;128:219–27.
56. Tokuyama H, Hagi T, Mattarollo SR, Morley J, Wang Q, So HF, et al. V gamma 9 V delta 2 T cell cytotoxicity against tumor cells is enhanced by monoclonal antibody drugs—rituximab and trastuzumab. Int J Cancer. 2008;122:2526–34.
57. Brandes M, Willimann K, Bioley G, Lévy N, Eberl M, Luo M, et al. Cross-presenting human gammadelta T cells induce robust $CD8^+$ alphabeta T cell responses. Proc Natl Acad Sci U S A. 2009;106:2307–12.
58. Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human gammadelta T cells. Science. 2005;309:264–8.
59. Ismaili J, Olislagers V, Poupot R, Fournié JJ, Goldman M. Human gamma delta T cells induce dendritic cell maturation. Clin Immunol. 2002;103:296–302.
60. Burnham RE, Zoine JT, Story JY, Garimalla SN, Gibson G, Rae A, et al. Characterization of donor variability for gammadelta T cell *ex vivo* expansion and development of an allogeneic gammadelta T cell immunotherapy. Front Med (Lausanne). 2020;7:588453.
61. Story JY, Zoine JT, Burnham RE, Hamilton JAG, Spencer HT, Doering CB, et al. Bortezomib enhances cytotoxicity of *ex vivo*-expanded gamma delta T cells against acute myeloid leukemia and T-cell acute lymphoblastic leukemia. Cytotherapy. 2021;23:12–24.

62. Airoldi I, Bertaina A, Prigione I, Zorzoli A, Pagliara D, Cocco C, et al. γδ T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-αβ$^+$/CD19$^+$ lymphocytes. Blood. 2015;125:2349–58.
63. Bertaina A, Roncarolo MG. Graft engineering and adoptive immunotherapy: new approaches to promote immune tolerance after hematopoietic stem cell transplantation. Front Immunol. 2019;10:1342.
64. Lamb LS Jr, Henslee-Downey PJ, Parrish RS, Godder K, Thompson J, Lee C, et al. Increased frequency of TCR gamma delta + T cells in disease-free survivors following T cell-depleted, partially mismatched, related donor bone marrow transplantation for leukemia. J Hematother. 1996;5:503–9.
65. Lamb LS Jr, Gee AP, Hazlett LJ, Musk P, Parrish RS, O'Hanlon TP, et al. Influence of T cell depletion method on circulating gammadelta T cell reconstitution and potential role in the graft-versus-leukemia effect. Cytotherapy. 1999;1:7–19.
66. Godder KT, Henslee-Downey PJ, Mehta J, Park BS, Chiang KY, Abhyankar S, et al. Long term disease-free survival in acute leukemia patients recovering with increased γδ T cells after partially mismatched related donor bone marrow transplantation. Bone Marrow Transplant. 2007;39:751–7.
67. Perko R, Kang G, Sunkara A, Leung W, Thomas PG, Dallas MH. Gamma delta T cell reconstitution is associated with fewer infections and improved event-free survival after hematopoietic stem cell transplantation for pediatric leukemia. Biol Blood Marrow Transplant. 2015;21:130–6.
68. Zheng J, Liu Y, Lau YL, Tu W. γδ-T cells: an unpolished sword in human anti-infection immunity. Cell Mol Immunol. 2013;10:50–7.
69. Lim WA, June CH. The principles of engineering immune cells to treat cancer. Cell. 2017;168:724–40.
70. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol. 2020;17:147–67.
71. van de Donk NWCJ, Usmani SZ, Yong K. CAR T-cell therapy for multiple myeloma: state of the art and prospects. Lancet Haematol. 2021;8:e446–61.
72. Pehlivan KC, Duncan BB, Lee DW. CAR-T cell therapy for acute lymphoblastic leukemia: transforming the treatment of relapsed and refractory disease. Curr Hematol Malig Rep. 2018;13:396–406.
73. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. Front Immunol. 2019;10:128.
74. Fleischer LC, Spencer HT, Raikar SS. Targeting T cell malignancies using CAR-based immunotherapy: challenges and potential solutions. J Hematol Oncol. 2019;12:141.
75. Fan M, Li M, Gao L, Geng S, Wang J, Wang Y, et al. Chimeric antigen receptors for adoptive T cell therapy in acute myeloid leukemia. J Hematol Oncol. 2017;10:151.
76. Torikai H, Reik A, Soldner F, Warren EH, Yuen C, Zhou Y, et al. Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. Blood. 2013;122:1341–9.
77. Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood. 2012;119:5697–705.
78. Provasi E, Genovese P, Lombardo A, Magnani Z, Liu PQ, Reik A, et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med. 2012;18:807–15.
79. Sommer C, Boldajipour B, Kuo TC, Bentley T, Sutton J, Chen A, et al. Preclinical evaluation of allogeneic CAR T cells targeting BCMA for the treatment of multiple myeloma. Mol Ther. 2019;27:1126–38.
80. Rasaiyaah J, Georgiadis C, Preece R, Mock U, Qasim W. TCRαβ/CD3 disruption enables CD3-specific antileukemic T cell immunotherapy. JCI Insight. 2018;3 :e99442.
81. Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. Cancer Res. 2015;75:3853–64.

82. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543:113–7.

83. Georgiadis C, Preece R, Nickolay L, Etuk A, Petrova A, Ladon D, et al. Long terminal repeat CRISPR-CAR-coupled "universal" T cells mediate potent anti-leukemic effects. Mol Ther. 2018;26:1215–27.

84. Benyamine A, Le Roy A, Mamessier E, Gertner-Dardenne J, Castanier C, Orlanducci F, et al. BTN3A molecules considerably improve Vγ9Vδ2T cells-based immunotherapy in acute myeloid leukemia. Oncoimmunology. 2016;5:e1146843.

85. Kunzmann V, Wilhelm M. Anti-lymphoma effect of gammadelta T cells. Leuk Lymphoma. 2005;46:671–80.

86. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood. 2000;96:384–92.

87. Simões C, Silva I, Carvalho A, Silva S, Santos S, Marques G, et al. Quantification and phenotypic characterization of peripheral blood Vdelta1 + T cells in chronic lymphocytic leukemia and monoclonal B cell lymphocytosis. Cytometry B Clin Cytom. 2019;96:164–8.

88. Siegers GM, Dhamko H, Wang XH, Mathieson AM, Kosaka Y, Felizardo TC, et al. Human Vdelta1 gammadelta T cells expanded from peripheral blood exhibit specific cytotoxicity against B-cell chronic lymphocytic leukemia-derived cells. Cytotherapy. 2011;13:753–64.

89. Almeida AR, Correia DV, Fernandes-Platzgummer A, da Silva CL, da Silva MG, Anjos DR, et al. Delta one T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept. Clin Cancer Res. 2016;22:5795–804.

90. Di Lorenzo B, Sim ões AE, Caiado F, Tieppo P, Correia DV, Carvalho T, et al. Broad cytotoxic targeting of acute myeloid leukemia by polyclonal delta one T cells. Cancer Immunol Res. 2019;7:552–8.

91. Valés-Gómez M, Chisholm SE, Cassady-Cain RL, Roda-Navarro P, Reyburn HT. Selective induction of expression of a ligand for the NKG2D receptor by proteasome inhibitors. Cancer Res. 2008;68:1546–54.

92. Niu C, Jin H, Li M, Zhu S, Zhou L, Jin F, et al. Low-dose bortezomib increases the expression of NKG2D and DNAM-1 ligands and enhances induced NK and gammadelta T cell-mediated lysis in multiple myeloma. Oncotarget. 2017;8:5954–64.

93. Bhat J, Kouakanou L, Peters C, Yin Z, Kabelitz D. Immunotherapy with human gamma delta T cells-synergistic potential of epigenetic drugs? Front Immunol. 2018;9:512.

94. Satwani P, Bavishi S, Saha A, Zhao F, Ayello J, van de Ven C, et al. Upregulation of NKG2D ligands in acute lymphoblastic leukemia and non-Hodgkin lymphoma cells by romidepsin and enhanced *in vitro* and *in vivo* natural killer cell cytotoxicity. Cytotherapy. 2014;16:1431–40.

95. Wu X, Tao Y, Hou J, Meng X, Shi J. Valproic acid upregulates NKG2D ligand expression through an ERK-dependent mechanism and potentially enhances NK cell-mediated lysis of myeloma. Neoplasia. 2012;14:1178–89.

96. de Weerdt I, Hofland T, Lameris R, Endstra S, Jongejan A, Moerland PD, et al. Improving CLL Vgamma9Vdelta2-T-cell fitness for cellular therapy by *ex vivo* activation and ibrutinib. Blood. 2018;132:2260–72.

97. Braza MS, Klein B, Fiol G, Rossi JF. γδ T-cell killing of primary follicular lymphoma cells is dramatically potentiated by GA101, a type II glycoengineered anti-CD20 monoclonal antibody. Haematologica. 2011;96:400–7.

98. Gertner-Dardenne J, Bonnafous C, Bezombes C, Capietto AH, Scaglione V, Ingoure S, et al. Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies. Blood. 2009;113:4875–84.

99. Choi BD, Yu X, Castano AP, Bouffard AA, Schmidts A, Larson RC, et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat Biotechnol. 2019;37:1049–58.

100. Zhou S, Liu M, Ren F, Meng X, Yu J. The landscape of bispecific T cell engager in cancer treatment. Biomark Res. 2021;9:38.
101. Goebeler ME, Bargou RC. T cell-engaging therapies—BiTEs and beyond. Nat Rev Clin Oncol. 2020;17:418–34.
102. Chen YH, Wang Y, Liao CH, Hsu SC. The potential of adoptive transfer of gamma9delta2 T cells to enhance blinatumomab's antitumor activity against B-cell malignancy. Sci Rep. 2021;11:12398.
103. Schiller CB, Braciak TA, Fenn NC, Seidel UJ, Roskopf CC, Wildenhain S, et al. CD19-specific triplebody SPM-1 engages NK and gammadelta T cells for rapid and efficient lysis of malignant B-lymphoid cells. Oncotarget. 2016;7:83392–408.
104. Ganesan R, Chennupati V, Ramachandran B, Hansen MR, Singh S, Grewal IS. Selective recruitment of γδ T cells by a bispecific antibody for the treatment of acute myeloid leukemia. Leukemia. 2021;35:2274–84.
105. Mardiana S, Gill S. CAR T cells for acute myeloid leukemia: state of the art and future directions. Front Oncol. 2020;10:697.
106. Deniger DC, Switzer K, Mi T, Maiti S, Hurton L, Singh H, et al. Bispecific T-cells expressing polyclonal repertoire of endogenous gammadelta T-cell receptors and introduced CD19-specific chimeric antigen receptor. Mol Ther. 2013;21:638–47.
107. Rozenbaum M, Meir A, Aharony Y, Itzhaki O, Schachter J, Bank I, et al. Gamma-delta CAR-T cells show CAR-directed and independent activity against leukemia. Front Immunol. 2020;11:1347.
108. Fleischer LC, Becker SA, Ryan RE, Fedanov A, Doering CB, Spencer HT. Non-signaling chimeric antigen receptors enhance antigen-directed killing by gammadelta T cells in contrast to alphabeta T cells. Mol Ther Oncolytics. 2020;18:149–60.
109. Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, et al. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. Blood. 2003;102:200–6.
110. Laurent G, de Micheaux SL, Solal-Celigny P, Soubeyran P, Delwail V, Ghesquières H, et al. Phase I/II study of IPH1101, γδ T cell agonist, combined with rituximab, in low grade follicular lymphoma patients. Blood. 2009;114:1649.
111. Kunzmann V, Smetak M, Kimmel B, Weigang-Koehler K, Goebeler M, Birkmann J, et al. Tumor-promoting versus tumor-antagonizing roles of gammadelta T cells in cancer immunotherapy: results from a prospective phase I/II trial. J Immunother. 2012;35:205–13.
112. Bertaina A, Zorzoli A, Petretto A, Barbarito G, Inglese E, Merli P, et al. Zoledronic acid boosts gammadelta T-cell activity in children receiving alphabeta$^+$ T and CD19$^+$ cell-depleted grafts from an HLA-haplo-identical donor. Oncoimmunology. 2017;6:e1216291.
113. Merli P, Algeri M, Galaverna F, Milano GM, Bertaina V, Biagini S, et al. Immune modulation properties of zoledronic acid on TcRgammadelta T-lymphocytes after TcRalphabeta/CD19-depleted haploidentical stem cell transplantation: an analysis on 46 pediatric patients affected by acute leukemia. Front Immunol. 2020;11:699.
114. Xu Y, Xiang Z, Alnaggar M, Kouakanou L, Li J, He J, et al. Allogeneic Vgamma9Vdelta2 T-cell immunotherapy exhibits promising clinical safety and prolongs the survival of patients with late-stage lung or liver cancer. Cell Mol Immunol. 2021;18:427–39.
115. Alnaggar M, Xu Y, Li J, He J, Chen J, Li M, et al. Allogenic Vγ9Vδ2 T cell as new potential immunotherapy drug for solid tumor: a case study for cholangiocarcinoma. J Immunother Cancer. 2019;7:36.
116. Abe Y, Muto M, Nieda M, Nakagawa Y, Nicol A, Kaneko T, et al. Clinical and immunological evaluation of zoledronate-activated Vγ9γδ T-cell-based immunotherapy for patients with multiple myeloma. Exp Hematol. 2009;37:956–68.
117. Wilhelm M, Smetak M, Schaefer-Eckart K, Kimmel B, Birkmann J, Einsele H, et al. Successful adoptive transfer and *in vivo* expansion of haploidentical gammadelta T cells. J Transl Med. 2014;12:45.