

REVIEW

Open Access



Gamma delta T cells and their immunotherapeutic potential in cancer

Stephen G. Cieslak^{1,2} and Reza Shahbazi^{1,2,3,4*}

Abstract

Gamma–delta ($\gamma\delta$) T cells are a unique subset of T lymphocytes that play diverse roles in immune responses, bridging innate and adaptive immunity. With growing interest in their potential for cancer immunotherapy, a comprehensive and inclusive exploration of $\gamma\delta$ T cell families, their development, activation mechanisms, functions, therapeutic implications, and current treatments is essential. This review aims to provide an inclusive and thorough discussion of these topics. Through our discussion, we seek to uncover insights that may harbor innovative immunotherapeutic strategies. Beginning with an overview of $\gamma\delta$ T cell families including $V\delta 1$, $V\delta 2$, and $V\delta 3$, this review highlights their distinct functional properties and contributions to anti-tumor immunity. Despite $\gamma\delta$ T cells exhibiting both anti-tumor and pro-tumor activities, our review elucidates strategies to harness the anti-tumor potential of $\gamma\delta$ T cells for therapeutic benefit. Moreover, our paper discusses the structural intricacies of the $\gamma\delta$ T cell receptor and its significance in tumor recognition. Additionally, this review examines conventional and emerging $\gamma\delta$ T cell therapies, encompassing both non-engineered and engineered approaches, with a focus on their efficacy and safety profiles in clinical trials. From multifunctional capabilities to diverse tissue distribution, $\gamma\delta$ T cells play a pivotal role in immune regulation and surveillance. By analyzing current research findings, this paper offers insights into the dynamic landscape of $\gamma\delta$ T cell–based immunotherapies, underscoring their promise as a potent armamentarium against cancer. Furthermore, by dissecting the complex biology of $\gamma\delta$ T cells, we learn valuable information about the anti-cancer contributions of $\gamma\delta$ T cells, as well as potential targets for immunotherapeutic interventions.

Keywords $\gamma\delta$ T cells, $\gamma\delta$ T cell receptors, Immunotherapy, Cancer, Immunology, Oncology, CAR

*Correspondence:

Reza Shahbazi
rshahbaz@iu.edu

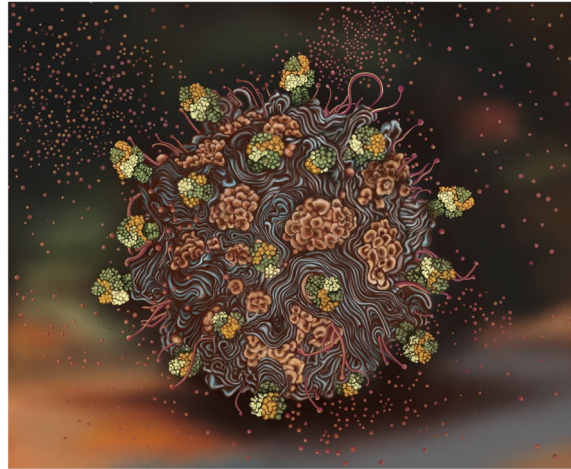
Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Graphical abstract

Gamma–delta T cell in action.

**Introduction**

Gamma–delta ($\gamma\delta$) T cells, a unique subset of T lymphocytes, have emerged as pivotal players in the orchestration of immune responses, wielding a diverse array of functions that extend beyond conventional alpha–beta ($\alpha\beta$) T cells. With an unconventional T cell receptor (TCR) repertoire and distinct tissue distribution, $\gamma\delta$ T cells serve as versatile guardians of immunity, bridging innate and adaptive immune responses. While other reviews discuss various aspects of $\gamma\delta$ T cells and their roles in cancer immunotherapy, our review article provides a comprehensive, inclusive overview of $\gamma\delta$ T cell families and subtypes, development, activation, functions, therapeutic implications in cancer immunology, and structure, as well as current therapies developed in academic, clinical, and corporate settings.

For instance, V δ 1 T cells are important for anti-tumor immunity and play a role in responding to solid tumors through cytotoxic activity and recognition of both peptide and non-peptide antigens. V δ 2 T cells, mainly located in the blood, activate anti-tumor immunity in cancers typically by responding to phosphoantigens, while V δ 3 T cells respond to oxidative stress markers in cancer, as well as both traditional and non-traditional antigens. Through elucidating the mechanisms governing $\gamma\delta$ T cell biology and the intricacies of each $\gamma\delta$ T cell family, we seek to unveil novel insights that may pave the way for innovative immunotherapeutic strategies and interventions.

While $\gamma\delta$ T cells have shown great promise in pre-clinical and clinical studies, their role in cancer remains

complex due to their ability to exert both beneficial and detrimental effects on tumor progression. This dual functionality has led to challenges in optimizing $\gamma\delta$ T cell-based therapies. The ability of $\gamma\delta$ T cells to recognize and respond to tumor-associated antigens opens new avenues for cancer treatment, particularly when engineered or activated to enhance their anti-tumor potential. Overall, this review delves into conventional and novel approaches to $\gamma\delta$ T cell therapy, including engineered therapies, focusing on their therapeutic efficacy and safety in clinical trials. By synthesizing current research, this review highlights the growing potential of $\gamma\delta$ T cells as a powerful tool in the fight against cancer.

 $\gamma\delta$ T cell biology

$\gamma\delta$ T cells and $\alpha\beta$ T cells develop from common thymic progenitors but differ in various functional aspects, such as faster activation and reduced propensity of $\gamma\delta$ T cells to induce graft-versus-host disease (GvHD). Unlike $\alpha\beta$ T cells, $\gamma\delta$ T cells do not rely on traditional major histocompatibility complex (MHC)-mediated antigen presentation, and some subsets show adaptive responses similar to $\alpha\beta$ T cells. For example, V δ 1 T cells, largely found in peripheral tissues, play critical roles in tissue balance and immunity, exhibiting cytotoxic activities through natural killer receptors (NKR), particularly in cancer contexts like multiple myeloma and colorectal cancer. V δ 2 T cells, primarily present in the blood, are involved in anti-tumor immunity and can be activated by phosphoantigens or aminobisphosphonates, promoting cytotoxicity in cancers such as multiple myeloma and renal cell carcinoma.

Vδ3 T cells are functionally and locationally similar to Vδ1 T cells; however, Vδ3 T cells can secrete helper T cell cytokines and play a role in oxidative stress recognition in cancer settings. Moreover, γδ T cells demonstrate both anti-tumor and pro-tumor properties, with roles in immune surveillance and cancer progression, potentially offering novel therapeutic avenues through their innate receptor activation, bypassing traditional antigen-specific mechanisms.

Although γδ T cells and αβ T cells develop from shared thymic progenitors, γδ T cells exhibit fundamental differences from αβ T cells (Fig. 1), such as faster functional activation at peripheral sites and a markedly reduced tendency to induce GvHD [1]. Moreover, γδ T cells do not rely on classic MHC-mediated antigen presentation and can even normally develop without MHC-II (Fig. 1) [1–3]. Additionally, γδ T cells are fundamentally similar to natural killer (NK) cells in that γδ T cells recognize neoplastic or infected cells through multiple receptor–ligand interactions, quickly responding in an innate-like manner without requiring prior exposure [1,

4, 5]. However, a subset of γδ T cells have been observed to clonally expand during primary infections and effectuate adaptive immune responses upon subsequential challenge, resembling the behavior of αβ T cells [1, 6]. Furthermore, in the intestines, γδ T cells exhibit protective and multifunctional memory [6]. Consequently, these characteristics position γδ T cells at the interface between αβ T lymphocytes and NK cells (Fig. 1) [1].

Vδ1 T cell biology

Vδ1 T cells are produced in the human thymus after Vδ2 T cells (Fig. 2), typically appearing around 4–6 months after birth [1, 7]. While Vδ1 T cells make up only about one-third of circulating γδ T cells in healthy adults (Fig. 2), Vδ1 T cells are predominantly found in various peripheral tissues such as the liver, gut epithelium, spleen, and dermis, playing crucial roles in maintaining tissue balance [1, 8]. In intraepithelial gut tissue, Vγ4Vδ1 cells bind BTN3 and endothelial protein C receptor (EPCR), a stress-induced MHC-I–like molecule, via complementary-determining region 3 of the Vγ4 chain, where

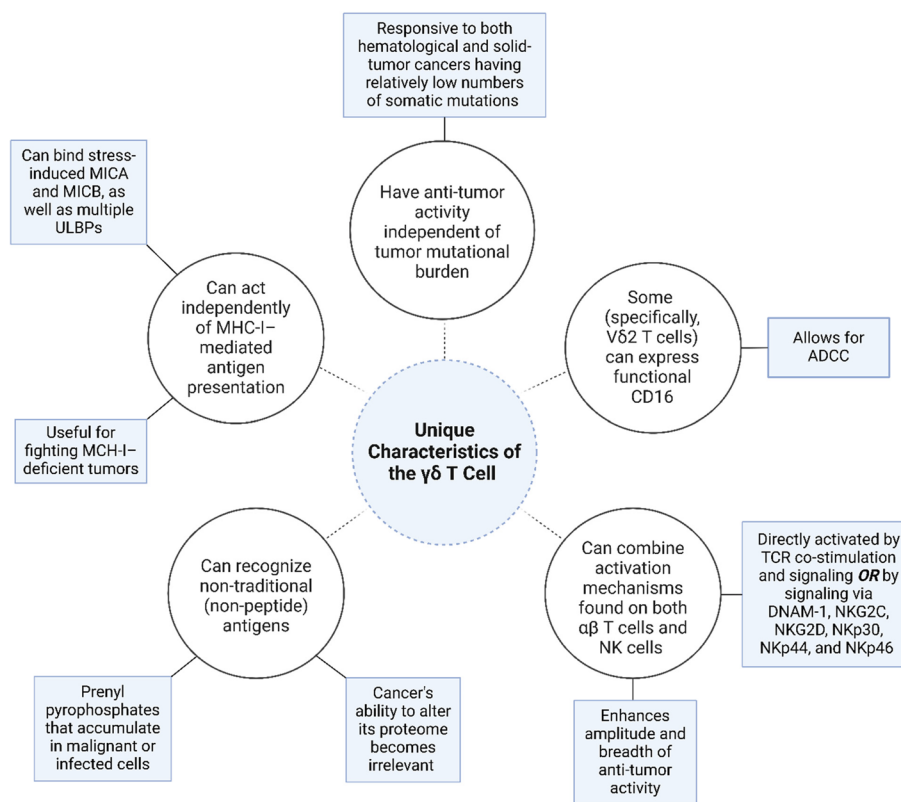


Fig. 1 The unique characteristics of γδ T cells. γδ T cells are unique in that γδ T cells can act independently of MHC-I-mediated antigen presentation, have anti-tumor activity independent of tumor mutational burden, combine activation mechanisms found on conventional T cells and NK cells, recognize non-traditional antigens, and express functional CD16, allowing for ADCC. ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; DNAM-1, DNAX accessory molecule 1; NKG2C, natural killer group 2C; NKG2D, natural killer group 2D; MHC-I, major histocompatibility complex 1; MICA, MHC class I chain-related protein A; MICB, MHC class I chain-related protein B; ULBP, UL-16 binding protein; NK, natural killer; TCR, T cell receptor; Nkp, natural killer protein. Created with BioRender.com

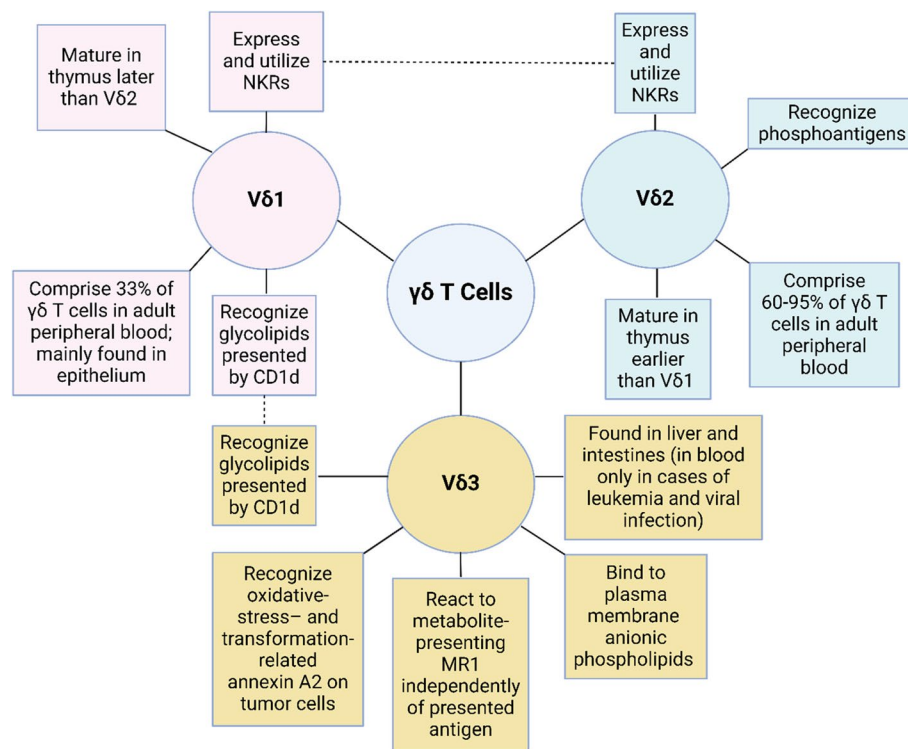


Fig. 2 The differences between $\gamma\delta$ T cell families. $\gamma\delta$ T cells have three functional families: V δ 1, V δ 2, and V δ 3. V δ 1 T cells are mostly tissue-resident, making up only 33% of $\gamma\delta$ T cells in the peripheral blood. Moreover, V δ 1 T cells mature in the thymus later than V δ 2 T cells. V δ 2 T cells are mostly circulatory, comprising 65–90% of $\gamma\delta$ T cells in the peripheral blood. These $\gamma\delta$ T cells are known for their ability to recognize phosphoantigens. Both V δ 2 and V δ 1 T cells can express and utilize NKRs. V δ 3 T cells are not well known but are mainly found in the liver and intestines responding to cellular metabolites and oxidative stress markers. However, in cases of leukemia and viral infection, V δ 3 T cells can be found in the peripheral blood. Notably, both V δ 3 and V δ 1 T cells can recognize glycolipids presented by CD1d. Dotted lines connect common characteristics between two $\gamma\delta$ T cell families. CD, cluster of differentiation; NKR, natural killer receptor; MHC-I, major histocompatibility complex 1; MR1, MHC class I-related protein 1. Created with BioRender.com

V γ 4 binds to BTNL3 with 3.6–sixfold higher affinity than to EPCR [9–12]. While V γ 4–EPCR binding can be inhibited by the markedly higher binding affinity of V γ 4 to BTNL3, BTNL3 is significantly downregulated in colorectal cancer (CRC) whereas EPCR is commonly overexpressed in CRC and multiple other cancers [10–17]. On the other hand, some V δ 1 T cell subtypes, such as V γ 2V δ 1 and V γ 3V δ 1, do not bind to BTNL3 [11]. Other V δ 1 T cell subtypes, such as V γ 5V δ 1 and V γ 4V δ 1, can bind to sulfatide, a glycolipid presented by CD1d (Fig. 3) [18]. Moreover, a non-prevalent and lesser-known V δ 1 T cell subtype, V γ 9V δ 1, can bind EPHA2 once EPHA2 is coordinately recognized by ephrin-A [19]. Interestingly, EPHA2 expression is upregulated during AMP-activated protein kinase (AMPK)-dependent metabolic cancer cell reprogramming and co-expression of AMPK and EPHA2 in CRC tumors is correlated with higher CD3⁺ T cell infiltration [19]. V γ 9V δ 1 TCRs can also bind distal to the metabolite binding cleft of the metabolite-presenting MHC class I-related protein 1 (MR1) [20].

Akin to V δ 2 T cells, V δ 1 T cells express innate NKRs (Fig. 2) [1]. For instance, when natural killer group 2D (NKG2D) is engaged by ligands like MHC class I chain-related protein A (MICA) and UL-16 binding proteins (ULBPs), V δ 1 T cells release perforin and granzyme B, with DNAX accessory molecule 1 (DNAM-1/CD225) also contributing to V δ 1 cytotoxic function [1, 21–24]. This phenomenon is especially observed in the strong response of V δ 1 T cells against primary multiple myeloma cells [23]. Another example is when V δ 1 T cells from B-cell chronic lymphocytic leukemia patients bind ULBP3 expressed on leukemia cells and are subsequently upregulated by trans-retinoic acid [24]. In specific contexts, V δ 1 T cells express NKRs from the natural cytotoxicity receptor family, such as NKp46 in the intestinal epithelium, enhancing V δ 1 cytotoxicity against CRC cells [1, 25]. Moreover, stimulation of V δ 1 T cells with IL-2 or IL-15 in vitro induces NKp30 and NKp44, augmenting V δ 1 anti-tumor activity against various leukemias [1, 21, 26]. Furthermore, in the clinical context of CRC samples lacking MHC-I and mismatch repair mechanisms,

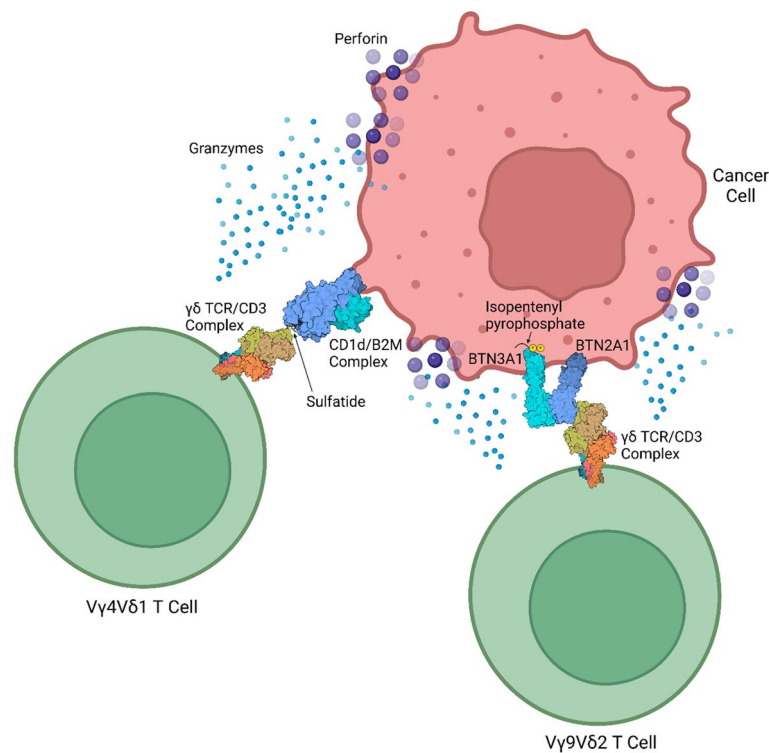


Fig. 3 Common $\gamma\delta$ T cells interacting with cancer cell. Phosphoantigens, such as isopentenyl pyrophosphate, do not directly interact with $V\gamma 9V\delta 2$ TCRs but instead bind to the BTN3A1 intracellular domain, thus allowing BTN3A1 to interact with BTN2A1, which can then bind to the $V\gamma 9$ chain. On the other hand, $V\gamma 4V\delta 1$ TCRs can bind to sulfatide – a glycolipid presented by CD1d. BTN3A1, butyrophilin subfamily 3 member A1; BTN2A1, butyrophilin subfamily 2 member A1; CD, cluster of differentiation; TCR, T cell receptor; B2M, β_2 microglobulin. Created with BioRender.com

abundant $V\delta 1$ and $V\delta 3$ T cells expressing PD-1 and NKRrs (particularly NKG2D), which play crucial roles in the efficacy of immune-checkpoint inhibitors, have been observed [1, 27].

$V\delta 2$ T cell biology

In humans and in certain non-rodent species, the first $\gamma\delta$ T cells formed are $V\delta 2$ T cells (Fig. 2) [1, 7, 28]. These cells originate from the early rearrangement of the $V\delta 2$ gene during fetal development, typically between weeks 5–6 in the fetal liver and weeks 8–15 in the fetal thymus [1, 7, 28]. However, over time and due to sequential infections during childhood, $V\delta 2$ T cells expand and eventually constitute a significant portion (60–95%) of $\gamma\delta$ T cells in adult peripheral blood (Fig. 2) [1]. Interestingly, in the tumor microenvironment (TME), mevalonate pathway dysregulation can result in the accumulation of the lower-affinity phosphoantigen isopentenyl pyrophosphate (IPP), thus facilitating activation and chemotaxis of $V\delta 2$ T cells [1, 29–32].

When IL-2 is present, stimulation by phosphoantigens results in the selective and swift expansion of $V\delta 2$ T cells *ex vivo*, which exhibit anti-tumor activity *in vitro*, as is observed in cases of multiple myeloma and renal cell

carcinoma [1, 33–36]. Moreover, IPP-activated $CD56^+$ $\gamma\delta$ T cells exhibit strong anti-tumor activity against squamous cell carcinoma (SCC) [37]. Furthermore, aminobisphosphonates like pamidronate and zoledronate have been clinically shown to promote $V\delta 2$ activation and expansion *in vivo*, as well as $V\delta 2$ -mediated killing of multiple myeloma and chronic myelogenous leukemia cells [1, 38–41]. These compounds inhibit farnesyl pyrophosphatase synthase, interfering with the mevalonate pathway and leading to IPP accumulation, which facilitates $V\delta 2$ T cell cytotoxicity and chemotaxis [1, 30, 31]. Recent studies propose using agonistic monoclonal antibodies targeting butyrophilin subfamily 3 member A1 (BTN3A1) to enhance $V\delta 2$ T cell activation and cytotoxicity against tumor cells [1, 42–45]. However, whether diverse antigens can bind to the $V\delta 2$ chain of the $V\gamma 9V\delta 2$ TCR remains elusive [1]. Nevertheless, it has been suggested that various ligands binding to different regions of the same $\gamma\delta$ TCR could enable $\gamma\delta$ T cells to mediate both adaptive and innate responses [1, 9].

Apart from TCR-mediated recognition, $V\delta 2$ T cells express surface NKRrs that facilitate cytotoxic function upon recognizing ligands on target cells (Fig. 2) [1]. For example, the C-type lectin-like receptor NKG2D

interacts with stress-induced MICA and MICB, in addition to multiple ULBPs, activating V δ 2 T cells [1, 46, 47]. Moreover, DNAM-1/CD225 interacts with nectin-2 and nectin-like 5/CD155, promoting hepatocellular carcinoma cell lysis [1, 48]. Additionally, these cells can express functional CD16, enabling antibody-dependent cellular cytotoxicity (ADCC) against tumor cells targeted with antibody-based therapies (Fig. 1), such as trastuzumab, which binds HER2 on breast cancers, and rituximab, which binds CD20 on B-cell lymphomas [1, 49–52]. Lastly, and contrary to other $\gamma\delta$ T cells, V δ 2 T cells possess the ability to cross-present antigens similarly to dendritic cells, thereby inducing CD8⁺ $\alpha\beta$ T cell responses [1, 53].

V δ 3 T cell biology

V δ 3 T cells are rarely found in the peripheral blood of healthy individuals but are notable in the liver and intestines; nevertheless, V δ 3 T cells can be found in circulation during leukemia and viral infections (Fig. 2) [1, 54–57]. These $\gamma\delta$ T cells share functional characteristics with V δ 1 T cells, such as the ability to bind glycolipids presented by CD1d (Fig. 2) [1, 58]. Notably, V δ 3 T cells can secrete T_H1, T_H2, and T_H17 cytokines [58]. However, one V δ 3 TCR clone has exhibited reactivity to annexin A2 on tumor cells (Fig. 2), an intracellular protein that becomes upregulated during cellular transformation or oxidative stress, binds to anionic phospholipids in the cell membrane, and can be brought to the cell surface [1, 59]. Furthermore, certain V δ 3 TCR clones independently react to MR1 by binding atypical positions on or beneath the antigen-binding cleft of MR1 (Fig. 2) [1, 60, 61]. Some researchers have suggested an inherent MR1 autoreactivity of certain V δ 3 TCRs based on this unique mode of ligand recognition [1, 61]. Additionally, V δ 1⁻/V δ 2⁻ T cells have been identified in duodenal biopsy specimens and human blood that react with MR1 tetramers in a metabolite-independent manner (Fig. 2), indicating potential therapeutic implications [1, 61].

Anti-tumor properties

Research on the roles of pleiotropic $\gamma\delta$ T cells in human and mouse tumor immunology is extensive [1, 62, 63]. Similar to other T cell subsets, studies have emphasized the diverse functions of $\gamma\delta$ T cells in the intestinal TME, showcasing a dynamic interplay between pro-tumor and anti-tumor $\gamma\delta$ T cells [1, 63–67]. However, this functional duality has primarily been elucidated through mouse studies, where $\gamma\delta$ T cells have a higher propensity than human $\gamma\delta$ T cells to generate IL-17A, a cytokine associated with breast tumor metastasis and growth [1, 63, 68–70]. Contrarily, human $\gamma\delta$ thymocytes inherently lean toward differentiating into $\gamma\delta$ T cells upon stimulation

with IL-2 or IL-15, and are consequently more inclined to exhibit anti-tumor effects within the TME [1, 71]. This process occurs through IL-2-mediated hyperphosphorylation of ERK1/2, AKT, and STAT5, where ERK1/2 is responsible for the differentiation of $\gamma\delta$ thymocytes into IFN- γ ⁺ or TNF- α ⁺ $\gamma\delta$ T cells [71]. It is also important to note that $\alpha\beta$ T cells can facilitate $\gamma\delta$ T cell function through the release of IL-2 and activation of the transcription factor T-bet [72].

Studies using syngeneic mouse models have clearly demonstrated the non-redundant role of $\gamma\delta$ T cells in controlling prostate and spontaneous B-cell lymphoma tumor growth [1, 73–76]. Notably, the anti-tumor effector functions detected in mice are also manifest in human $\gamma\delta$ T cells upon in vitro activation [1, 36, 37, 39–41]. These functions include the secretion of T_H1 cytokines such as TNF- α and IFN- γ , the release of cytolytic granules containing granzymes A and B and perforin, and the expression of death receptor ligands like TRAIL and FASL, which can induce tumor cell apoptosis [1, 36, 37, 39–41]. Importantly, research has shown that IFN- γ secretion is inhibited by LAG-3, TIM-3, and PD-1, while TNF- α secretion is inhibited by TIM-3 [72, 77–81]. Moreover, B7-H3 on cancer cells can suppress IFN- γ expression in V δ 2 T cells by blocking T-bet [72, 82]. It is therefore plausible that at least some anti-tumor signaling pathways of $\gamma\delta$ T cells work by inhibiting LAG-3, PD-1, and/or TIM-3; however, as discussed previously, it is known that $\alpha\beta$ T cells can facilitate $\gamma\delta$ T cell activity through the activation of T-bet [72]. Nevertheless, the TCR primarily determines $\gamma\delta$ T cell activation and subsequent T_H1 cytokine secretion, although NKR2 are critical for the powerful cytotoxicity of $\gamma\delta$ T cells (Fig. 1) [1, 83]. Akin to NK cells, NKG2D activation prompts $\gamma\delta$ T cells to release cytolytic granules, leading to in vitro cytotoxic activity against various tumor cell types, including those derived from CRC, SCC, and human renal cell carcinoma [1, 22, 36, 37, 84]. NKG2D also regulates soluble TRAIL production in IL-2-activated human $\gamma\delta$ T cells, which triggers apoptosis in lung cancer cell lines [1, 85]. Whether this mechanism extends to $\gamma\delta$ T cells stimulated through other methods is uncertain [1]. Interestingly, whereas V δ 2 T cells can execute ADCC via CD16 expression (Fig. 1), V δ 1 T cells do not rely on CD16-dependent ADCC; instead, these cells mainly utilize granzyme B and perforin secretion to induce cytotoxicity, as has been demonstrated against neuroblastomas [1, 50, 51, 86].

In addition to directly targeting cancer cells, $\gamma\delta$ T cells play a role in coordinating anti-tumor responses at various levels [1]. IFN- γ secretion by $\gamma\delta$ T cells augments overall cytotoxicity by amplifying IFN- γ release by $\alpha\beta$ T cells and increasing expression of MHC-I on cancer cells, which facilitate recognition of cancer cells by CD8⁺

T cells [1, 73, 87]. Furthermore, $\gamma\delta$ T cells expressing CD137L/4-1BBL can co-stimulate NK cells, increasing ADCC and direct cytotoxicity against tumor cells [1, 88]. Additionally, V δ 2 T cells have the ability to cross-present MHC-I- and MHC-II-restricted peptides, functioning as antigen-presenting cells that efficiently trigger $\alpha\beta$ T cell proliferation and activation, as well as induce peptide-specific CD8⁺ T cell responses [1, 89–91]. Previous opsonization of cancer cells with tumor-specific antibodies (also known as “licensing”) is necessary for this mechanism to occur [1, 92]. Notably, $\gamma\delta$ T cells can also enhance antibody production by B cells in both humans and mice by inducing differentiation of T_{FH} cells [1, 93–97]. In humans, a subset of CXCR5-expressing V δ 2 T cells promotes antibody class switching and production through IL-4 and IL-10 secretion, as well as through (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP)-mediated upregulation of IL-21R [1, 93, 94]. Taken together, the diverse functions of $\gamma\delta$ T cells highlight the significance of $\gamma\delta$ T cells as anti-tumor effectors [1].

Pro-tumor properties

In mouse models of cancer, studies have consistently elucidated the pro-tumor potential of IL-17-producing $\gamma\delta$ T cells [1, 63, 68, 70, 98]. For example, mouse CD27⁻ $\gamma\delta$ T cells that secrete IL-17A facilitate ovarian cancer growth by mobilizing pro-tumor small peritoneal macrophages, whereas IL-17-devoid $\gamma\delta$ T cells are beneficial in ovarian cancer [98, 99]. However, in human peripheral blood, IL-17⁺ $\gamma\delta$ T cells are nearly absent [1, 71]. Furthermore, a pan-cancer transcriptomic analysis that identified $\gamma\delta$ T cells as strongly associated with a favorable prognosis found no correlation between $\gamma\delta$ T cells and IL-17 expression in the TME [1, 100]. Nonetheless, other studies have reported observing tumor-infiltrating IL-17⁺ $\gamma\delta$ T cells in CRC, SCC, HPV-associated uterine cervical SCC, and gallbladder cancer patients, particularly in advanced stages of the disease [1, 101–106]. This observation aligns with research describing temporal segregation and dynamic interplay between pro-tumor and anti-tumor intestinal $\gamma\delta$ T cell subsets in humans and mice [1, 67]. These subsets, defined by enrichment for IL-17 expression and cytolytic markers, respectively, show that the pro-tumor subset amasses during CRC progression [1, 37, 66, 67].

IL-17-producing $\gamma\delta$ T cells are responsible for several pro-tumor actions (especially in hepatocellular carcinomas and mammary cancers) including directly triggering malignant cell migration by upregulating MTA1 and proliferation by activating the IL-6/STAT3 signaling pathway, recruiting tumorigenic neutrophils, mobilizing polymorphonuclear myeloid-derived suppressor

cells (PMN-MDSCs) to suppress anti-tumor cytotoxic activity of and IFN- γ production by V δ 2 T cells, increasing endothelial permeability, and facilitating vascular endothelial growth factor (VEGF)-dependent angiogenesis – all of which sustain an immunosuppressive TME [1, 104–113]. Interestingly, the expansion and activation of IL-17⁺ $\gamma\delta$ T cells may be facilitated by commensal microbiota, as demonstrated in a lung adenocarcinoma mouse model [1, 114, 115]. Furthermore, disruption of the epithelial barrier in human CRC due to tumor growth allows bacterial invasion [1, 106]. Subsequent phagocytosis and antigen presentation by dendritic cells promote IL-17⁺ $\gamma\delta$ T cell polarization, as well as production of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF), correlating with the TME-based accumulation of immunosuppressive PMN-MDSCs [1, 106]. Additionally, IL-17⁺ $\gamma\delta$ T cells in mice facilitate neutrophil recruitment, in which neutrophils suppress pro-tumoral IL-17⁺ $\gamma\delta$ T cells by inducing oxidative stress and establishing a multifaceted regulatory crosstalk between these immune populations [1, 68, 116–118]. This regulatory crosstalk, known as the “ $\gamma\delta$ T cell–IL17A–neutrophil axis” drives immunosuppression even to the point of conferring resistance to high-dose anti-VEGFR2 (vascular endothelial growth factor receptor 2) breast cancer therapy [118].

The possible pro-tumor functions of $\gamma\delta$ T cells may not solely rely on IL-17 production [1, 119, 120]. Additional potential mediators include PD-L1, galectin 1, and galectin 9 which suppress effector T cells when interacting with PD-1 and glycosylated receptors, respectively [1, 119, 120]. Specifically, PD-L1 and galectin 9 expression by tumor-infiltrating $\gamma\delta$ T cells have been shown to functionally exhaust and inhibit $\alpha\beta$ T cells in pancreatic cancer mouse models [1, 119]. Moreover, pancreatic cancer patients were observed to have increased levels of PD-L1 and galectin 9 in circulating $\gamma\delta$ T cells, with even higher levels in tumor-infiltrating $\gamma\delta$ T cells, compared to healthy individuals [1, 119]. Another possible pro-tumor pathway involves IL-4 and IL-10 secretion, which is connected with V δ 1 T cells exhibiting a regulatory phenotype and inhibiting V δ 2 T cell function [1, 121, 122]. Regulatory $\gamma\delta$ T cells can infiltrate human breast tumors and inhibit dendritic cell and $\alpha\beta$ T cell activity, as well as suppress adaptive and innate immunity, potentially by inducing senescence [1, 123, 124]. However, interaction with Toll-like receptor 8 ligand in mice and in vitro reversed this effect [1, 123, 124]. Furthermore, in the breast cancer TME, a CD73-expressing V δ 1 T cell subset was detected, in which cells produce adenosine, IL-8, and IL-10, implying an immunosuppressive phenotype [1, 125]. Nevertheless, a recent study showed that $\gamma\delta$ T cells, primarily V δ 1 T cells, that infiltrate triple-negative

breast cancers produce an abundance of IFN- γ and are positively associated with overall survival and progression-free survival [1, 126]. These discoveries suggest that despite the existence of minor immunosuppressive $\gamma\delta$ T cell subsets, these subsets are typically surpassed by anti-tumor $\gamma\delta$ T cells [1]. Additionally, $\gamma\delta$ T cells usually produce higher amounts of anti-tumor cytokines (such as TNF and IFN- γ) than pro-tumor cytokines (for instance: IL-4, IL-10, IL-17, IL-22, and IL-35) within the TME, reflecting their prognostic value in cancer patients [1, 102, 104].

Therapeutic implications

Existing cancer immunotherapies heavily rely on the presence of tumor-associated antigens and/or neoantigens, which aligns with the functioning of B cells and $\alpha\beta$ T cells [12, 127, 128]. Unfortunately, cancers inherently have significant epigenetic variability and genomic instability, leading to flaws in antigen presentation and/or the suppression of neoantigens [12, 129–135]. Consequently, immune evasion and resistance to current immunotherapies occur; however, neoantigens are not the sole pathway to immunological recognition in cancer [12, 129, 131–135]. While genomic instability may impede antigen-specific $\alpha\beta$ T cell immunosurveillance, this instability also prompts immunological stress ligand expression on cancer cells like the MULT1/H60/RAE-1 families in mice and ULBP/MIC families in humans [12, 136, 137]. These ligands, which are regulated by the DNA damage response pathway, interact with the innate activating receptor NKG2D, which is expressed by $\gamma\delta$ T cells, CD8⁺ $\alpha\beta$ T cells, and NK cells [12, 136]. Additionally, $\gamma\delta$ T cells express various other innate activating receptors like NKG2A, NKG2C, NKp30, NKp46, and DNAM-1 whose ligands are often present on stressed neoplastic cells [21, 25, 26, 48, 99, 138–141]. Interestingly, NKG2A inhibits NKG2C effector functions of V δ 2 T cells while conferring on V δ 2 T cells the highest anti-tumor effector functions [138, 140]. Moreover, recent research highlighted a unique subset of V δ 1 T cells in human intestinal epithelium expressing NKp30, NKp46, NKG2C, and NKG2D, with powerful NKp46-dependent cytolytic responses against CRC [12, 25]. Notably, the presence of V δ 1 T cells in CRC correlated significantly with lower-stage disease [12]. Furthermore, $\gamma\delta$ T cells can be directly activated by innate receptors without requiring simultaneous antigen-specific TCR signaling, unlike most $\alpha\beta$ T cells (Fig. 1) [12, 26, 126]. Nevertheless, $\gamma\delta$ T cells can still be activated through the $\gamma\delta$ TCR, although not via conventional MHC engagement but rather by detecting self-encoded molecules associated with cellular distress and tissue health [12]. Upon activation, these cells primarily generate anti-tumor cytokines like IFN- γ , release cytotoxic granules,

and eliminate cancer cells [1, 12, 37]. Consequently, $\gamma\delta$ T cells may offer cancer immunosurveillance without the use of antigen-specific adaptive $\alpha\beta$ T cells [12].

$\gamma\delta$ TCR structure

Human $\gamma\delta$ T cells have distinct innate and adaptive functions that are determined by their TCR sequences, which result from somatic recombination. Specifically, the TCR γ and δ loci undergo recombination processes, where V δ 1–3 and V γ 2–5, V γ 8, and V γ 9 genes are key to $\gamma\delta$ T cell diversity, resulting in certain chain pairings that are more common in specific tissues. These chain pairings are divided into three major families: V δ 1, V δ 2, and V δ 3. V δ 1 TCRs exhibit extensive diversity, contributing to unique adult repertoires, while V δ 2 T cells, with a semi-invariant repertoire, largely respond to phosphoantigens via interactions with BTN3A1. V δ 3 T cells, with a limited TCR repertoire, are also implicated in potential therapeutic applications due to their unique antigen binding capabilities. Notwithstanding the differences between $\gamma\delta$ TCR families, every $\gamma\delta$ TCR structure interacts with a CD3 subunit to form an octameric complex, initiating signaling upon antigen engagement.

The distinct functions of human $\gamma\delta$ T cells, whether innate or adaptive, are linked to specific cell subsets with different developmental origins, as evidenced by their diverse TCR sequences resulting from somatic recombination [1]. For instance, the TCR δ repertoire in human skin is distinct and restricted from that in the peripheral blood [142]. Comparable to the TCR β (*TRB*) locus, the TCR δ (*TRD*) locus undergoes recombination of joining (J), diversity (D), and variable (V) segments [1]. There are eight *TRDV* genes (V δ 1–8), with V δ 1–3 being specific to $\gamma\delta$ T cells and V δ 4–8 being shared with the TCR α locus [1]. Among the eight V δ variants, V δ 1, V δ 2, and V δ 3 are notably the most utilized gene segments, thus serving as key markers for classifying $\gamma\delta$ T cell families [1, 142–144]. These genes can rearrange with three *TRDD* (D1–3) and four *TRDJ* genes (J1–4) [1]. On the other hand, although the TCR γ (*TRG*) locus, akin to the TCR α (*TRA*) locus, undergoes VJ recombination, only six of the 14 *TRGV* genes are functional (V γ 2–5, V γ 8, and V γ 9), and can recombine with five *TRGJ* genes (J1, J2, JP, JP1, and JP2) [1]. Following recombination, the transcribed and spliced V(D)J gene segments are combined with constant exons (C in *TRD* and either C1 or C2 in *TRG*), resulting in unique $\gamma\delta$ TCR heterodimers expressed on the cell surface [1, 145]. While a vast number of distinct $\gamma\delta$ TCR sequences (10^{17} – 10^{18}) could theoretically be generated due to V(D)J recombination diversity, certain rearrangements and chain pairings are significantly more prevalent, influenced not only by biases in recombination

but also by the selection of functional T cell clonotypes, such as various oligoclonal populations prevailing in different tissues and circulation [1, 62]. Interestingly, deep sequencing of TCR β and TCR γ repertoires suggests that TCR β rearranges after $\alpha\beta$ and $\gamma\delta$ T cell commitment [146].

A thorough analysis of the human $\gamma\delta$ TCR reveals that the $\gamma\delta$ TCR structure is every bit as intriguing as the genetic events preceding its expression. Specifically, TCR γ and TCR δ chains interact with three dimeric CD3 subunits – CD3 $\epsilon\gamma$, CD3 $\epsilon'\delta$, and CD3 $\zeta\zeta'$ [147–149]. These three CD3 subunits and the $\gamma\delta$ TCR form an octameric complex [147–149]. Moreover, TCR γ and TCR δ subunits each consist of an extracellular domain (ECD) with membrane-distal $V\gamma/V\delta$ and membrane-proximal $C\gamma/C\delta$ subdomains, a membrane-proximal connecting peptide (MPCP), a transmembrane helix (TMH), and a short cytoplasmic tail (Fig. 4) [149]. Likewise, not only do CD3 γ , CD3 δ , CD3 ϵ , and CD3 ϵ' subunits each possess a connecting peptide, ECD, and TMH, but also a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) (Fig. 4) [149]. Contrarily, CD3 ζ and CD3 ζ' each lack an ECD and MPCP but have a single TMH and three cytoplasmic ITAMs (Fig. 4) [147, 149]. Together, the ECDs of the $\gamma\delta$ TCR form the ECD of the octameric complex; the MPCPs of the $\gamma\delta$ TCR and the connecting peptides

of the CD3 $\epsilon\gamma$ and CD3 $\epsilon'\delta$ subunits connect the $\gamma\delta$ TCR ECDs to the ECDs of the CD3 $\epsilon\gamma$ and CD3 $\epsilon'\delta$ subunits, which CD3 ECDs form the membrane-proximal domain of the octameric complex; and the TMHs of the $\gamma\delta$ TCR and CD3 subunits CD3 $\epsilon\gamma$, CD3 $\epsilon'\delta$, and CD3 $\zeta\zeta'$ form the transmembrane domain of the octameric complex (Fig. 4) [149]. Upon antigen engagement, the octameric complex initiates phosphorylation of the cytoplasmic ITAMs of the CD3 subunits, facilitating downstream events [149–152]. Although all human $\gamma\delta$ TCRs follow general structural and functional patterns, notable structural differences do occur.

In $V\delta 1$ TCRs, the diversity in $V(D)J$ gene rearrangement and pairing of $V\gamma$ and $V\delta 1$ chains are more extensive compared to $V\delta 2$ TCRs, with four prevailing $V\gamma/V\delta$ combinations: $V\gamma 2V\delta 1$, $V\gamma 3V\delta 1$, $V\gamma 4V\delta 1$, and $V\gamma 5V\delta 1$ [1, 7, 144, 149]. Whereas $V\gamma 3V\delta 1$, $V\gamma 4V\delta 1$, and the uncommon $V\gamma 9V\delta 1$ TCRs exist as monomers, $V\gamma 2V\delta 1$ and $V\gamma 5V\delta 1$ TCRs exist as dimers [149]. In newborns, the $V\delta 1$ TCR repertoire is broad and without prevalence of specific clonotypes, while adults typically exhibit TCR privacy – the phenomenon where specific dominant $V\delta 1$ clones provide a repertoire of TCRs unique to each individual [1, 153, 154]. This privacy is mainly attributed to the *TRD* genes/ δ chains, whereas the *TRG* gene/ γ chain repertoire tends to be shared among individuals [1, 154,

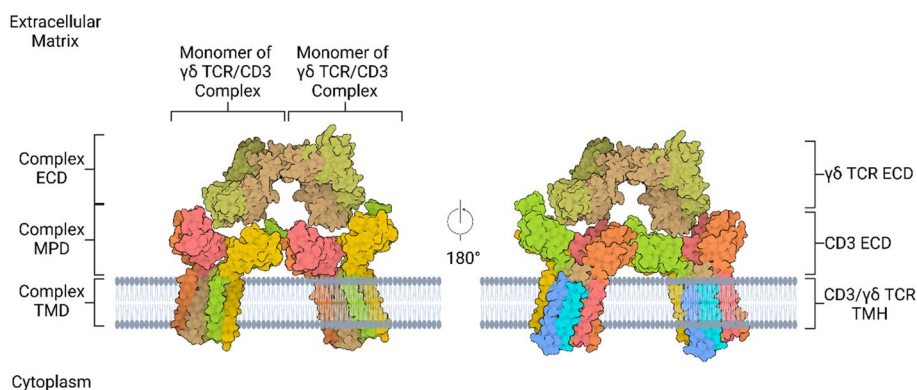


Fig. 4 Dimer of $\gamma\delta$ TCR/CD3 complexes. $\gamma\delta$ TCRs exist in octameric complexes with CD3 proteins; in $V\gamma 2V\delta 1$ and $V\gamma 5V\delta 1$ T cells, these octameric complexes form dimers with each other. Most distally from the plasma membrane, the membrane-distal $V\gamma/V\delta$ (narrower brown blob/narrower olive-green blob) and membrane-proximal $C\gamma/C\delta$ (wider brown blob/wider olive-green blob) subdomains of the $\gamma\delta$ TCR ECDs form the ECD of the octameric complex. Closer to the plasma membrane, the MPCPs of the $\gamma\delta$ TCR and the CPs of the CD3 $\epsilon\gamma$ (CD3 ϵ = light green blob; CD3 γ = gold blob) and CD3 $\epsilon'\delta$ (CD3 ϵ' = pink blob; CD3 δ = orange blob) subunits, which CD3 ECDs form the MPD of the octameric complex. Within the plasma membrane, the TMHs of the $\gamma\delta$ TCR (TCR γ = brown helix; TCR δ = olive-green helix) and CD3 subunits CD3 $\epsilon\gamma$ (CD3 ϵ = light green helix; CD3 γ = gold helix), CD3 $\epsilon'\delta$ (CD3 ϵ' = pink helix; CD3 δ = orange helix), and CD3 $\zeta\zeta'$ (CD3 ζ = periwinkle helix; CD3 ζ' = cyan helix) form the TMD of the octameric complex. Intracellularly, the short cytoplasmic tails (various colored helices) of the CD3 subunits contain ITAMs, which are phosphorylated upon antigen recognition by the $\gamma\delta$ TCR. CD, cluster of differentiation; TCR, T cell receptor; ECD, extracellular domain; MPCP, membrane-proximal connecting peptide; CP, connecting peptide; MPD, membrane-proximal domain; TMD, transmembrane domain; TMH, transmembrane helix; ITAM, immunoreceptor tyrosine-based activation motif. Created with BioRender.com

155]. Consequently, the selection and expansion of long-lived V δ 1 T cells with advantageous TCRs during adulthood resemble a memory-like process, suggesting an adaptive immunosurveillance role [1, 153, 154]. Furthermore, it is generally accepted that TCR ligand recognition facilitates clonotype selection [1]. Notwithstanding private TCR repertoires, degenerate recognition could cause diverse TCRs to react to the same conserved ligands [1]. V δ 1 TCRs can recognize various ligands, including lipid antigens, MICA, MICB, and MHC-like proteins such as CD1c and CD1d, although the *in vivo* impact of these interactions remains unclear [1, 18, 156–160].

Contrary to V δ 1 T cells, V δ 2 T cells exhibit a semi-invariant TCR repertoire that can be shared among individuals, resulting almost exclusively from the pairing of the V δ 2 and V γ 9 TCR chains; hence, V δ 2 T cells are often referred to as V γ 9V δ 2 cells, although previously, as V γ 2V δ 2 cells [1, 146, 161, 162]. Moreover, V γ 9V δ 2 TCRs are known to exist as monomers and can respond robustly to unconventional antigens called phosphoantigens (Fig. 1), such as non-peptidic prenyl pyrophosphates, which accumulate in malignant or infected cells [1, 149, 163]. Notably, phosphoantigens do not directly interact with V δ 2 TCRs but instead bind to the butyrophilin subfamily 3 member A1 (BTN3A1/CD277) intracellular domain, causing BTN3A1 to undergo a conformational change [1, 43, 164–167]. This change enables BTN3A1 to interact with BTN2A1, which can then bind to the V δ 2 TCR V γ 9 chain (Fig. 3) [1, 168, 169]. However, the recognition of prenyl pyrophosphates is dependent upon all complementarity-determining regions of the TCR [163]. While V δ 2 T cells are well known for responding to non-peptide antigens, their TCRs can also recognize peptide antigens such as MSH2, the α and β subunits of the F1-ATPase, and delipidated APOA1, which serves as a stabilizing bridge between the β subunit of the F1-ATPase and the V γ 9V δ 2 TCR [160, 170, 171]. Interestingly, although the F1 ATPase is a mitochondrial protein and MSH2, a nuclear protein, both are expressed ectopically in various cancer cells [170, 171].

Similar to V δ 2 T cells, V δ 3 T cells also exist as monomers and exhibit a semi-invariant TCR repertoire that can be shared among individuals, albeit resulting almost exclusively from the pairing of the V δ 3 and V γ 8 TCR chains [1, 149]. Consequently, V δ 3 T cells are often called V γ 8V δ 3 cells [1]. Additionally, as previously mentioned, V δ 3 TCRs can bind to areas outside the antigen binding cleft of some antigens, indicating possible therapeutic uses [1, 60, 61]. Thus, understanding the specific TCR clonotypes that recognize malignant antigens is crucial for optimizing synthetic TCR engineering in immunotherapy [1].

Standard $\gamma\delta$ T cell therapies

For almost 20 years, $\gamma\delta$ T cells have been used in experimental cancer therapies, either by activating *in vivo* $\gamma\delta$ T cells or through the administration of V δ 2-enriched autologous or allogenic peripheral blood mononuclear cells (PBMCs), often combined with other adjunct treatments. The most widely studied therapy involves activating *in vivo* $\gamma\delta$ T cells with zoledronate and/or IL-2, showing positive results in various cancers, including prostate, breast, and melanoma, with V δ 2 T cells expanding at tumor sites. Another approach combines zoledronate and IL-2 with engraftment of V δ 2-enriched PBMCs, used for solid cancers like colorectal and pancreatic cancers – although, results have been mixed – with some patients showing tumor progression despite localized reductions. Additionally, phosphoantigens, such as 2-methyl-3-butenyl-1-pyrophosphate (2M3B1PP), have been used to expand $\gamma\delta$ T cells, yielding promising results in extending tumor doubling times; nevertheless, some compounds like bromohydrin pyrophosphate (BrHPP) caused adverse effects. Other methods, such as using irreversible electroporation or anti-BTN3A antibodies, have also been tested, with some success in improving survival and enhancing V δ 2 T cell activity at tumor sites.

Since 2007, $\gamma\delta$ T cells have been used in various experimental cancer therapies, either through the targeting of *in vivo* $\gamma\delta$ T cells with stimulators and/or the administration of $\gamma\delta$ T cells as co-treatments with adjunct therapies (summarized in Table 1) [12]. Of these therapies, the most widely studied is the use of *in vivo* $\gamma\delta$ T cells activated with zoledronate and/or IL-2, with or without letrozole as an adjunct, for use in prostate, renal cell, and breast cancers, as well as melanoma and neuroblastoma [12, 172–178]. This regimen was well tolerated, resulting in better expansion and maintenance of V δ 2 T cells with less V δ 2 T cells found in circulation [12, 172–178]. Potentially, reduced levels of V δ 2 T cells in circulation denotes higher levels of V δ 2 T cells at tumor sites [12]. The second most commonly studied therapy involves combining the stimulation of *in vivo* $\gamma\delta$ T cells using zoledronate and IL-2 with the subsequent engraftment of V δ 2-enriched autologous PBMCs, with or without zoledronate or gemcitabine as an adjunct, to combat mixed solid cancers like colorectal, gastric, pancreatic, and non-small cell lung cancers [12, 179–186]. Although this treatment model resulted in elevated plasma IFN- γ and increased V δ 2 T cell activity at tumor sites, only one patient showed partial reductions of lung lesions and two patients showed reductions of local ascites, with all three of these patients experiencing new metastases at distant sites [12, 179–186]. Whereas some zoledronate-supplemented therapies were tested on multiple different

Table 1 Standard $\gamma\delta$ T cell therapies.

Year(s)	Technology	Cancer(s)	Effects/Status	Citation(s)
2007, 2011	2M3B1PP and IL-2 followed by V δ 2-enriched autologous PBMC adoptive cell therapy with/without zoledronate	Renal cell carcinoma	Tumor doubling time prolonged	[12, 192, 193]
2007, 2010–2013, 2016, 2018	Zoledronate with/without IL-2, with/without letrozole	Prostate, breast, and renal cell cancers; melanoma; and neuroblastoma	Well tolerated, less V δ 2 $\gamma\delta$ T cells in blood (potentially more at tumor), better expansion and maintenance of V δ 2 $\gamma\delta$ T cells	[12, 172–178]
2008, 2010	BrHPP and IL-2 followed by nothing or V δ 2-enriched autologous PBMC adoptive cell therapy	Renal cell carcinoma and mixed solid cancers	Tachyphylaxis with repeated dosing, disseminated intravascular coagulation	[12, 190, 191]
2010–2011, 2013–2014, 2017, 2020	Zoledronate and IL-2 followed by V δ 2-enriched autologous PBMC adoptive cell therapy with/without gemcitabine	Non-small cell lung, colorectal, gastric, pancreatic, and mixed solid cancers	Elevated plasma IFN- γ ; increased V δ 2 T cell activity at tumor site; partial reductions of lung lesions (1 patient) and local ascites (2 patients), all with new metastases	[12, 179–186]
2013	Zoledronate and IL-2 followed by V δ 2-enriched autologous PBMC adoptive cell therapy with autologous expanded NK cells and $\alpha\beta$ T cells at unspecified ratio post-radiofrequency ablation	Hepatocellular carcinoma	Improvement in progression-free survival	[12, 187]
2019	Zoledronate and undisclosed cytokines followed by V δ 2-enriched allogenic PBMC adoptive cell therapy	Cholangiocarcinoma	Well tolerated	[12, 188]
2020	Zoledronate and IL-2 followed by V δ 2-enriched allogenic PBMC adoptive cell therapy with irreversible electroporation	Pancreatic cancer	Modest improvement in survival when V δ 2 treatment is followed by irreversible electroporation	[12, 194]
2021	Zoledronate, IL-2, IL-15, and vitamin C followed by V δ 2-enriched allogenic PBMC adoptive cell therapy with irreversible electroporation, iodine-125, and/or cryoablation	Lung and liver cancers	One case of complete response in patient who also had concurrent iodine-125 therapy	[12, 189]
2021	Anti-BTN3A agonist antibody	Mixed solid cancers	Less V δ 2 T cells in blood (potentially more at tumor)	[12, 42]
2023	Gastric cancer exosomal THBS1 to enhance production of IFN- γ , TNF- α , perforin, and granzyme B in vitro, and elevate killing of gastric cancer cells by V δ 2 T cells in vivo	Gastric cancer	Successful preclinical trials	[195, 196]

2M3B1PP 2-methyl-3-butenyl-1-pyrophosphate, PBMC peripheral blood mononuclear cell, BrHPP bromohydrin pyrophosphate, IL interleukin, NK natural killer, BTN3A butyrophilin subfamily 3 member A1, THBS1 thrombospondin 1, IFN interferon, TNF tumor necrosis factor

cancers, others were specific to one or two cancer types [12]. For example, in vivo $\gamma\delta$ T cells expanded with zoledronate and IL-2 followed by infusion of V δ 2-enriched autologous PBMCs, using autologous expanded NK and $\alpha\beta$ T cells post-radiofrequency ablation as an adjunct, yielded an improvement in progression-free survival in hepatocellular carcinoma patients [12, 187]. Another example involves the use of in vivo $\gamma\delta$ T cells activated with zoledronate and various cytokines, as well as subsequent V δ 2-enriched allogenic PBMC engraftment, which was well tolerated in cholangiocarcinoma patients [12, 188]. However, stimulation of in vivo $\gamma\delta$ T cells with zoledronate, IL-2, IL-15, and vitamin C followed by infusion of V δ 2-enriched allogenic PBMCs, using irreversible electroporation, iodine-125, and/or cryoablation as adjunct(s) to treat lung and liver cancers resulted in only one case of complete response, with this complete response coming from a patient who received the iodine-125 adjunct therapy [12, 189].

Additionally, phosphoantigens have been used to expand in vivo $\gamma\delta$ T cells for uses in treating renal cell carcinoma and other solid tumor cancers [12, 89, 190–193]. Specifically, administration of 2M3B1PP and IL-2 preceding V δ 2-enriched autologous PBMC adoptive cell therapy, with low-dose IL-2 and/or zoledronate as adjunct(s), facilitated prolongation of tumor doubling time [12, 192, 193]. Interestingly, BrHPP and IL-2, followed by either no additional treatment or V δ 2-enriched autologous PBMC adoptive cell therapy with low-dose IL-2 as an adjunct only caused adverse effects, such as disseminated intravascular coagulation or tachyphylaxis with repeated dosing [12, 190, 191].

In other cases, neither zoledronate, IL-2, nor phosphoantigens were used to activate in vivo $\gamma\delta$ T cells. For instance, irreversible electroporation followed by adoptive cell therapy with V δ 2-enriched allogenic PBMCs facilitated a modest improvement in survival for pancreatic cancer patients [12, 194]. Also, the use of anti-BTN3A agonist antibodies to combat various solid tumors resulted in less circulating V δ 2 T cells (again, suggesting the attraction of more V δ 2 T cells to the tumor sites) [12, 42]. Furthermore, the use of gastric cancer exosomal THBS1 to enhance production of IFN- γ , TNF- α , perforin, and granzyme B in vitro, to ultimately elevate the killing of gastric cancer cells by V δ 2 T cells in vivo, has yielded successful preclinical trials [195, 196].

Non-CAR $\gamma\delta$ T cell engineering

Engineered $\gamma\delta$ T cells have been developed to target specific cancers, including both non-chimeric antigen receptor (non-CAR)- and chimeric antigen receptor (CAR)-based approaches. One early non-CAR method used polysaccharide K (PSK) to stimulate $\gamma\delta$ T cells,

showing anti-tumor effects in HER2⁺ breast cancer models. In subsequent years, other non-CAR approaches enhanced $\gamma\delta$ T cell efficacy, such as inhibiting CD3 ϵ for better tumor killing and using monoclonal antibodies against CTLA-4 and PD-1 to treat melanoma, yielding positive preclinical results. More recently, non-CAR $\gamma\delta$ T cells targeting peripheral blood cancers were developed, such as an anti-CD19 non-CAR $\gamma\delta$ T cell, which showed strong responses in lymphoma treatment without significant immune exhaustion. The latest innovation, which involves engineering V δ 2 T cells to produce synthetic opsonins, targets osteosarcoma with enhanced cytotoxicity, improves tumor control, and reduces persistence in blood, thus showing promise for osteosarcoma therapy.

Since over a decade, there has been a drive to engineer enhanced $\gamma\delta$ T cells specific to certain types of cancers. Among these engineered $\gamma\delta$ T cells are non-CAR $\gamma\delta$ T cells, which are typically made via expansion followed by stimulation or inhibition of certain $\gamma\delta$ T cell surface markers, as opposed to the engineering of a CAR (summarized in Table 2). One of the first types of these non-CAR $\gamma\delta$ T cells was developed in 2013 at the University of Washington [197]. In this study, expanded $\gamma\delta$ T cells were stimulated with PSK from the *Trametes versicolor* (turkey tail) mushroom, which resulted in IFN- γ production and upregulation of CD25, CD69, and CD107a [197, 198]. Moreover, these PSK-stimulated $\gamma\delta$ T cells reciprocally activated dendritic cells and in the absence of dendritic cells, co-activated other $\gamma\delta$ T cells with TCR cross-linking in vitro [197]. Furthermore, PSK treatment stimulated $\gamma\delta$ T cells among tumor-infiltrating lymphocytes in vivo, contributing to the anti-tumor effects of PSK [197]. Overall, these non-CAR $\gamma\delta$ T cells were successful in both in vitro and in vivo preclinical trials against HER2⁺ breast cancer [197]. Another non-CAR $\gamma\delta$ T cell type was engineered in 2014 and 2018 by inhibiting CD3 ϵ on expanded V δ 2 T cells with anti-CD3 ϵ antibodies [199, 200]. The inhibition of CD3 ϵ enhanced $\gamma\delta$ TCR signaling through stabilization of the active CD3 conformation, as well as activation and tumor-killing efficacy of these $\gamma\delta$ T cells – by an unknown mechanism, although independent of Nck recruitment to the $\gamma\delta$ TCR – leading to an increased $\gamma\delta$ T cell-mediated release of cytotoxic granules and cancer cell lysis, thus denoting successful in vitro and in vivo preclinical trials against B-cell lymphoma and pancreatic ductal adenocarcinoma [199, 200]. Additionally, in 2022, a non-CAR $\gamma\delta$ T cell was developed via the inhibition of CTLA-4 and PD-1 on V δ 2 T cells using monoclonal antibodies to increase $\gamma\delta$ T cell infiltration and killing of melanoma cells [201]. This study yielded successful preclinical trials [201]. Compared to V δ 2 T cells engineered with the isotype control (IgG) antibody, anti-CTLA-4/anti-PD-1 V δ 2 T cells showed a

Table 2 Engineered non-CAR $\gamma\delta$ T cell therapies

Year(s)	Technology	Cancer(s)	Effects/Status	Citation(s)
2013	Stimulation of IFN- γ and upregulation of CD25, CD69, and CD107a in $\gamma\delta$ T cells using polysaccharide K from mushrooms	HER2 ⁺ breast cancer	Successful in vitro and in vivo preclinical trials	[197]
2014, 2018	Inhibition of CD3 ϵ on V δ 2 T cells with anti-CD3 ϵ antibodies	B-cell lymphoma and pancreatic ductal adenocarcinoma	Successful in vitro and in vivo preclinical trials	[199, 200]
2018, 2023	Engineered human anti-CD19 antibody and fused its Fab fragment to $\gamma\delta$ TCR constant chain with adding scFv/CD28 co-stimulatory molecule to anti-CD19 fragment	Relapsed/refractory diffuse large B-cell and primary CNS lymphomas	Successful preclinical and phase 1 clinical trials; induced rapid complete responses and durable remissions; good safety profile	[202, 203]
2022	Inhibition of CTLA-4 and PD-1 on V δ 2 T cells using mAbs to increase $\gamma\delta$ T cell infiltration and killing of melanoma cells	Melanoma	Successful in preclinical trials	[201]
2023	CD20-directed V δ 2 T cells (generated using antibody-cell conjugation)	Relapsed/refractory B-cell lymphoma	Successful in vitro and in vivo preclinical trials	[204]
2024	V δ 2 T cells modified to secrete stIL15 and scFv-Fc fusion proteins targeting GD2 (stIL15-OPS- $\gamma\delta$ T cells)	Osteosarcoma	Successful in vitro and in vivo preclinical trials	[205]

IFN interferon, CD cluster of differentiation, scFv single-chain variable fragment, Fc crystallizable fragment, CTLA-4 cytotoxic T lymphocyte-associated protein 4, PD-1 programmed cell death protein 1, mAb monoclonal antibody, stIL15 interleukin-15 receptor α -interleukin-15, OPS opsonin, GD2 ganglioside G2

marked increase in melanoma spheroid infiltration and reduction of spheroid volume, indicating an improved killing efficacy of melanoma cells [201].

More recently, the development of non-CAR $\gamma\delta$ T cells has been more focused on peripheral blood cancers. For example, in 2023, a special non-CAR $\gamma\delta$ T cell was engineered where the F_{ab} fragment of a human anti-CD19 antibody was fused to the $\gamma\delta$ TCR constant chain with the addition of the single-chain variable fragment (scFv) of the anti-CD19 antibody and a CD28 co-stimulatory molecule to the anti-CD19 F_{ab} fragment [202, 203]. This anti-CD19 non-CAR $\gamma\delta$ T cell was successful in combating relapsed/refractory diffuse large B-cell lymphoma and primary CNS lymphoma [202, 203]. Specifically, preclinical and phase 1 clinical trials reported that this anti-CD19 non-CAR $\gamma\delta$ T cell induced rapid complete responses and durable remissions with a good safety profile [202, 203]. Interestingly, the anti-CD19 non-CAR yielded the same efficacy but reduced exhaustion markers, such as PD-1, LAG-3, and TIM-3, and cytokine release compared with the anti-CD19 CAR [202, 203]. The decreases in cytokine release and immune exhaustion markers while maintaining tumor-killing efficacy is ideal, as such indicates a milder immune response. Additionally, in 2023, CD20-directed V δ 2 T cells were generated using antibody-cell conjugation (ACC) with rituximab, as opposed to using CAR technology [204]. These anti-CD20 $\gamma\delta$ T cells were successful in both in vitro and in vivo preclinical trials against relapsed/refractory B-cell lymphoma [204]. In this case, T cell activation and increased cytotoxicity against CD20-expressing cancer cells was

achieved through antigen recognition of the ACC-linked anti-CD20 antibody [204].

An exciting and most recent development involves the engineering of V δ 2 T cells to produce synthetic opsonins in the form of mitogenic IL-15R α -IL-15 (stIL15) and scFv-Fc fusion proteins [205]. Specifically, these $\gamma\delta$ T cells were designed to secrete opsonins targeting GD2 (stIL15-OPS- $\gamma\delta$ T cells) for treating osteosarcoma [205]. stIL15-OPS- $\gamma\delta$ T cells promote bystander activity of myeloid and other lymphoid cells, while exhibiting enhanced cytotoxicity independent of transgene expression [205]. Compared to unmodified $\gamma\delta$ T cells, stIL15-OPS- $\gamma\delta$ T cells facilitate improved in vivo control of subcutaneous osteosarcoma tumors as well as reduced osteosarcoma persistence in the blood [205]. Moreover, whereas stIL15-OPS- $\gamma\delta$ T cells were effective against patient-derived osteosarcomas both in vitro and in vivo, efficacy was enhanced with the addition of zoledronate [205]. Notably, stIL15-OPS- $\gamma\delta$ T cells favor osteosarcoma-homing and ADCC, recruiting other immune cells to promote antibody-dependent cellular phagocytosis and ADCC against antigen-positive tumor cells [205]. Taken together, by combining bystander activation and direct cytolysis, stIL15-OPS- $\gamma\delta$ T cells serve as a promising allogeneic cell therapy to combat osteosarcoma [205].

CAR $\gamma\delta$ T cell engineering

CAR $\gamma\delta$ T cells have been engineered to target specific cancers, incorporating CARs for both activation and antigen binding. For example, glypican-3-specific CAR V δ 1

T cells with co-expressed IL-15 demonstrated enhanced anti-tumor activity against hepatocellular carcinoma, showing robust expansion and in vivo efficacy. Moreover, engineered anti-CD20 CAR V δ 1 T cells yielded high response rates and a favorable safety profile in clinical trials against lymphoma, with no significant GvHD. Another advancement was the creation of anti-mesothelin CAR V δ 2 T cells, which showed effective targeting of ovarian cancer both in vitro and in vivo, with increased IL-15 expression further enhancing anti-tumor effects. More recently, the development of anti- $\alpha\beta$ 6 CAR G115 V δ 2 T cells for pancreatic, triple-negative breast cancers, and chronic myelogenous leukemia demonstrated promising dual-specific cancer immunotherapy potential, while anti-CD22 CAR $\gamma\delta$ T cells proved effective in eradicating B-cell acute lymphoblastic leukemia (B-ALL).

In the past decade, there also has been a drive to develop CAR $\gamma\delta$ T cells. These engineered $\gamma\delta$ T cells have chimeric antigen receptors, which denotes receptors with both activation and antigen-binding functions. As is the case with some non-CAR $\gamma\delta$ T cells, there are CAR $\gamma\delta$ T cells that have been engineered to be specific for certain types of cancers (summarized in Table 3). For example, in 2021, V δ 1 T cells were engineered using a glypican-3-specific CAR and soluble IL-15 to combat hepatocellular carcinoma [206, 207]. These anti-glypican-3 CAR V δ 1 T cells co-expressing secreted IL-15 displayed robust expansion from PBMCs [206, 207]. Upon expansion, these anti-glypican-3 CAR V δ 1 cells exhibited minimal inhibitory receptor expression manifested by robust in vitro anti-tumor activity against hepatocellular carcinoma cells, even when in the presence of soluble glypican-3 [206, 207]. Furthermore, co-expression of secreted IL-15 increased proliferation and maintained long-term cytotoxicity of these anti-glypican-3 CAR V δ 1 T cells

[206, 207]. Importantly, anti-glypican-3 CAR V δ 1 cells with secreted IL-15 co-expression augmented in vivo anti-tumor activity compared to those without secreted IL-15 co-expression [206, 207]. Neither case resulted in xenogeneic GvHD [206, 207].

A second example of engineered, anti-cancer CAR $\gamma\delta$ T cells is the CD20-directed CAR V δ 1 T cell, designed in 2022 for combating various lymphomas, such as large B-cell, diffuse large B-cell, high-grade B-cell, mantle cell, and Burkitt lymphomas [208, 209]. This anti-CD20 CAR V δ 1 T cell was engineered using a novel, fully human anti-CD20 monoclonal antibody and expresses multiple chemokine receptors and NKRs [209]. Moreover, these experimental anti-CD20 CAR V δ 1 cells exhibited anti-tumor activity both in vitro and in vivo during preclinical trials, without any xenogeneic GvHD [209]. Furthermore, in a phase 1 clinical trial of 11 patients, one patient developed grade 1 cytokine release syndrome and another, grade 2 [208]. A third patient developed grade 1 immune effector cell-associated neurotoxicity syndrome; however, this adverse effect resolved within 24 hours [208]. Additionally, there were no dose-limiting toxicity or GvHD events [208]. The best objective response rate (ORR) and CR rate were 78% [208]. Of the four patients who received prior anti-CD19 CAR T cell therapies, both the ORR and CR rate were 100% [208]. Upon completion of the trial, of the seven patients who achieved CR, one died while in complete remission, two progressed, and four were still in CR [208]. Overall, the anti-CD20 CAR V δ 1 T cells showed a favorable safety profile, an encouraging CR rate, and sustained durability [208].

Another example of an anti-cancer CAR $\gamma\delta$ T cell is the anti-mesothelin CAR V δ 2 T cell enhanced with increased CD16 expression to augment ADCC, with or without additional increased IL-15 expression, for targeting

Table 3 Engineered CAR $\gamma\delta$ T cell therapies

Year(s)	Technology	Cancer(s)	Effects/Status	Citation(s)
2021	V δ 1 T cells engineered using glypican-3-specific CAR and soluble IL-15	Hepatocellular carcinoma	Successful preclinical in vitro and in vivo studies	[206, 207]
2022	CD20-directed CAR-V δ 1 T cells	Diffuse large B-cell, large B-cell, high-grade B-cell, mantle cell, and Burkitt lymphomas	Successful preclinical and phase 1 clinical trials	[208, 209]
2023	Increasing CD16 on V δ 2 T cells to enhance ADCC using anti-mesothelin CAR engineering with or without increased IL-15 expression	Ovarian cancer	Successful preclinical in vitro and in vivo studies (even better with increased IL-15 expression)	[210]
2024	Modification of G115 $\gamma\delta$ TCR to recognize $\alpha\beta$ 6-expressing tumor cells (CAR V δ 2 T cell)	Triple-negative breast and pancreatic cancers	Successful in vitro preclinical trial	[211, 212]
2024	$\gamma\delta$ anti-CD22 CART cell (both V δ 1 and V δ 2)	B-cell acute lymphoblastic leukemia	Completely successful preclinical trials; now in clinical trials	[213]

CAR, chimeric antigen receptor, IL interleukin, CD cluster of differentiation, ADCC antibody-dependent cellular cytotoxicity, TCR T cell receptor

ovarian cancers [210]. These CAR $\gamma\delta$ T cells exhibit pronounced in vitro anti-tumor activity against multiple ovarian cancer lines and can kill tumor-associated macrophages [210]. Moreover, anti-mesothelin CAR V δ 2 T cells are efficacious and safe in subcutaneous and intraperitoneal in vivo ovarian cancer models [210]. Interestingly, these anti-mesothelin CAR $\gamma\delta$ T cells perform even better when IL-15 expression is increased, leading to profound anti-tumor effects both in vitro and in vivo [210].

Most recently, an anti- $\alpha\beta$ 6 CAR G115 V δ 2 T cell was developed with a modification in the $\gamma\delta$ TCR to recognize $\alpha\beta$ 6- and phosphoantigen-expressing tumor cells, such as those found in pancreatic and triple-negative breast cancers, as well as chronic myelogenous leukemia (CML), respectively [211, 212]. G115 is a clonal V δ 2 TCR that can confer responsiveness to phosphoantigens when genetically inserted into $\alpha\beta$ T cell genomes [211]. To broaden the cancer specificity of G115, a tumor-binding dodecamer peptide was selected from the foot-and-mouth disease virus and inserted into the complementarity-determining region 3 of the TCR δ 2 chain [211]. This dodecamer peptide, known as the A20 peptide, binds with high selectivity and affinity to the epithelial-selective integrin $\alpha\beta$ 6, which is expressed in various solid tumors [211]. Consequently, the anti- $\alpha\beta$ 6 CAR G115 V δ 2 T cell is able to kill both $\alpha\beta$ 6- and phosphoantigen-expressing tumor cells, with enhanced cytolytic activity against K562 CML cells [211, 212]. Moreover, anti- $\alpha\beta$ 6 CAR G115 V δ 2 cell activation resulted in IFN- γ release in the presence of either $\alpha\beta$ 6 or phosphoantigen [211]. Thus, given these successful in vitro preclinical trials, anti- $\alpha\beta$ 6 CAR G115 V δ 2 T cells show promise as a novel dual-specific cancer immunotherapy [211].

An additional most recent development involves the anti-CD22 CAR $\gamma\delta$ T cell for the treatment of B-ALL [213]. Interestingly, there was a major difference in performance based on whether these anti-CD22 CAR $\gamma\delta$ T cells were purified via positive or negative selection [213]. Specifically, positive selection resulted in a significantly increased in vitro secretion of IL-2 and IFN- γ but a decreased in vitro tumor killing rate [213]. Conversely, negative selection resulted in reduced cytokine levels in response to CD22⁺ B-ALL, but a quicker ex vivo tumor killing rate with significant enrichment of anti-CD22 CAR V δ 1 and V δ 2 T cells [213]. Nevertheless, whether purified through positive or negative selection, anti-CD22 CAR $\gamma\delta$ T cells exhibited similar in vivo killing efficacy against B-ALL cells [213]. Furthermore, when anti-CD22 CAR $\gamma\delta$ T cells were developed with both positive and negative selection, the B-ALL cells were efficiently eradicated upon treatment [213].

Corporate clinical trials

At least eight pharmaceutical companies are currently conducting clinical trials to test engineered $\gamma\delta$ T cell therapies for cancer treatment. Takeda Pharmaceutical Company is exploring allogeneic V δ 1 T cell therapies for acute myeloid leukemia, which are currently in phase 1 clinical trials. IN8bio is testing various $\gamma\delta$ T cell technologies, including genetically modified autologous and allogeneic therapies for glioblastoma and leukemia, with some in phase 1 and phase 2 clinical trials. Acepodia is developing anti-CD20 and anti-EGFR $\gamma\delta$ T cell therapies for various cancers, which are presently in phase 1 clinical trials. Other companies, such as TC BioPharm, CytoMed Therapeutics, Kiromic Biopharma, Lava Therapeutics, and Adicet Bio, are advancing various $\gamma\delta$ T cell therapies, including but not limited to CAR-engineered therapies targeting solid and hematological cancers, with multiple therapies progressing through phase 1, 2, and 3 clinical trials.

Although some clinical trials have been previously discussed in this review, this section describes clinical trials conducted strictly by pharmaceutical companies, the specific details of which are undisclosed due to the necessity of maintaining trade secrets. There are currently at least eight companies that have engineered and are clinically testing $\gamma\delta$ T cells for the purpose of treating cancer (summarized in Table 4). One of these companies – the Tokyo- and Osaka-based Takeda Pharmaceutical Company Ltd. – which acquired GammaDelta Therapeutics Ltd. in 2021 and Adaptate Biotherapeutics in 2022, is testing allogeneic V δ 1 T cell therapy platforms, including both blood-derived and tissue-derived platforms, as well as early-stage cell therapy programs for the treatment of relapsed/refractory acute myeloid leukemia [214–216]. Currently, these experimental therapies are in phase 1 clinical trials [216].

Another company, IN8bio, which is based in Birmingham, Alabama and New York, New York, has multiple experimental $\gamma\delta$ T cell technologies undergoing clinical trials [217]. For example, DeltEx Drug Resistant Immunotherapy (DRI) Auto (INB-200) is an autologous, genetically-modified $\gamma\delta$ T cell designed for combating glioblastoma and is in phase 1 clinical trials [217]. DeltEx Allo is an allogenic, donor-derived $\gamma\delta$ T cell that is administered to patients following hematopoietic bone marrow transplantation for combating leukemia, which is also in phase 1 clinical trials [217]. Similar to DeltEx DRI Auto (INB-200), DeltEx DRI Auto (INB-400) is likewise an autologous, genetically-modified $\gamma\delta$ T cell used for combating glioblastoma, but is specifically modified to be resistant to alkylating chemotherapy and is in phase 2 clinical trials [217].

Table 4 Corporate clinical trials for $\gamma\delta$ T cell therapies

Company	Technology Pipeline	Cancer(s)	Citation(s)
Takeda Pharmaceutical Company Ltd. ^a	Allogeneic V δ 1 T cell therapy platforms, including both blood-derived and tissue-derived platforms, as well as early-stage cell therapy programs (phase 1 clinical trials)	Relapsed/refractory acute myeloid leukemia	[214–216]
IN8bio	DeltEx DRI Auto (INB-200) (phase 1 clinical trials) DeltEx Allo (phase 1 clinical trials) DeltEx DRI Auto (INB-400) (phase 2 clinical trials)	Glioblastoma and leukemia	[217]
Acepodia	CD20-targeting, ACC- $\gamma\delta$ 2 T cell therapy (phase 1 clinical trials) EGFR-targeting, ACC- $\gamma\delta$ 2 T cell therapy (entering clinical trials)	Chronic lymphocytic leukemia; diffuse large B-cell, follicular, and mantle cell lymphomas; and colorectal, non-small cell lung, and triple-negative breast cancers	[218]
TC BioPharm ^b	Unmodified, allogeneic $\gamma\delta$ T cells (finished phase 1 clinical trials)	Acute myeloid leukemia	[219]
CytoMed Therapeutics Ltd	NKG2D–CAR $\gamma\delta$ T cell therapy (phase 1 clinical trials) Unmodified $\gamma\delta$ T cell therapy (clinical trial application)	Relapsed/refractory solid tumor cancers	[220]
Kiromic Biopharma	Universal, non-engineered $\gamma\delta$ T cell therapy in combination with standard anti-tumor modality) (phase 1 clinical trials began Q4 2022) Anti–PD-L1 CAR $\gamma\delta$ T cell therapy in combination with standard anti-tumor modality or as stand-alone (phase 2 clinical trials began Q2 2023) Anti-mesothelin CAR $\gamma\delta$ T cell therapy in combination with standard anti-tumor modality or as stand-alone (began phase 3 clinical trials Q4 2023)	Solid tumor cancers	[221]
Adicet Bio	Anti-CD20 CAR $\gamma\delta$ T cell (phase 1 clinical trials ongoing)	Relapsed/refractory non-Hodgkin and mantle cell lymphomas	[222]
Lava Therapeutics ^{c,d}	Anti-PSMA V δ 2 T cell (phase 1 clinical trials) Anti-EGFR V δ 2 T cell (phase 1 clinical trials)	Metastatic castration-resistant prostate and solid tumor cancers	[223]

DRI drug-resistant immunotherapy, *CD* cluster of differentiation, *ACC* antibody-cell conjugation, *EGFR* epidermal growth factor receptor, *NKG2D* natural killer group 2D, *CAR* chimeric antigen receptor, *Q* quarter, *PD-L1* programmed cell death protein ligand 1, *PSMA* prostate-specific membrane antigen

^a Acquired GammaDelta Therapeutics Ltd. in 2021 and Adaptate Biotherapeutics in 2022

^b Partnered with ÚHKT, Institute of Hematology and Blood Transfusion (Prague, Czech Republic)

^c Clinical collaboration with Merck & Co. as of January 25, 2024 for anti-PSMA V δ 2 T cell

^d Partnered with Pfizer for anti-EGFR V δ 2 T cell

Moreover, Acepodia, which is based in Alameda, California and Taiwan, has engineered a CD20-targeting, ACC V δ 2 T cell therapy for treating chronic lymphocytic leukemia and diffuse large B-cell, follicular, and mantle cell lymphomas [218]. This anti-CD20, ACC V δ 2 T cell is currently in phase 1 clinical trials [218]. Another experimental therapy undergoing testing by Acepodia is the epidermal growth factor receptor (EGFR)-targeting, ACC V δ 2 T cell therapy for use in combating colorectal, non-small cell lung, and triple-negative breast cancers [218]. At present, this therapy is entering clinical trials [218].

Furthermore, the Scotland-based TC BioPharm has developed unmodified, allogeneic $\gamma\delta$ T cells for treating acute myeloid leukemia [219]. This therapy was designed in partnership with the Czechia-based ÚHKT (Institute of Hematology and Blood Transfusion) and has finished phase 1 clinical trials [219]. Additionally, the

Singapore- and Malaysia-based CytoMed Therapeutics Ltd. has engineered a NKG2D CAR $\gamma\delta$ T cell therapy, which is in phase 1 clinical trials, and an unmodified $\gamma\delta$ T cell therapy, which is under application for clinical trials [220]. Both of these therapies are designed to target relapsed/refractory solid tumors [220].

Whereas most companies focus on a mix of solid tumor and hematological cancers, Kiromic Biopharma, based in Houston, Texas, focuses solely on off-the-shelf, allogeneic $\gamma\delta$ T cell therapies for solid tumor cancers, as these cancers comprise around 90% of all cancers [221]. For instance, Deltacel is a universal, non-engineered, $\gamma\delta$ T cell therapy designed to be used in combination with a standard anti-tumor modality [221]. Phase 1 clinical trials for Deltacel began in the fourth quarter of 2022 [221]. Procel, an anti PD-L1 CAR $\gamma\delta$ T cell technology, was designed to be used as a stand-alone treatment as well as

in combination with a standard anti-tumor modality, and began phase 2 clinical trials in the second quarter of 2023 [221]. Another therapy intended for use either in combination with a standard anti-tumor modality or as a stand-alone treatment is Isocel, which is an anti-mesothelin CAR $\gamma\delta$ T cell therapy that began phase 3 clinical trials during the fourth quarter of 2023 [221].

Additionally, Lava Therapeutics, based in Philadelphia, Pennsylvania and the Netherlands, has developed an anti-PSMA (prostate-specific membrane antigen) V δ 2 T cell for combating metastatic castration-resistant prostate cancer [222]. This experimental therapy is in phase 1 clinical trials and is marked by an ongoing clinical collaboration with Merck & Co. as of January 25, 2024 [222]. Another therapy undergoing phase 1 clinical trials is the anti-EGFR V δ 2 T cell for the treatment of solid tumors [222]. These clinical trials are being conducted under a partnership with Pfizer [222]. A different direction is currently being explored by Adicet Bio, which is based in Redwood City, California and Boston, Massachusetts [223]. Adicet Bio is using an anti-CD20 CAR $\gamma\delta$ T cell for mantle cell lymphoma, which is currently in phase 1 clinical trials [223]. Moreover, this anti-CD20 CAR $\gamma\delta$ T cell has been granted Fast Track designation by the United States Food and Drug Administration for clinical testing as a treatment for relapsed/refractory B-cell non-Hodgkin lymphoma [223].

Conclusions and future perspectives

In conclusion, the vast body of evidence presented in this review underscores the pivotal role of $\gamma\delta$ T cells as indispensable orchestrators of immune responses. From diverse tissue distribution to multifunctional capabilities, $\gamma\delta$ T cells stand out as key players in immune surveillance and regulation. By delving into the intricate biology of $\gamma\delta$ T cells, we have gained valuable insights into the contributions of $\gamma\delta$ T cells to human health and the combating of disease, as well as potential targets for therapeutic interventions.

However, despite the significant progress made in elucidating the roles of $\gamma\delta$ T cells, several challenges remain to be overcome, presenting exciting avenues for future research. One of the challenges currently facing researchers is that $\gamma\delta$ T cell co-treatment effectiveness is still somewhat unknown and shows mixed results. For example, these treatments are, at best, only partially effective and at worst, either ineffective or laced with dangerous side effects. Another challenge is that the stimulation or inhibition of $\gamma\delta$ T cell surface markers to enhance $\gamma\delta$ T cell performance is not specific, which, if not carefully executed, may result in autoimmune problems. Additionally, to generate cancer-specific $\gamma\delta$ T cells, one must find unique cancer cellular surface antigens that are absent on

non-cancer cells. This process is challenging because of its time-consuming nature and high monetary costs, as these antigens must be thoroughly investigated for every type and subtype of cancer; otherwise, severe adverse effects may occur. Furthermore, any treatment method that targets a protein on a cancer cell risks the challenge of the cancer cell gaining resistance to that treatment.

Future research directions should focus on addressing the above-mentioned challenges. Addressing these challenges will answer important questions, such as how to develop cancer-specific $\gamma\delta$ T cells safely, efficiently, and economically, to provide affordable solutions with negligible risk to any cancer patient. As we continue to unravel the complexities of $\gamma\delta$ T cell biology, we anticipate that our expanding knowledge will fuel the development of innovative immunotherapeutic strategies, ultimately benefiting patients across a spectrum of diseases, especially cancer.

Acknowledgements

This work was primarily supported by funds to R.S. from the Indiana University, Brown Center for Immunotherapy, Showalter Trust Award, The 100 Voices of Hope Award, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, and The Tumor Microenvironment & Metastasis (TMM) research program. We especially thank the very talented graphic designer and artist Safa Rezaei Benam for her precious contributions to the artwork in this study.

Authors' contributions

S.C. wrote the draft and prepared the figures. R.S. supervised the project, contributed to writing, and edited the manuscript. All authors reviewed and approved the final manuscript.

Funding

This work was primarily supported by funds to R.S. from the Indiana University Brown Center for Immunotherapy, the Showalter Trust Award, the 100 Voices of Hope Award, and the IUSCCC Tumor Microenvironment & Metastasis grant.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have reviewed and approved the final manuscript and consent to its publication.

Competing interests

The authors declare no competing interests.

Author details

¹Division of Hematology/Oncology, Department of Medicine, Indiana University, Indianapolis, IN, USA. ²Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN, USA. ³Tumor Microenvironment & Metastasis, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN, USA. ⁴Brown Center for Immunotherapy, Indiana University, Indianapolis, IN, USA.

Received: 18 December 2024 Accepted: 7 March 2025

Published online: 28 March 2025

References

- Mensurado S, Blanco-Domínguez R, Silva-Santos B. The emerging roles of $\gamma\delta$ T cells in cancer immunotherapy. *Nat Rev Clin Oncol*. 2023;20:178–91. <https://doi.org/10.1038/s41571-022-00722-1>.
- Bigby M, et al. Most gamma delta T cells develop normally in the absence of MHC class II molecules. *J Immunol*. 1993;151:4465–75.
- Deseke M, Prinz I. Ligand recognition by the $\gamma\delta$ TCR and discrimination between homeostasis and stress conditions. *Cell Mol Immunol*. 2020;17:914–24. <https://doi.org/10.1038/s41423-020-0503-y>.
- Mokuno Y, et al. Expression of toll-like receptor 2 on gamma delta T cells bearing invariant V gamma 6/V delta 1 induced by Escherichia coli infection in mice. *J Immunol*. 2000;165:931–40. <https://doi.org/10.4049/jimmunol.165.2.931>.
- Silva-Santos B, Serre K, Norell H. $\gamma\delta$ T cells in cancer. *Nat Rev Immunol*. 2015;15:683–91. <https://doi.org/10.1038/nri3904>.
- Sheridan BS, et al. $\gamma\delta$ T cells exhibit multifunctional and protective memory in intestinal tissues. *Immunity*. 2013;39:184–95. <https://doi.org/10.1016/j.immuni.2013.06.015>.
- Krangel MS, Yssel H, Brocklehurst C, Spits H. A distinct wave of human T cell receptor gamma/delta lymphocytes in the early fetal thymus: evidence for controlled gene rearrangement and cytokine production. *J Exp Med*. 1990;172:847–59. <https://doi.org/10.1084/jem.172.3.847>.
- Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat Rev Immunol*. 2002;2:336–45. <https://doi.org/10.1038/nri797>.
- Melandri D, et al. The $\gamma\delta$ TCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. *Nat Immunol*. 2018;19:1352–65. <https://doi.org/10.1038/s41590-018-0253-5>.
- Willcox CR, et al. Cytomegalovirus and tumor stress surveillance by binding of a human $\gamma\delta$ T cell antigen receptor to endothelial protein C receptor. *Nat Immunol*. 2012;13:872–9. <https://doi.org/10.1038/ni.2394>.
- Willcox CR, et al. Butyrophilin-like 3 Directly Binds a Human V γ 4(+) T Cell Receptor Using a Modality Distinct from Clonally-Restricted Antigen. *Immunity*. 2019;51:813–825.e814. <https://doi.org/10.1016/j.immuni.2019.09.006>.
- Zlatareva I, Wu Y. Local $\gamma\delta$ T cells: translating promise to practice in cancer immunotherapy. *Br J Cancer*. 2023;129:393–405. <https://doi.org/10.1038/s41416-023-02303-0>.
- Di Marco Barros, R. et al. Epithelia Use Butyrophilin-like Molecules to Shape Organ-Specific $\gamma\delta$ T Cell Compartments. *Cell*. 2016;167:203–218. e217, <https://doi.org/10.1016/j.cell.2016.08.030>
- Ducros E, et al. Endothelial protein C receptor expressed by ovarian cancer cells as a possible biomarker of cancer onset. *Int J Oncol*. 2012;41:433–40. <https://doi.org/10.3892/ijo.2012.1492>.
- Heng W, Mu CY, Chen C, Huang JA, Wang ZY. Endothelial cell protein C receptor (EPCR) is expressed by lung carcinoma and correlated with clinical parameters. *Clin Lab*. 2013;59:375–80. <https://doi.org/10.7754/clin.lab.2012.120214>.
- Lal N, et al. Endothelial protein C receptor is overexpressed in colorectal cancer as a result of amplification and hypomethylation of chromosome 20q. *J Pathol Clin Res*. 2017;3:155–70. <https://doi.org/10.1002/cjp2.70>.
- Lebrero-Fernández C, et al. Altered expression of Butyrophilin (BTN) and BTN-like (BTNL) genes in intestinal inflammation and colon cancer. *Immun Inflamm Dis*. 2016;4:191–200. <https://doi.org/10.1002/iid3.105>.
- Luoma AM, et al. Crystal structure of V δ 1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human $\gamma\delta$ T cells. *Immunity*. 2013;39:1032–42. <https://doi.org/10.1016/j.immuni.2013.11.001>.
- Harly, C. et al. Human $\gamma\delta$ T cell sensing of AMPK-dependent metabolic tumor reprogramming through TCR recognition of EphA2. *Sci Immunol*. 6, <https://doi.org/10.1126/sciimmunol.aba9010> (2021).
- Hoque M, et al. Structural characterization of two $\gamma\delta$ TCR/CD3 complexes. *Nat Commun*. 2025;16:318. <https://doi.org/10.1038/s41467-024-55467-5>.
- Almeida AR, 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. <https://doi.org/10.1158/1078-0432.Ccr-16-0597>.
- Bauer S, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*. 1999;285:727–9. <https://doi.org/10.1126/science.285.5428.727>.
- 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. <https://doi.org/10.3109/14653249.2012.700766>.
- Poggi A, et al. Vdelta1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by trans-retinoic acid. *Cancer Res*. 2004;64:9172–9. <https://doi.org/10.1158/0008-5472.Can-04-2417>.
- Mikulak, J. et al. NKp46-expressing human gut-resident intraepithelial V δ 1 T cell subpopulation exhibits high antitumor activity against colorectal cancer. *JCI Insight*. 2019;4, <https://doi.org/10.1172/jci.insight.125884>.
- Correa DV, et al. Differentiation of human peripheral blood V δ 1+ T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. *Blood*. 2011;118:992–1001. <https://doi.org/10.1182/blood-2011-02-339135>.
- de Vries, N. L. et al. $\gamma\delta$ T cells are effectors of immune checkpoint blockade in mismatch repair-deficient colon cancers with antigen presentation defects. *bioRxiv*. 2021.2010.2014.464229, <https://doi.org/10.1101/2021.10.14.464229> (2021).
- McVay LD, Carding SR. Extrathymic origin of human gamma delta T cells during fetal development. *J Immunol*. 1996;157:2873–82.
- Ashihara E, et al. Isopentenyl pyrophosphate secreted from Zoledronate-stimulated myeloma cells, activates the chemotaxis of $\gamma\delta$ T cells. *Biochem Biophys Res Commun*. 2015;463:650–5. <https://doi.org/10.1016/j.bbrc.2015.05.118>.
- Benzaïd I, et al. High phosphoantigen levels in bisphosphonate-treated human breast tumors promote Vgamma9Vdelta2 T-cell chemotaxis and cytotoxicity in vivo. *Cancer Res*. 2011;71:4562–72. <https://doi.org/10.1158/0008-5472.Can-10-3862>.
- Gober HJ, et al. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med*. 2003;197:163–8. <https://doi.org/10.1084/jem.20021500>.
- Tanaka Y, et al. Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. *Nature*. 1995;375:155–8. <https://doi.org/10.1038/375155a0>.
- Burjanadzé M, et al. In vitro expansion of gamma delta T cells with anti-myeloma cell activity by Phosphostim and IL-2 in patients with multiple myeloma. *Br J Haematol*. 2007;139:206–16. <https://doi.org/10.1111/j.1365-2141.2007.06754.x>.
- Tanaka Y, et al. Synthesis of pyrophosphate-containing compounds that stimulate Vgamma2Vdelta2 T cells: application to cancer immunotherapy. *Med Chem*. 2007;3:85–99. <https://doi.org/10.2174/15734060779317544>.
- Wang RN, et al. Optimized protocols for $\gamma\delta$ T cell expansion and lentiviral transduction. *Mol Med Rep*. 2019;19:1471–80. <https://doi.org/10.3892/mmr.2019.9831>.
- Viey E, et al. Phosphostim-activated gamma delta T cells kill autologous metastatic renal cell carcinoma. *J Immunol*. 2005;174:1338–47. <https://doi.org/10.4049/jimmunol.174.3.1338>.
- Alexander AA, et al. Isopentenyl pyrophosphate-activated CD56+ gamma{delta} T lymphocytes display potent antitumor activity toward human squamous cell carcinoma. *Clin Cancer Res*. 2008;14:4232–40. <https://doi.org/10.1158/1078-0432.Ccr-07-4912>.
- Hoeres T, Smetak M, Pretscher D, Wilhelm M. Improving the Efficiency of V γ 9V δ 2 T-Cell Immunotherapy in Cancer. *Front Immunol*. 2018;9:800. <https://doi.org/10.3389/fimmu.2018.00800>.
- D'Asaro M, et al. V gamma 9V delta 2 T lymphocytes efficiently recognize and kill zoledronate-sensitized, imatinib-sensitive, and imatinib-resistant chronic myelogenous leukemia cells. *J Immunol*. 2010;184:3260–8. <https://doi.org/10.4049/jimmunol.0903454>.
- Kunzmann V, et al. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood*. 2000;96:384–92.
- Mattarollo SR, Kenna T, Nieda M, Nicol AJ. Chemotherapy and zoledronate sensitize solid tumour cells to Vgamma9Vdelta2 T cell cytotoxicity. *Cancer Immunol Immunother*. 2007;56:1285–97. <https://doi.org/10.1007/s00262-007-0279-2>.

42. De Gassart, A. et al. Development of ICT01, a first-in-class, anti-BTN3A antibody for activating V γ 9V δ 2 T cell-mediated antitumor immune response. *Sci Transl Med*. 2021;13:eabj0835. <https://doi.org/10.1126/scitranslmed.abj0835>.
43. Harly C, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human $\gamma\delta$ T-cell subset. *Blood*. 2012;120:2269–79. <https://doi.org/10.1182/blood-2012-05-430470>.
44. Payne KK, et al. BTN3A1 governs antitumor responses by coordinating $\alpha\beta$ and $\gamma\delta$ T cells. *Science*. 2020;369:942–9. <https://doi.org/10.1126/science.aay2767>.
45. Starick, L. et al. Butyrophilin 3A (BTN3A, CD277)-specific antibody 20.1 differentially activates V γ 9V δ 2 TCR clonotypes and interferes with phosphoantigen activation. *Eur J Immunol*. 2017;47:982–992. <https://doi.org/10.1002/eji.201646818>.
46. Rincon-Orozco B, et al. Activation of V gamma 9V delta 2 T cells by NKG2D. *J Immunol*. 2005;175:2144–51. <https://doi.org/10.4049/jimmunol.175.4.2144>.
47. Wrobel P, 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. <https://doi.org/10.1111/j.1365-3083.2007.01963.x>.
48. Toutirais O, et al. DNAX accessory molecule-1 (CD226) promotes human hepatocellular carcinoma cell lysis by Vgamma9Vdelta2 T cells. *Eur J Immunol*. 2009;39:1361–8. <https://doi.org/10.1002/eji.200838409>.
49. Angelini DF, et al. FcgammaRIII discriminates between 2 subsets of Vgamma9Vdelta2 effector cells with different responses and activation pathways. *Blood*. 2004;104:1801–7. <https://doi.org/10.1182/blood-2004-01-0331>.
50. Gertner-Dardenne J, et al. Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies. *Blood*. 2009;113:4875–84. <https://doi.org/10.1182/blood-2008-08-172296>.
51. Tokuyama H, et al. V γ 9V δ 2 T cell cytotoxicity against tumor cells is enhanced by monoclonal antibody drugs—Rituximab and trastuzumab. *Int J Cancer*. 2008;122:2526–34. <https://doi.org/10.1002/ijc.23365>.
52. Capietto AH, Martinet L, Fournier JJ. Stimulated $\gamma\delta$ T cells increase the in vivo efficacy of trastuzumab in HER-2+ breast cancer. *J Immunol*. 2011;187:1031–8. <https://doi.org/10.4049/jimmunol.1100681>.
53. Brandes M, et al. Cross-presenting human gammadelta T cells induce robust CD8+ alpha beta T cell responses. *Proc Natl Acad Sci U S A*. 2009;106:2307–12. <https://doi.org/10.1073/pnas.0810059106>.
54. Bartkowiak J, Kulczyk-Wojdala D, Blonski JZ, Robak T. Molecular diversity of gammadelta T cells in peripheral blood from patients with B-cell chronic lymphocytic leukaemia. *Neoplasma*. 2002;49:86–90.
55. Déchanet J, et al. Implication of gammadelta T cells in the human immune response to cytomegalovirus. *J Clin Invest*. 1999;103:1437–49. <https://doi.org/10.1172/jci5409>.
56. Dunne MR, et al. Persistent changes in circulating and intestinal $\gamma\delta$ T cell subsets, invariant natural killer T cells and mucosal-associated invariant T cells in children and adults with coeliac disease. *PLoS ONE*. 2013;8: e76008. <https://doi.org/10.1371/journal.pone.0076008>.
57. Kenna T, et al. Distinct subpopulations of gamma delta T cells are present in normal and tumor-bearing human liver. *Clin Immunol*. 2004;113:56–63. <https://doi.org/10.1016/j.clim.2004.05.003>.
58. Mangan BA, et al. Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human V δ 3 T cells. *J Immunol*. 2013;191:30–4. <https://doi.org/10.4049/jimmunol.1300121>.
59. Marlin R, et al. Sensing of cell stress by human $\gamma\delta$ TCR-dependent recognition of annexin A2. *Proc Natl Acad Sci U S A*. 2017;114:3163–8. <https://doi.org/10.1073/pnas.1621052114>.
60. Le Nours J, et al. A class of $\gamma\delta$ T cell receptors recognize the underside of the antigen-presenting molecule MR1. *Science*. 2019;366:1522–7. <https://doi.org/10.1126/science.aav3900>.
61. Rice, M. T. et al. Recognition of the antigen-presenting molecule MR1 by a V δ 3(+) $\gamma\delta$ T cell receptor. *Proc Natl Acad Sci U S A*. 2021;118. <https://doi.org/10.1073/pnas.2110288118>.
62. Sebestyen Z, Prinz I, Déchanet-Merville J, Silva-Santos B, Kuball J. Translating gammadelta ($\gamma\delta$) T cells and their receptors into cancer cell therapies. *Nat Rev Drug Discov*. 2020;19:169–84. <https://doi.org/10.1038/s41573-019-0038-z>.
63. Silva-Santos B, Mensurado S, Coffelt SB. $\gamma\delta$ T cells: pleiotropic immune effectors with therapeutic potential in cancer. *Nat Rev Cancer*. 2019;19:392–404. <https://doi.org/10.1038/s41568-019-0153-5>.
64. Chitadze G, Oberg HH, Wesch D, Kabelitz D. The Ambiguous Role of $\gamma\delta$ T Lymphocytes in Antitumor Immunity. *Trends Immunol*. 2017;38:668–78. <https://doi.org/10.1016/j.it.2017.06.004>.
65. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med*. 2010;363:1938–48. <https://doi.org/10.1056/NEJMra1001389>.
66. Mensurado S, Silva-Santos B. Battle of the $\gamma\delta$ T cell subsets in the gut. *Trends Cancer*. 2022;8:881–3. <https://doi.org/10.1016/j.trecan.2022.08.006>.
67. Reis BS, et al. TCR-V γ δ usage distinguishes protumor from antitumor intestinal $\gamma\delta$ T cell subsets. *Science*. 2022;377:276–84. <https://doi.org/10.1126/science.abj8695>.
68. Coffelt SB, et al. IL-17-producing $\gamma\delta$ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature*. 2015;522:345–8. <https://doi.org/10.1038/nature14282>.
69. Papotto PH, Ribot JC, Silva-Santos B. IL-17(+) $\gamma\delta$ T cells as kick-starters of inflammation. *Nat Immunol*. 2017;18:604–11. <https://doi.org/10.1038/ni.3726>.
70. Rei M, Pennington DJ, Silva-Santos B. The emerging Protumor role of $\gamma\delta$ T lymphocytes: implications for cancer immunotherapy. *Cancer Res*. 2015;75:798–802. <https://doi.org/10.1158/0008-5472.Can-14-3228>.
71. Ribot JC, Ribeiro ST, Correia DV, Sousa AE, Silva-Santos B. Human $\gamma\delta$ 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. <https://doi.org/10.4049/jimmunol.1303119>.
72. Gao Z, et al. Gamma delta T-cell-based immune checkpoint therapy: attractive candidate for antitumor treatment. *Mol Cancer*. 2023;22:31. <https://doi.org/10.1186/s12943-023-01722-0>.
73. Gao Y, et al. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. *J Exp Med*. 2003;198:433–42. <https://doi.org/10.1084/jem.20030584>.
74. Girardi M, et al. Regulation of cutaneous malignancy by gammadelta T cells. *Science*. 2001;294:605–9. <https://doi.org/10.1126/science.1063916>.
75. Liu Z, et al. Protective immunosurveillance and therapeutic antitumor activity of gammadelta T cells demonstrated in a mouse model of prostate cancer. *J Immunol*. 2008;180:6044–53. <https://doi.org/10.4049/jimmunol.180.9.6044>.
76. Street SE, et al. Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. *J Exp Med*. 2004;199:879–84. <https://doi.org/10.1084/jem.20031981>.
77. Hoeres T, Holzmann E, Smetak M, Birkmann J, Wilhelm M. PD-1 signaling modulates interferon- γ production by Gamma Delta ($\gamma\delta$) T-Cells in response to leukemia. *Oncoimmunology*. 2019;8:1550618. <https://doi.org/10.1080/2162402x.2018.1550618>.
78. Jin Z, et al. Higher TIGIT(+)CD226(-) $\gamma\delta$ T Cells in Patients with Acute Myeloid Leukemia. *Immunol Invest*. 2022;51:40–50. <https://doi.org/10.1080/08820139.2020.1806868>.
79. Schofield L, et al. Synergistic effect of IL-12 and IL-18 induces TIM3 regulation of $\gamma\delta$ T cell function and decreases the risk of clinical malaria in children living in Papua New Guinea. *BMC Med*. 2017;15:114. <https://doi.org/10.1186/s12916-017-0883-8>.
80. Yang R, et al. Bispecific Antibody PD-L1 x CD3 Boosts the Anti-Tumor Potency of the Expanded V γ 2V δ 2 T Cells. *Front Immunol*. 2021;12: 654080. <https://doi.org/10.3389/fimmu.2021.654080>.
81. Yang, Z. Z. et al. Expression of LAG-3 defines exhaustion of intratumoral PD-1(+) T cells and correlates with poor outcome in follicular lymphoma. *Oncotarget*. 2017;8:61425–61439. <https://doi.org/10.18632/oncotarget.18251>.
82. Lu H, et al. B7–H3 inhibits the IFN- γ -dependent cytotoxicity of V γ 9V δ 2 T cells against colon cancer cells. *Oncoimmunology*. 2020;9:1748991. <https://doi.org/10.1080/2162402x.2020.1748991>.
83. Simões AE, Di Lorenzo B, Silva-Santos B. Molecular Determinants of Target Cell Recognition by Human $\gamma\delta$ T Cells. *Front Immunol*. 2018;9:929. <https://doi.org/10.3389/fimmu.2018.00929>.
84. Todaro M, et al. Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. *J Immunol*. 2009;182:7287–96. <https://doi.org/10.4049/jimmunol.0804288>.

85. Dokouhaki P, et al. NKG2D regulates production of soluble TRAIL by ex vivo expanded human $\gamma\delta$ T cells. *Eur J Immunol*. 2013;43:3175–82. <https://doi.org/10.1002/eji.201243150>.
86. Fisher JP, et al. Neuroblastoma killing properties of V δ 2 and V δ 2-negative $\gamma\delta$ T cells following expansion by artificial antigen-presenting cells. *Clin Cancer Res*. 2014;20:5720–32. <https://doi.org/10.1158/1078-0432.Ccr-13-3464>.
87. Riond J, Rodriguez S, Nicolau M, L., al Saati T, & Gairin J, E. In vivo major histocompatibility complex class I (MHCI) expression on MHCIlow tumor cells is regulated by gammadelta T and NK cells during the early steps of tumor growth. *Cancer Immun*. 2009;9:10.
88. Maniar A, et al. Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. *Blood*. 2010;116:1726–33. <https://doi.org/10.1182/blood-2009-07-234211>.
89. Altvater B, et al. Activated human $\gamma\delta$ T cells induce peptide-specific CD8+ T-cell responses to tumor-associated self-antigens. *Cancer Immunol Immunother*. 2012;61:385–96. <https://doi.org/10.1007/s00262-011-1111-6>.
90. Brandes M, Willmann K, Moser B. Professional antigen-presentation function by human gammadelta T Cells. *Science*. 2005;309:264–8. <https://doi.org/10.1126/science.1110267>.
91. Holmen Olofsson G, et al. V γ 9V δ 2 T Cells Concurrently Kill Cancer Cells and Cross-Present Tumor Antigens. *Front Immunol*. 2021;12: 645131. <https://doi.org/10.3389/fimmu.2021.645131>.
92. Himoudi N, et al. Human $\gamma\delta$ T lymphocytes are licensed for professional antigen presentation by interaction with opsonized target cells. *J Immunol*. 2012;188:1708–16. <https://doi.org/10.4049/jimmunol.1102654>.
93. Bansal RR, Mackay CR, Moser B, Eberl M. IL-21 enhances the potential of human $\gamma\delta$ T cells to provide B-cell help. *Eur J Immunol*. 2012;42:110–9. <https://doi.org/10.1002/eji.201142017>.
94. Caccamo N, et al. CXCR5 identifies a subset of Vgamma9Vdelta2 T cells which secrete IL-4 and IL-10 and help B cells for antibody production. *J Immunol*. 2006;177:5290–5. <https://doi.org/10.4049/jimmunol.177.8.5290>.
95. Rampoldi, F., Ullrich, L. & Prinz, I. Revisiting the Interaction of $\gamma\delta$ T-Cells and B-Cells. *Cells*. 2020;9: <https://doi.org/10.3390/cells9030743>.
96. Rezende RM, et al. $\gamma\delta$ T cells control humoral immune response by inducing T follicular helper cell differentiation. *Nat Commun*. 2018;9:3151. <https://doi.org/10.1038/s41467-018-05487-9>.
97. Wen L, et al. Germinal center formation, immunoglobulin class switching, and autoantibody production driven by “non alpha/beta” T cells. *J Exp Med*. 1996;183:2271–82. <https://doi.org/10.1084/jem.183.5.2271>.
98. Rei M, et al. Murine CD27(-) V γ 6(+) $\gamma\delta$ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. *Proc Natl Acad Sci U S A*. 2014;111:E3562–3570. <https://doi.org/10.1073/pnas.1403424111>.
99. Foord, E., Arruda, L. C. M., Gaballa, A., Klynning, C. & Uhlin, M. Characterization of ascites- and tumor-infiltrating $\gamma\delta$ T cells reveals distinct repertoires and a beneficial role in ovarian cancer. *Sci Transl Med*. 2021;13: <https://doi.org/10.1126/scitranslmed.abb0192>.
100. Gentles AJ, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. 2015;21:938–45. <https://doi.org/10.1038/nm.3909>.
101. Chen X, et al. Distribution and functions of $\gamma\delta$ T cells infiltrated in the ovarian cancer microenvironment. *J Transl Med*. 2019;17:144. <https://doi.org/10.1186/s12967-019-1897-0>.
102. Lo Presti, E. et al. Squamous Cell Tumors Recruit $\gamma\delta$ T Cells Producing either IL17 or IFN γ Depending on the Tumor Stage. *Cancer Immunol Res*. 2017;5:397–407. <https://doi.org/10.1158/2326-6066.Cir-16-0348>
103. Meraviglia S, et al. Distinctive features of tumor-infiltrating $\gamma\delta$ T lymphocytes in human colorectal cancer. *Oncoimmunology*. 2017;6: e1347742. <https://doi.org/10.1080/2162402x.2017.1347742>.
104. Patil RS, et al. IL17 producing $\gamma\delta$ T cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. *Int J Cancer*. 2016;139:869–81. <https://doi.org/10.1002/ijc.30134>.
105. Van Hede D, et al. Human papillomavirus oncoproteins induce a reorganization of epithelial-associated $\gamma\delta$ T cells promoting tumor formation. *Proc Natl Acad Sci U S A*. 2017;114:E9056–e9065. <https://doi.org/10.1073/pnas.1712883114>.
106. Wu P, et al. $\gamma\delta$ T17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity*. 2014;40:785–800. <https://doi.org/10.1016/j.immuni.2014.03.013>.
107. Benevides L, et al. IL17 Promotes Mammary Tumor Progression by Changing the Behavior of Tumor Cells and Eliciting Tumorigenic Neutrophils Recruitment. *Cancer Res*. 2015;75:3788–99. <https://doi.org/10.1158/0008-5472.Can-15-0054>.
108. Guo N, et al. Interleukin-17 Promotes Migration and Invasion of Human Cancer Cells Through Upregulation of MTA1 Expression. *Front Oncol*. 2019;9:546. <https://doi.org/10.3389/fonc.2019.00546>.
109. Hu Z, et al. IL-17 Activates the IL-6/STAT3 Signal Pathway in the Proliferation of Hepatitis B Virus-Related Hepatocellular Carcinoma. *Cell Physiol Biochem*. 2017;43:2379–90. <https://doi.org/10.1159/000484390>.
110. Kulig P, et al. IL17A-Mediated Endothelial Breach Promotes Metastasis Formation. *Cancer Immunol Res*. 2016;4:26–32. <https://doi.org/10.1158/2326-6066.Cir-15-0154>.
111. Ma S, et al. IL-17A produced by $\gamma\delta$ T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res*. 2014;74:1969–82. <https://doi.org/10.1158/0008-5472.Can-13-2534>.
112. Sacchi A, et al. Myeloid-Derived Suppressor Cells Specifically Suppress IFN- γ Production and Antitumor Cytotoxic Activity of V δ 2 T Cells. *Front Immunol*. 2018;9:1271. <https://doi.org/10.3389/fimmu.2018.01271>.
113. Wakita D, et al. Tumor-infiltrating IL-17-producing gammadelta T cells support the progression of tumor by promoting angiogenesis. *Eur J Immunol*. 2010;40:1927–37. <https://doi.org/10.1002/eji.200940157>.
114. Jin C, et al. Commensal Microbiota Promote Lung Cancer Development via $\gamma\delta$ T Cells. *Cell*. 2019;176:998–1013.e1016. <https://doi.org/10.1016/j.cell.2018.12.040>.
115. Papotto PH, Yilmaz B, Silva-Santos B. Crosstalk between $\gamma\delta$ T cells and the microbiota. *Nat Microbiol*. 2021;6:1110–7. <https://doi.org/10.1038/s41564-021-00948-2>.
116. Kalyan S, Kabelitz D. When neutrophils meet T cells: beginnings of a tumultuous relationship with underappreciated potential. *Eur J Immunol*. 2014;44:627–33. <https://doi.org/10.1002/eji.201344195>.
117. Mensurado S, et al. Tumor-associated neutrophils suppress pro-tumoral IL-17+ $\gamma\delta$ T cells through induction of oxidative stress. *PLoS Biol*. 2018;16: e2004990. <https://doi.org/10.1371/journal.pbio.2004990>.
118. Zhang Z, et al. “ $\gamma\delta$ T Cell-IL17A-Neutrophil” Axis Drives Immunosuppression and Confers Breast Cancer Resistance to High-Dose Anti-VEGFR2 Therapy. *Front Immunol*. 2021;12: 699478. <https://doi.org/10.3389/fimmu.2021.699478>.
119. Daley D, et al. $\gamma\delta$ T Cells Support Pancreatic Oncogenesis by Restraining $\alpha\beta$ T Cell Activation. *Cell*. 2016;166:1485–1499.e1415. <https://doi.org/10.1016/j.cell.2016.07.046>.
120. Rutkowski MR, et al. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. *Cancer Cell*. 2015;27:27–40. <https://doi.org/10.1016/j.ccr.2014.11.009>.
121. Hao J, et al. Regulatory role of V γ 1 $\gamma\delta$ T cells in tumor immunity through IL-4 production. *J Immunol*. 2011;187:4979–86. <https://doi.org/10.4049/jimmunol.1101389>.
122. Mao Y, et al. A new effect of IL-4 on human $\gamma\delta$ T cells: promoting regulatory V δ 1 T cells via IL-10 production and inhibiting function of V δ 2 T cells. *Cell Mol Immunol*. 2016;13:217–28. <https://doi.org/10.1038/cmi.2015.07>.
123. Peng G, et al. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity*. 2007;27:334–48. <https://doi.org/10.1016/j.immuni.2007.05.020>.
124. Ye J, et al. Tumor-derived $\gamma\delta$ regulatory T cells suppress innate and adaptive immunity through the induction of immunosenescence. *J Immunol*. 2013;190:2403–14. <https://doi.org/10.4049/jimmunol.1202369>.
125. Chabab G, et al. Identification of a regulatory V δ 1 gamma delta T cell subpopulation expressing CD73 in human breast cancer. *J Leukoc Biol*. 2020;107:1057–67. <https://doi.org/10.1002/jlb.3ma0420-278r>.
126. Wu, Y. et al. An innate-like V δ 1(+) $\gamma\delta$ T cell compartment in the human breast is associated with remission in triple-negative breast cancer. *Sci Transl Med*. 2019;11: <https://doi.org/10.1126/scitranslmed.aax9364>.
127. Dijkstra KK, Wu Y, Swanton C. The Effects of Clonal Heterogeneity on Cancer Immunosurveillance. *Annual Review of Cancer*

- Biology. 2023;7:131–47. <https://doi.org/10.1146/annurev-cancer-rbio-061521-101910>.
128. Wu Y, Biswas D, Swanton C. Impact of cancer evolution on immune surveillance and checkpoint inhibitor response. *Semin Cancer Biol.* 2022;84:89–102. <https://doi.org/10.1016/j.semcancer.2021.02.013>.
 129. Allen AG, et al. A highly efficient transgene knock-in technology in clinically relevant cell types. *Nat Biotechnol.* 2024;42:458–69. <https://doi.org/10.1038/s41587-023-01779-8>.
 130. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* 2022;12:31–46. <https://doi.org/10.1158/2159-8290.Cd-21-1059>.
 131. Rizvi, N. A. *et al.* Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015;348:124–128. <https://doi.org/10.1126/science.aaa1348>.
 132. Rosenthal R, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature.* 2019;567:479–85. <https://doi.org/10.1038/s41586-019-1032-7>.
 133. Sade-Feldman M, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun.* 2017;8:1136. <https://doi.org/10.1038/s41467-017-01062-w>.
 134. Shim JH, et al. HLA-corrected tumor mutation burden and homologous recombination deficiency for the prediction of response to PD-(L)1 blockade in advanced non-small-cell lung cancer patients. *Ann Oncol.* 2020;31:902–11. <https://doi.org/10.1016/j.annonc.2020.04.004>.
 135. Snyder A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med.* 2014;371:2189–99. <https://doi.org/10.1056/NEJMoa1406498>.
 136. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature.* 2005;436:1186–90. <https://doi.org/10.1038/nature03884>.
 137. Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. *Annu Rev Immunol.* 2013;31:413–41. <https://doi.org/10.1146/annurev-immunol-032712-095951>.
 138. Angelini DF, et al. NKG2A inhibits NKG2C effector functions of $\gamma\delta$ T cells: implications in health and disease. *J Leukoc Biol.* 2011;89:75–84. <https://doi.org/10.1189/jlb.0710413>.
 139. Barrow AD, Martin CJ, Colonna M. The Natural Cytotoxicity Receptors in Health and Disease. *Front Immunol.* 2019;10:909. <https://doi.org/10.3389/fimmu.2019.00909>.
 140. Cazzetta V, et al. NKG2A expression identifies a subset of human V δ 2 T cells exerting the highest antitumor effector functions. *Cell Rep.* 2021;37: 109871. <https://doi.org/10.1016/j.celrep.2021.109871>.
 141. Fuchs A, Colonna M. The role of NK cell recognition of nectin and nectin-like proteins in tumor immunosurveillance. *Semin Cancer Biol.* 2006;16:359–66. <https://doi.org/10.1016/j.semcancer.2006.07.002>.
 142. Holtmeier W, et al. The TCR-delta repertoire in normal human skin is restricted and distinct from the TCR-delta repertoire in the peripheral blood. *J Invest Dermatol.* 2001;116:275–80. <https://doi.org/10.1046/j.1523-1747.2001.01250.x>.
 143. Kabelitz D, Kalyan S, Oberg HH, Wesch D. Human V δ 2 versus non-V δ 2 $\gamma\delta$ T cells in antitumor immunity. *Oncoimmunology.* 2013;2: e23304. <https://doi.org/10.4161/onci.23304>.
 144. Kalyan S, Kabelitz D. Defining the nature of human $\gamma\delta$ T cells: a biographical sketch of the highly empathetic. *Cell Mol Immunol.* 2013;10:21–9. <https://doi.org/10.1038/cmi.2012.44>.
 145. Lefranc MP. IMGT, the International ImMunoGeneTics Information System. *Cold Spring Harb Protoc.* 2011;595–603:2011. <https://doi.org/10.1101/pdb.top115>.
 146. Sherwood, A. M. *et al.* Deep sequencing of the human TCR γ and TCR β repertoires suggests that TCR β rearranges after $\alpha\beta$ and $\gamma\delta$ T cell commitment. *Sci Transl Med.* 2011;3:90ra61. <https://doi.org/10.1126/scitranslmed.3002536>.
 147. Morath A, Schamel WW. $\alpha\beta$ and $\gamma\delta$ T cell receptors: Similar but different. *J Leukoc Biol.* 2020;107:1045–55. <https://doi.org/10.1002/jlb.2mr1219-233r>.
 148. Siegers GM, et al. Different composition of the human and the mouse gammadelta T cell receptor explains different phenotypes of CD3 γ and CD3 δ immunodeficiencies. *J Exp Med.* 2007;204:2537–44. <https://doi.org/10.1084/jem.20070782>.
 149. Xin W, et al. Structures of human $\gamma\delta$ T cell receptor–CD3 complex. *Nature.* 2024;630:222–9. <https://doi.org/10.1038/s41586-024-07439-4>.
 150. Chien YH, Meyer C, Bonneville M. $\gamma\delta$ T cells: first line of defense and beyond. *Annu Rev Immunol.* 2014;32:121–55. <https://doi.org/10.1146/annurev-immunol-032713-120216>.
 151. Vantourout P, Hayday A. Six-of-the-best: unique contributions of $\gamma\delta$ T cells to immunology. *Nat Rev Immunol.* 2013;13:88–100. <https://doi.org/10.1038/nri3384>.
 152. Zeng X, et al. $\gamma\delta$ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response. *Immunity.* 2012;37:524–34. <https://doi.org/10.1016/j.immuni.2012.06.011>.
 153. Davey MS, et al. Clonal selection in the human V δ 1 T cell repertoire indicates $\gamma\delta$ TCR-dependent adaptive immune surveillance. *Nat Commun.* 2017;8:14760. <https://doi.org/10.1038/ncomms14760>.
 154. Ravens S, et al. Human $\gamma\delta$ T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat Immunol.* 2017;18:393–401. <https://doi.org/10.1038/ni.3686>.
 155. Di Lorenzo B, Ravens S, Silva-Santos B. High-throughput analysis of the human thymic V δ 1(+) T cell receptor repertoire. *Sci Data.* 2019;6:115. <https://doi.org/10.1038/s41597-019-0118-2>.
 156. Spada FM, et al. Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. *J Exp Med.* 2000;191:937–48. <https://doi.org/10.1084/jem.191.6.937>.
 157. Uldrich AP, et al. CD1d-lipid antigen recognition by the $\gamma\delta$ TCR. *Nat Immunol.* 2013;14:1137–45. <https://doi.org/10.1038/ni.2713>.
 158. Groh V, et al. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc Natl Acad Sci U S A.* 1999;96:6879–84. <https://doi.org/10.1073/pnas.96.12.6879>.
 159. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. *Science.* 1998;279:1737–40. <https://doi.org/10.1126/science.279.5357.1737>.
 160. Hu Y, et al. $\gamma\delta$ T cells: origin and fate, subsets, diseases and immunotherapy. *Signal Transduct Target Ther.* 2023;8:434. <https://doi.org/10.1038/s41392-023-01653-8>.
 161. Willcox CR, Davey MS, Willcox BE. Development and Selection of the Human V γ 9V δ 2(+) T-Cell Repertoire. *Front Immunol.* 2018;9:1501. <https://doi.org/10.3389/fimmu.2018.01501>.
 162. Chen, Z. W. Multifunctional immune responses of HMBPP-specific V γ 2V δ 2 T cells in M. tuberculosis and other infections. *Cell Mol Immunol.* 2013;10:58–64. <https://doi.org/10.1038/cmi.2012.46>.
 163. Wang H, Fang Z, Morita CT. Vgamma2Vdelta2 T Cell Receptor recognition of prenyl pyrophosphates is dependent on all CDRs. *J Immunol.* 2010;184:6209–22. <https://doi.org/10.4049/jimmunol.1000231>.
 164. Gu S, et al. Phosphoantigen-induced conformational change of butyrophilin 3A1 (BTN3A1) and its implication on V γ 9V δ 2 T cell activation. *Proc Natl Acad Sci U S A.* 2017;114:E7311–e7320. <https://doi.org/10.1073/pnas.1707547114>.
 165. Peigné CM, et al. The Juxtamembrane Domain of Butyrophilin BTN3A1 Controls Phosphoantigen-Mediated Activation of Human V γ 9V δ 2 T Cells. *J Immunol.* 2017;198:4228–34. <https://doi.org/10.4049/jimmunol.1601910>.
 166. Sandstrom, A. *et al.* The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human V γ 9V δ 2 T cells. *Immunity.* 2014;40:490–500. <https://doi.org/10.1016/j.immuni.2014.03.003>.
 167. Yang Y, et al. A Structural Change in Butyrophilin upon Phosphoantigen Binding Underlies Phosphoantigen-Mediated V γ 9V δ 2 T Cell Activation. *Immunity.* 2019;50:1043–1053.e1045. <https://doi.org/10.1016/j.immuni.2019.02.016>.
 168. Hsiao CC, Nguyen K, Jin Y, Vinogradova O, Wiemer AJ. Ligand-induced interactions between butyrophilin 2A1 and 3A1 internal domains in the HMBPP receptor complex. *Cell Chem Biol.* 2022;29:985–995.e985. <https://doi.org/10.1016/j.chembiol.2022.01.004>.
 169. Rigau, M. *et al.* Butyrophilin 2A1 is essential for phosphoantigen reactivity by $\gamma\delta$ T cells. *Science.* 2020;367. <https://doi.org/10.1126/science.aay5516>.
 170. Dai Y, Chen H, Mo C, Cui L, He W. Ectopically expressed human tumor biomarker MutS homologue 2 is a novel endogenous ligand that is recognized by human $\gamma\delta$ T cells to induce innate anti-tumor/virus

- immunity. *J Biol Chem.* 2012;287:16812–9. <https://doi.org/10.1074/jbc.M111.327650>.
171. Scotet E, et al. Tumor recognition following Vgamma9Vdelta2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. *Immunity.* 2005;22:71–80. <https://doi.org/10.1016/j.immuni.2004.11.012>.
 172. Dieli F, et al. Targeting human gamma delta T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. *Cancer Res.* 2007;67:7450–7. <https://doi.org/10.1158/0008-5472.Can-07-0199>.
 173. Kunzmann V, et al. Tumor-promoting versus tumor-antagonizing roles of $\gamma\delta$ T cells in cancer immunotherapy: results from a prospective phase I/II trial. *J Immunother.* 2012;35:205–13. <https://doi.org/10.1097/CJ.I.0b013e318245bb1e>.
 174. Lang JM, et al. Pilot trial of interleukin-2 and zoledronic acid to augment $\gamma\delta$ T cells as treatment for patients with refractory renal cell carcinoma. *Cancer Immunol Immunother.* 2011;60:1447–60. <https://doi.org/10.1007/s00262-011-1049-8>.
 175. Meraviglia S, et al. In vivo manipulation of Vgamma9Vdelta2 T cells with zoledronate and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. *Clin Exp Immunol.* 2010;161:290–7. <https://doi.org/10.1111/j.1365-2249.2010.04167.x>.
 176. Pressey, J. G. et al. In vivo expansion and activation of $\gamma\delta$ T cells as immunotherapy for refractory neuroblastoma: A phase 1 study. *Medicine (Baltimore).* 2016;95:e4909 <https://doi.org/10.1097/md.0000000000004909>.
 177. Sugie T, et al. Zoledronic acid-induced expansion of $\gamma\delta$ T cells from early-stage breast cancer patients: effect of IL-18 on helper NK cells. *Cancer Immunol Immunother.* 2013;62:677–87. <https://doi.org/10.1007/s00262-012-1368-4>.
 178. Sugie T, et al. Combined effects of neoadjuvant letrozole and zoledronic acid on $\gamma\delta$ T cells in postmenopausal women with early-stage breast cancer. *Breast.* 2018;38:114–9. <https://doi.org/10.1016/j.breast.2017.12.017>.
 179. Aoki T, et al. Adjuvant combination therapy with gemcitabine and autologous $\gamma\delta$ T-cell transfer in patients with curatively resected pancreatic cancer. *Cytotherapy.* 2017;19:473–85. <https://doi.org/10.1016/j.jcyt.2017.01.002>.
 180. Izumi T, et al. Ex vivo characterization of $\gamma\delta$ T-cell repertoire in patients after adoptive transfer of V γ 9V δ 2 T cells expressing the interleukin-2 receptor β -chain and the common γ -chain. *Cytotherapy.* 2013;15:481–91. <https://doi.org/10.1016/j.jcyt.2012.12.004>.
 181. Kakimi, K. et al. Adoptive transfer of zoledronate-expanded autologous V γ 9V δ 2 T-cells in patients with treatment-refractory non-small-cell lung cancer: a multicenter, open-label, single-arm, phase 2 study. *J Immunother Cancer.* 2020;8 <https://doi.org/10.1136/jitc-2020-001185>.
 182. Nakajima J, et al. A phase I study of adoptive immunotherapy for recurrent non-small-cell lung cancer patients with autologous gammadelta T cells. *Eur J Cardiothorac Surg.* 2010;37:1191–7. <https://doi.org/10.1016/j.ejcts.2009.11.051>.
 183. Nicol AJ, et al. Clinical evaluation of autologous gamma delta T cell-based immunotherapy for metastatic solid tumours. *Br J Cancer.* 2011;105:778–86. <https://doi.org/10.1038/bjc.2011.293>.
 184. Noguchi A, et al. Zoledronate-activated V γ 9 δ 2 T cell-based immunotherapy is feasible and restores the impairment of $\gamma\delta$ T cells in patients with solid tumors. *Cytotherapy.* 2011;13:92–7. <https://doi.org/10.3109/14653249.2010.515581>.
 185. Sakamoto M, et al. Adoptive immunotherapy for advanced non-small cell lung cancer using zoledronate-expanded $\gamma\delta$ Tcells: a phase I clinical study. *J Immunother.* 2011;34:202–11. <https://doi.org/10.1097/CJ.I.0b013e318207ecfb>.
 186. Wada I, et al. Intraperitoneal injection of in vitro expanded V γ 9V δ 2 T cells together with zoledronate for the treatment of malignant ascites due to gastric cancer. *Cancer Med.* 2014;3:362–75. <https://doi.org/10.1002/cam4.196>.
 187. Cui J, et al. Combination of radiofrequency ablation and sequential cellular immunotherapy improves progression-free survival for patients with hepatocellular carcinoma. *Int J Cancer.* 2014;134:342–51. <https://doi.org/10.1002/ijc.28372>.
 188. Alnaggar M, et al. Allogenic V γ 9V δ 2 T cell as new potential immunotherapy drug for solid tumor: a case study for cholangiocarcinoma. *J Immunother Cancer.* 2019;7:36. <https://doi.org/10.1186/s40425-019-0501-8>.
 189. Xu Y, et al. Allogeneic V γ 9V δ 2 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. <https://doi.org/10.1038/s41423-020-0515-7>.
 190. Bennouna J, et al. Phase-I study of Innacell gammadelta, an autologous cell-therapy product highly enriched in gamma9delta2 T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. *Cancer Immunol Immunother.* 2008;57:1599–609. <https://doi.org/10.1007/s00262-008-0491-8>.
 191. Bennouna J, et al. Phase I study of bromohydrin pyrophosphate (BrHPP, IPH 1101), a Vgamma9Vdelta2 T lymphocyte agonist in patients with solid tumors. *Cancer Immunol Immunother.* 2010;59:1521–30. <https://doi.org/10.1007/s00262-010-0879-0>.
 192. Kobayashi H, Tanaka Y, Yagi J, Minato N, Tanabe K. Phase I/II study of adoptive transfer of $\gamma\delta$ T cells in combination with zoledronic acid and IL-2 to patients with advanced renal cell carcinoma. *Cancer Immunol Immunother.* 2011;60:1075–84. <https://doi.org/10.1007/s00262-011-1021-7>.
 193. Kobayashi H, et al. Safety profile and anti-tumor effects of adoptive immunotherapy using gamma-delta T cells against advanced renal cell carcinoma: a pilot study. *Cancer Immunol Immunother.* 2007;56:469–76. <https://doi.org/10.1007/s00262-006-0199-6>.
 194. Lin M, et al. Irreversible electroporation plus allogenic V γ 9V δ 2 T cells enhances antitumor effect for locally advanced pancreatic cancer patients. *Signal Transduct Target Ther.* 2020;5:215. <https://doi.org/10.1038/s41392-020-00260-1>.
 195. Li J, et al. Gastric cancer derived exosomal THBS1 enhanced V γ 9V δ 2 T-cell function through activating RIG-I-like receptor signaling pathway in a N6-methyladenosine methylation dependent manner. *Cancer Lett.* 2023;576: 216410. <https://doi.org/10.1016/j.canlet.2023.216410>.
 196. Li J, et al. Gastric cancer-derived exosomal miR-135b-5p impairs the function of V γ 9V δ 2 T cells by targeting specificity protein 1. *Cancer Immunol Immunother.* 2022;71:311–25. <https://doi.org/10.1007/s00262-021-02991-8>.
 197. Inatsuka C, et al. Gamma delta T cells are activated by polysaccharide K (PSK) and contribute to the anti-tumor effect of PSK. *Cancer Immunol Immunother.* 2013;62:1335–45. <https://doi.org/10.1007/s00262-013-1436-4>.
 198. Lincoff, G. H. *Field Guide to Mushrooms.* 33 edn, 489 (Alfred A. Knopf, 1981).
 199. Juraske C, et al. Anti-CD3 Fab Fragments Enhance Tumor Killing by Human $\gamma\delta$ T Cells Independent of Nck Recruitment to the $\gamma\delta$ T Cell Antigen Receptor. *Front Immunol.* 2018;9:1579. <https://doi.org/10.3389/fimmu.2018.01579>.
 200. Oberg HH, et al. Novel bispecific antibodies increase $\gamma\delta$ T-cell cytotoxicity against pancreatic cancer cells. *Cancer Res.* 2014;74:1349–60. <https://doi.org/10.1158/0008-5472.Can-13-0675>.
 201. Ou L, et al. Preclinical platforms to study therapeutic efficacy of human $\gamma\delta$ T cells. *Clin Transl Med.* 2022;12: e814. <https://doi.org/10.1002/ctm2.814>.
 202. Li C, et al. Novel CD19-specific $\gamma\delta$ TCR-T cells in relapsed or refractory diffuse large B-cell lymphoma. *J Hematol Oncol.* 2023;16:5. <https://doi.org/10.1186/s13045-023-01402-y>.
 203. Xu Y, et al. A novel antibody-TCR (AbTCR) platform combines Fab-based antigen recognition with gamma/delta-TCR signaling to facilitate T-cell cytotoxicity with low cytokine release. *Cell Discov.* 2018;4:62. <https://doi.org/10.1038/s41421-018-0066-6>.
 204. Li, H. K. et al. A Novel Allogeneic Rituximab-Conjugated Gamma Delta T Cell Therapy for the Treatment of Relapsed/Refractory B-Cell Lymphoma. *Cancers (Basel).* 2023;15. <https://doi.org/10.3390/cancers15194844>.
 205. Fowler, D. et al. Payload-delivering engineered $\gamma\delta$ T cells display enhanced cytotoxicity, persistence, and efficacy in preclinical models of osteosarcoma. *Sci Transl Med.* 2024;16:eadg9814. <https://doi.org/10.1126/scitranslmed.adg9814>.
 206. Makkouk, A. et al. Off-the-shelf V δ 1 gamma delta T cells engineered with glypican-3 (GPC-3)-specific chimeric antigen receptor (CAR) and soluble IL-15 display robust antitumor efficacy against hepatocellular carcinoma. *J Immunother Cancer.* 2021;9 <https://doi.org/10.1136/jitc-2021-003441>.

207. Papadakos, S. P. *et al.* $\gamma\delta$ T Cells: A Game Changer in the Future of Hepatocellular Carcinoma Immunotherapy. *Int J Mol Sci.* 2024;25, <https://doi.org/10.3390/ijms25031381>.
208. Neelapu SS, *et al.* A Phase 1 Study of ADI-001: Anti-CD20 CAR-Engineered Allogeneic Gamma Delta 1 ($\gamma\delta$) T Cells in Adults with B-Cell Malignancies. *Blood.* 2022;140:4617–9. <https://doi.org/10.1182/blood-2022-157400>.
209. Nishimoto KP, *et al.* Allogeneic CD20-targeted $\gamma\delta$ T cells exhibit innate and adaptive antitumor activities in preclinical B-cell lymphoma models. *Clin Transl Immunology.* 2022;11: e1373. <https://doi.org/10.1002/cti2.1373>.
210. Lee D, *et al.* Unlocking the potential of allogeneic V δ 2 T cells for ovarian cancer therapy through CD16 biomarker selection and CAR/IL-15 engineering. *Nat Commun.* 2023;14:6942. <https://doi.org/10.1038/s41467-023-42619-2>.
211. Davies, D. M. *et al.* Engineering a Dual Specificity $\gamma\delta$ T-Cell Receptor for Cancer Immunotherapy. *Biology (Basel).* 2024;13, <https://doi.org/10.3390/biology13030196>.
212. Lozzio C, Lozzio B. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood.* 1975;45:321–34. <https://doi.org/10.1182/blood.V45.3.321.321>.
213. Song HW, *et al.* Manufacture of CD22 CART cells following positive versus negative selection results in distinct cytokine secretion profiles and $\gamma\delta$ T cell output. *Mol Ther Methods Clin Dev.* 2024;32: 101171. <https://doi.org/10.1016/j.omtm.2023.101171>.
214. Pharmaceuticals, T. *Takeda to Acquire GammaDelta Therapeutics to Accelerate Development of Allogeneic $\gamma\delta$ T Cell Therapies Addressing Solid Tumors*, <<https://www.takeda.com/newsroom/newsreleases/2021/takeda-to-acquire-gammadelta-therapeutics-to-accelerate-development-of-allogeneic-cell-therapies-addressing-solid-tumors/>> (2021).
215. Pharmaceuticals, T. *Takeda to Acquire Adaptate Biotherapeutics to Develop Novel Gamma Delta ($\Gamma\delta$) T Cell Engager Therapies Targeting Solid Tumors*, <<https://www.takeda.com/newsroom/newsreleases/2022/takeda-to-acquire-adaptate-biotherapeutics/>> (2022).
216. Pharmaceuticals, T. (2023).
217. IN8bio. *First Clinical Program to Have Successfully Genetically Modified $\Gamma\delta$ T Cells*, <<https://in8bio.com/pipeline/>> (2023).
218. Acepodia. *$\Gamma\delta$ T Cells-Acepodia: Powerful, Accessible Cell Therapies for Patients with Cancer*, <<https://www.acepodia.com/pipeline/#ACC>> (2020).
219. BioPharm, T. *Pipeline TC BioPharm*, <tcbiopharm.com/pipeline> (2024).
220. Cytomed. *Pipeline*, <w2.cytomed.sg/pipeline/> (2024).
221. Kiromic BioPharma, I. *Kiromic BioPharma Pipeline to Prioritize a New Gamma Delta T-Cell Product Candidate*, <ir.kiromic.com/news-releases/news-release-details/kiromic-biopharma-pipeline-prioritize-new-gamma-delta-t-cell> (2022).
222. Therapeutics, L. *Pipeline*, <www.lavatherapeutics.com/pipeline-programs/pipeline/> (2024).
223. Bio, A. *Building a Broad Pipeline in Autoimmune Diseases & Cancer*, <www.adicetbio.com/pipeline/> (2024).

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.