Am J Cancer Res 2024;14(4):1482-1500
www.ajcr.us /ISSN:2156-6976/ajcr0155453

Review Article

Controversial role of γδ T cells in colorectal cancer

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Received January 14, 2024; Accepted March 29, 2024; Epub April 15, 2024; Published April 30, 2024

Abstract: Colorectal cancer (CRC) is the third most frequent type of cancer, and the second leading cause of cancer-related deaths worldwide. Current treatments for patients with CRC do not substantially improve the survival and quality of life of patients with advanced CRC, thus necessitating the development of new treatment strategies. The emergence of immunotherapy has revitalized the field, showing great potential in advanced CRC treatment. Owing to the ability of tumor cells to evade the immune system through major histocompatibility complex shedding and heterogeneous and low antigen spreading, only a few patients respond to immunotherapy. γδ T cells have heterogeneous structures and functions, and their key roles in immune regulation, tumor immunosurveillance, and specific primary immune responses have increasingly been recognized. γδ T cells recognize and kill CRC cells efficiently, thus inhibiting tumor progress through various mechanisms. However, γδ T cells can potentially promote tumor development and metastasis. Thus, given this dual role in prognosis, these cells can act as either a "friend" or "foe" of CRC. In this review, we explore the characteristics of γδ T cells and their functions in CRC, highlighting their application in immunotherapy.

Keywords: Colorectal cancer, γδ T cells, tumor microenvironment, immunotherapy, T-cell receptors

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors, with an estimated 1.9 million new CRC cases and 935,000 CRC-related deaths in 2020, the third and second highest figures worldwide, respectively [1]. The 5-year survival rate for patients with stage I CRC is >90%, whereas that for patients with stage IV CRC is only 10% [2]. The main treatments for CRC are endoscopic resection, surgery, radiation therapy, and systemic therapy [3]. However, the efficacy of current treatment strategies for patients with intermediate to advanced CRC remains unsatisfactory. Therefore, more effective treatments are urgently needed for patients with advanced CRC. The emergence of immunotherapy has revitalized the field, showing great potential in advanced CRC treatment.

Unlike the effects of conventional cancer therapies that influence the proliferation, survival, and metabolic activities of tumor cells, the anti-tumor effects of immunotherapy are mainly manifested through the modulation of the tumor microenvironment (TME), restoration of anti-cancer immunity, and stimulation or suppression of the immune system [4]. The major factors affecting immunotherapy encompass the TME, tumor immunogenicity, major histocompatibility complex (MHC) dysfunction, irreversible T-cell failure, and tumor mutations [5]. In addition to malignant cells, the TME of CRC contains endothelial cells, stromal fibroblasts, and host-derived interacting cells, and the presence of immune cells is considered to be a strong predictor of clinical prognosis [6, 7].

Approximately, only 14% of patients with CRC who have mismatch repair defects or high microsatellite instability respond to immunothera-

py [8]. However, current common immune checkpoint inhibitors are nonfunctional in tumors with high mismatch repair capacity, microsatellite stability, or low microsatellite instability [9]. In the TME with low microsatellite instability, there are only a few functional tumor-infiltrating lymphocytes (TILs) and numerous immunosuppressive cells, which include T regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages [10]. Therefore, searching for new targets is imperative, and γδ T cells are attracting attention owing to their potent antitumor activity and unique role in immune surveillance, thereby making them an attractive tool for cancer immunotherapy.

Within the immune cells that comprise the TME, γδ T cells are an innate subpopulation of T lymphocytes that are of interest owing to their ability to mediate natural immunity as the first line of defense [11]. Based on function, γδ T cells are classified into different subpopulations, such as γδ T1, γδ T17, follicular helper γδ T (γδTfh), γδ Treg, and memory γδ T cells [12]. In oncology, γδ T cells constitute a strong positive prognostic indicator in most malignancies [13] and can infiltrate a variety of tumor tissues such as rectal, pancreatic, or breast cancer tissues [14]. While γδ T cells can directly kill tumor cells, several of these cells have been used clinically for treating certain malignancies; however, further research is needed to apply these cells against CRC.

γδ T cells in the intestine

γδ T cells constitute a subpopulation of peripheral blood T lymphocytes. The two polypeptide chains that make up the γδ T-cell receptors (TCRs): the γ and δ chains, both of which are non-covalently linked to CD3 molecules and influence the TME [15]. According to their TCR-δ chains, human γδ T cells can be divided into two major subpopulations. By fighting against invading intestinal pathogens, epithelial stress, and malignant transformation, γδ T cells perform key roles in mucosal tissue homeostasis and immune surveillance. γδ T cells act as a bridge between innate immunity and adaptive immunity, which are the first lines of defense against infection and malignancy [16]. In barrier sites including the intestine, γδ T cells are particularly abundant, with as much as 20% of intraepithelial CD3+ T cells in the human colon expressing γδ TCRs [17]. γδ T cells are primarily found in the intestinal epithelial mucosa and represent the first line of resistance towards pathogens. γδ T cells may play an important role in the pathogenesis of inflammatory bowel disease and colitis-associated cancer [18]. Normal colonic tissues are dominated by Vδ1 T cells, while Vδ2 T cells are less expressed. In patients with chronic inflammatory bowel disease, intestinal Vδ1 T cell frequency is lower, while that of Vδ2 T cells is high and correlates with the duration and severity of the inflammatory response [19]. Vδ1+ cells in colonic epithelial tissue can be powerful inflammatory cells, by producing IL-17 and other related cytokines, and suppressor cells that reduce the ability of the immune system to kill cancer cells [20]. Human γδ-intestinal epithelial lymphocytes (IELs) scan epithelial cells for the expression of MHC I-related genes, which are inducers of toxicity in γδ T cells, and account for approximately 40% of IELs [21]. Moreover, γδ IELs mediate the intestinal-resident bacteria, which can penetrate the intestinal mucosal epithelial cells, to stimulate early protective immunity [22]. Vδ1 T cells are mainly expressed in mucosal and epithelial tissues and account for the majority of human intestinal IELs, which proliferate mainly in peripheral tissues, including solid tumors [23]. Furthermore, human intestinal epithelial Vδ1T cells respond to tumor cells overexpressing MICA/B and ULBP through the synergistic action of TCR and NKG2D [24]. γδ IELs stimulate mucosal healing and recruit macrophages to the area of injury, preventing symbiotic cell infiltration after injury, limiting excessive inflammatory responses, and maintaining homeostasis by removing damaged epithelial cells [25]. A recent study demonstrated that a subpopulation of human intestinal intraepithelial Vδ1 T cells constitutively expressing NKp46 exerted higher anti-tumor activity, and NKp46 expression on intestinal intraepithelial lymphocytes correlated with high cytolytic capacity. In tumor-free tissues of patients with CRC, a lower frequency of NKp46pos/Vδ1 IELs increased the risk of metastasis [23]. γδ T cells possess a range of biological effects, which include killing tumor cells, engaging in immunomodulation, presenting antigens, and assisting in dendritic cell (DC) maturation [26]. As γδ T cells are not MHC-restricted, their activation does not require

antigen-presenting cells (APCs) for antigen processing and presentation; they are an antitumor immune complement to αβ T cells and can be activated rapidly in the early stages of immune response. Thus, these cells as a highly promising immune population to develop novel cancer immunotherapies [27]. Better control of tumor growth by γδ T cells than by conventional αβ T cells at the same quantity suggests that γδ T cells have a better ability to suppress tumor growth and target tumors for killing [28]. During TCR γδ rearrangement, Vδ2 and Vγ9 are nearly completely co-expressed to create Vγ9Vδ2 T cells, which are the predominant γδ T subtype in peripheral blood, accounting for approximately 90% of the total [29]. Brandes et al. [30] demonstrated that Vγ9Vδ2 T cells present antigens 100-fold more efficiently than αβ T lymphocytes or monocytes. These can recognize phosphoantigens and exhibit powerful anti-tumor effects on, among others, cholangiocellular carcinoma, primary glioblastoma, and breast cancer [31].

In an azoxymethane-induced CRC model, mice deficient in γδ T cells were more tumorigenic than mice deficient in αβ T cells, which suggests that γδ T cells can effectively function as primary tumor suppressors [28]. γδ T cells killed cancer cells through the production of chemokines and cytokines or direct engagement with cancer cells via death receptor signaling, and their greater ability to survive in hypoxic environments suggests that they may be favorable target cells for immunotherapy, particularly, against CRC [32]. Flow cytometry and transcriptome analysis have shown that most of the γδ T cells in CRC tissues constitute a higher number of cytotoxic Vδ1 cells [33]. Vδ1+ γδ T cells exhibit high anti-tumor activity against various cancers, such as chronic lymphocytic leukemia, multiple myeloma, breast cancer, and CRC [34]. In a study of γδ T cells in the CRC TME, γδ T cells were detected predominantly in paracancerous tissues and rarely in intratumoral tissues, and there was no significant increase in the number of T-cell subpopulations of Vδ1 and Vδ2 in CRC-infiltrating γδ T cells; however, the predominant subpopulation comprised Vδ1 T cells [35]. Vδ3+ T cells recognize CD1d molecules expressed on target cells with cytotoxic activity and release Th1-, Th2-, and Th17-like pro-inflammatory cytokines [36].

The plasticity or polarization is another crucial characteristic of γδ T cells, and the TME provides abundant cytokines and hypoxic conditions that contribute to the polarization of γδ T cells towards distinct effector subtypes, resulting in antitumor or tumor-promoting functions [37]. The antitumor function of γδ T cells is often inhibited by the TME, which contributes to their transformation into a subpopulation that promotes tumor progression [38]. Owing to their plasticity, γδ T cells could be regarded as “friends” or “foes” of CRC. We discuss this dual role in CRC immunotherapy, shifting the perspective of γδ T cell-based immunity toward their newly discovered immunosuppressive regulatory function.

Tumor cell recognition

γδ T cells recognize a variety of antigens in an MHC-dependent or -independent manner; they can be effective against tumors with low mutational load and MHC downregulation [39]. Unlike conventional αβ T cells, recognition of ligands by most γδ T cells does not require antigen presentation by human leukocyte antigen molecules [40]. The γδ TCR-recognition ligand is typically required to express stimulatory stress molecules on T lymphocytes to prevent self-reactions with negative effects [41]. BTN3A1 and BTN2A1 are immunoglobulin-like molecules with immunomodulatory functions that mediate the interaction of γδ T cells with phosphorus antigen (PAG) or can be directly recognized by the Vγ9Vδ2 TCR [42]. Furthermore, Vδ1TCR can recognize tumor cells by MICA [43]. In addition to TCR, γδ T cells express multiple NK cell receptors, such as NKp30, NKG2D, NKp44, tumor necrosis factor (TNF), and DNAM-1, which recognize tumor cells, although their expression is associated with certain conditions [44]. Masking of DNAM-1 on the surface of γδ T cells substantially inhibits their capacity to kill tumor cells [45]. When γδ T cells come in contact with tumor cells, the Vγ9+ subpopulation proliferates rapidly, and NKG2D expression by γδ T cells is upregulated [46]. Human γδ T cells exhibit direct cytotoxicity against different types of cancer cells, modulate anti-tumor cytokines, and interact with other immune cells to eliminate tumors [47].

Vγ9Vδ2 T cells recognize microbes and lipids conjugated to the non-classical MHC protein CD1d [48]. In the absence of MHC restriction, Vγ9Vδ2 T cells can recognize non-peptide phosphorylation end-products of isoprene biosynthesis with phosphorylated antigens [49]. The isoprene biosynthetic pathway is upregulated in tumor cells to ensure availability of energy and accumulation of PAG, leading to its recognition by Vγ9Vδ2 T cells [50]. Additionally, Vγ9Vδ2 T cells recognize specific ligands overexpressed by tumor cells through the interaction of NKG2D and DNAM-1 with their ligands [51]. These receptors fine-tune the activation threshold of γδ T cells and enhance recognition of tumor targets on γδ T cells, which induce immediate immune responses to tumor targets [52]. Vγ9Vδ2 T cells also recognize ApoA1 ectopically expressed on the tumor surface and Toll-like receptors, which together are involved in and contribute to the antitumor effects of Vγ9Vδ2 T cells [53]. Corvaisier et al. [54] found that colon tumors were frequently infiltrated by Vγ9Vδ2 T lymphocytes. Expansion of γδ T cells may be directly driven by intestinal bacterial products through an array of toll-like receptors that recognize pathogen-associated molecular patterns [55].

γδ T cells that infiltrate human CRC recognize stress-associated antigens expressed in transformed cells and react by immediate release of cytotoxic cytokines [56]. Moreover, in addition to the expressed tumor-associated antigens, γδ T cells are recognized by various intrinsic cytotoxic receptors expressed on the cell membrane [57].

Antitumor functions of γδ T cells

Activated γδ T cells display potent cytotoxic activity by releasing granzyme B and perforin, amplifying the immune response through membrane-bound TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligands, or producing TNF and interferon (IFN)-γ, thereby inhibiting tumor progression [58]. Activated NKp30, NKp44, and NKp46 were also expressed on human Vδ1+ T cells followed by cytokine activation and co-stimulation to enhance IFN-γ production [59]. γδ T cells exposed to IL-2/IL-12/IL-18 produce IFN-γ and express cytotoxins and FasL, resulting in potent anti-tumor activity [60]. Release of cytokines, such as TNF and IFN-γ, by γδ T cells activates T cells in the tumor site by acting as APCs [61]. These cytokines inhibit tumor growth through multiple mechanisms, such as downregulating the exposure of cyclin and cyclin-dependent protein kinase, thereby affecting the tumor cell cycle and inhibiting tumor cell proliferation and tumor angiogenesis, ultimately inhibiting the proliferation of tumor cells [62, 63].

Not limited to cytotoxicity, the activation of NKp30 on Vδ1+ T cells induces the production of CC chemokine ligands CCL3, CCL4, and CCL5, linking target recognition to the attraction of antigen-presenting cells, such as monocytes and conventional αβ T cells [64].

γδ T cells can also kill αβ-coated cancer cells via the cell surface CD16-like αβ-dependent cytotoxicity of NK cells [65]. Additionally, the accumulation of isopentenyl pyrophosphate (IPP) and dimethylallylpyrophosphate in cancer cells leads to aberrant expression of MICA/B and UL16-binding proteins recognizing the tumor cells, and this activates γδ T cells and enhances the antitumor efficacy [66]. MICA/B and ULBP are highly expressed in mismatch-repair-deficient CRC cell lines and tumor organs, and removal of these ligands reduced γδ T cell activation and cytotoxicity. This suggests a role for the activation receptor, NKG2D, in γδ T cell immunity to mismatch-repair-deficient tumors [67]. During tumorigenesis, Vγ9Vδ2 T cells in circulation are inactivated by excessive production of IPP in cancer cells, leading to the expression of inflammatory homing chemokine receptors and mediating infiltration toward tumor tissues and organs [68]. Vγ9Vδ2 cell activation with phosphoantigens is currently the most common approach in γδ T cell-based antitumor research and is a key approach in the development of antitumor immunotherapy drugs [69]. Zoledronate (ZOL) enhances IPP-induced γδ T cell activation by inhibiting the activity of the IPP-metabolizing enzyme farnesyl diphosphate synthase that increases IPP levels [70]. *In vitro*, Vγ9Vδ2 T also overregulates both MHC and costimulatory molecules in response to IPP stimulation. Such an APC-like phenotype permits Vγ9Vδ2 T cells to initiate CD4+ T cells, which are polarized in a Th1 antitumor direction [71]. CD3 monoclonal antibody-stimulated tumor-derived γδ T cells produce large amounts of IFN-γ and show strong cytotoxic activity against autologous and allogeneic gastrointestinal tumor cells [72]. In contrast,

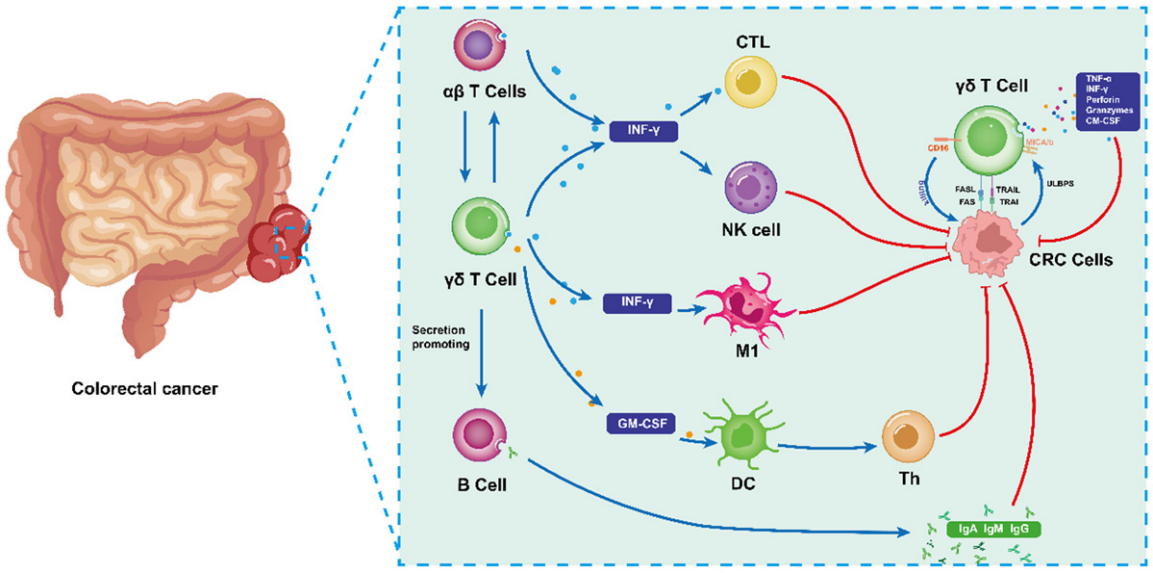

Figure 1. γδ T cells function against CRC cells.

the use of nitrogen-containing bisphosphonates increase IPP incubation in CRC stem cells and cancer cells sensitized to Vγ9Vδ2-induced cytotoxicity [73]. γδ T cells further facilitate the phagocytosis of tumor antigens through perforin and granzyme production and CD36 expression. These mechanisms directly induce toxicity in CRC cells, which subsequently lead to the induction of tumor antigen-specific CD8+ T cell responses [74]. Phosphoantigen expression enhances the toxicity of γδ T cells against tumor cells, which is partially mediated by the increased expression of hydroxymethylglutaryl CoA reductase [75]. γδ T cells recognize tumor cells and release perforin and granzyme B into the synaptic gap, which further activates caspases and destroys the DNA of tumor cells, leading to tumor cell mortality [76]. Antibody-dependent cytotoxicity is also an important mechanism of death induction that allows FcγRIIIA (CD16)-expressing Vδ2 T cells to kill colon cancer cells through antibody-dependent cytotoxicity [77]. Meraviglia et al. [78] showed that most γδ T cells in colorectal tumor and adjacent normal tissues comprised the Vδ1 T cell subtype and exhibited an effector phenotype. The production of IFN-γ is significantly regulated by Vδ1 T cells in tumor tissues of patients with CRC when compared with normal colonic tissues adjacent to the cancer, possibly due to the presence of certain identified inhibitory molecules in colonic tumor stem cells. The NKp46+ Vδ1 IEL subpopulation is rare in the intestinal tissue surrounding tumors in patients with CRC and the low frequency of NKp46+ Vδ1 IELs is associated with rapid progression of tumors to metastatic disease [23].

γδ T cells interact with B helper cells to produce immunoglobulin (Ig)A, IgM, and IgG antibodies while interacting with other immune cells to regulate the immune response [79]. Additionally, granulocyte-macrophage colony-stimulating factor (GM-CSF) production is associated with γδ T cells that maintain potent tumor-killing activity and produce immunomodulatory cytokines [80]. Upon binding to NKG2D, γδ T lymphocyte signals are activated, rapidly exerting their effector functions to destroy bacteria or infected cells by proliferating and producing pro-inflammatory and antibacterial cytokines, and releasing lytic enzymes in response to injury signals [81, 82]. At the level of PAG synthesis, valproic acid synergizes with ZOL to enhance γδ T cytotoxicity. It further affects the association between γδ T cells and tumor cells at the site of the NKG2D receptor/ligand axis [83].

γδ T cell-mediated TRAIL and Fas ligands form another death receptor signaling pathway. The combination of death effector domains with Fas-associated death domain-containing proteins leads to the activation of caspases and induction of target cell apoptosis [58] (**Figure 1**). *In vitro*-expanded Vδ1 T cells expressing Fas

ligands are highly toxic to colon cancer cells [84]. In a subpopulation of Vδ1 γδ T cells, NKp30 interacts with B7-H6 expressed on tumor cells for precise antitumor activity [85]. Additionally, IL-22 produced by γδ T cells acts as a regulator of the DNA damage response of enterocyte stem cells against environmental genotoxic factors, preventing malignant transformation and cancer development [86]. In a recent study, the expression of NKp46 on γδ IELs was associated with high antitumor activity against CRC [23]. Li et al. [87] found that the NKp46+ Vδ1 T cell subpopulation was able to restrict the metastatic spread of primary CRC to the liver and confirmed similar results in mice. Meraviglia et al. [78] showed that patients with CRC containing large numbers of γδ T cells had a remarkably higher 5-year disease-free survival rate, and γδ T cells were effective in controlling early-stage tumor growth. Vδ1 and Vδ2 TILs produced low levels of IFN-δ in colorectal tissues when compared with those in neighboring tissues; however, IL-17 was barely secreted [78]. In particular, an analysis of 585 patients with CRC showed that a high number of γδ T cells in the TIL and a reduced production of IFN-γ were associated with the likelihood of 5-year disease-free survival [78]. The unique mechanism underlying γδ T cell recognition of CRC cells, tumor invasion, and potent cytotoxicity suggests that γδ T cells are promising candidates for antitumor therapy.

Protumor activities of γδ T cells

Tumor-infiltrating γδ T cells promote tumor progression and metastasis by recruiting suppressor cells and inducing the apoptosis of antitumor immune cells [20]. IL-17 produced by γδ T cells promotes tumorigenesis and development through multiple downstream effects on tumor, endothelial, and other immune cells [88]. In a preclinical mouse model of CRC, Reis et al. [89] demonstrated that cytotoxic IFN-γ-producing Vγ7- and Vγ1-positive cells are essential for tumor surveillance and that IL-17-producing Vγ6+ T cells promote tumor proliferation.

In colon cancer, IL-17 is mainly produced by γδ T cells, in particular, 83% by Vδ1 T cells [90]. Inflammatory DCs in the tissues of patients with CRC produce IL-23 when irritated by bacterial products leaking from the colonic epithelial barrier, which drives the Vδ1+ γδ Th17 response and results in IL-17 production [91]. Even tumor-free intestinal tissue from patients with CRC contains IL-17-producing γδ T cells [20]. IL-17 can directly stimulate CRC cells and increase their migratory behavior via the PI3K/AKT signaling pathway [92, 93], which can directly stimulate the proliferation of intestinal epithelial cells by activating the MAPK signal transduction pathway and promote early CRC [94].

In CRC, due to the disruption of the tumor epithelial barrier and inflammatory DCs in the TME, the presence of microorganisms polarizes γδ T cells to produce cytokines, such as TNF-a, granulocyte colony-stimulating factor (GM-CSF), IL-17, and IL-8. These cytokines recruit MDSC into the TME, which regulates tumor cell development and induces Treg differentiation, underlining their functional plasticity shaped by the TME [20, 89].

Particularly, in human CRC, because IL-17-producing γδ T cells can act as pro-carcinogens, they are linked to worse survival and increased metastasis risk; more advanced the disease, the larger the tumor and the higher the risk of metastasis [95]. Additional evidence indicates that the toll-like receptor pathway plays an essential role upstream of IL-17-producing γδ T cells in inducing IL-1β and IL-23 production by cancer-related myeloid cells, as this process is initiated by colonic bacteria during carcinogen-induced CRC development [96]. Moreover, IL-17 induces M2 macrophage polarization [97].

γδ T cells can contribute to tumor growth through tumor cell-induced programmed death-1 (PD-1)/PD-L1 interactions and the inhibition of neutrophil and αβ Treg production. The TME regulation induced by hypoxia induces tumor cells to shed MICA/B to block the NKG2D-mediated cytotoxicity of γδ T cells [98]. Regulatory Vδ1+ T cells recruited by CXCL10 inhibit the activation of αβ T cells and DCs and trigger immune senescence, increasing the probability of tumor recurrence and metastasis [99]. Chen et al. [100] discovered a γδ T subpopulation showing high PD-1 expression that was considerably increased in CRC tissues. PD-1+ γδ T cells infiltrating these tumors expressed tissue-resident, activation, and depletion markers and maintained some level of granzyme B (GzmB)- and perforin-secretion capacity.

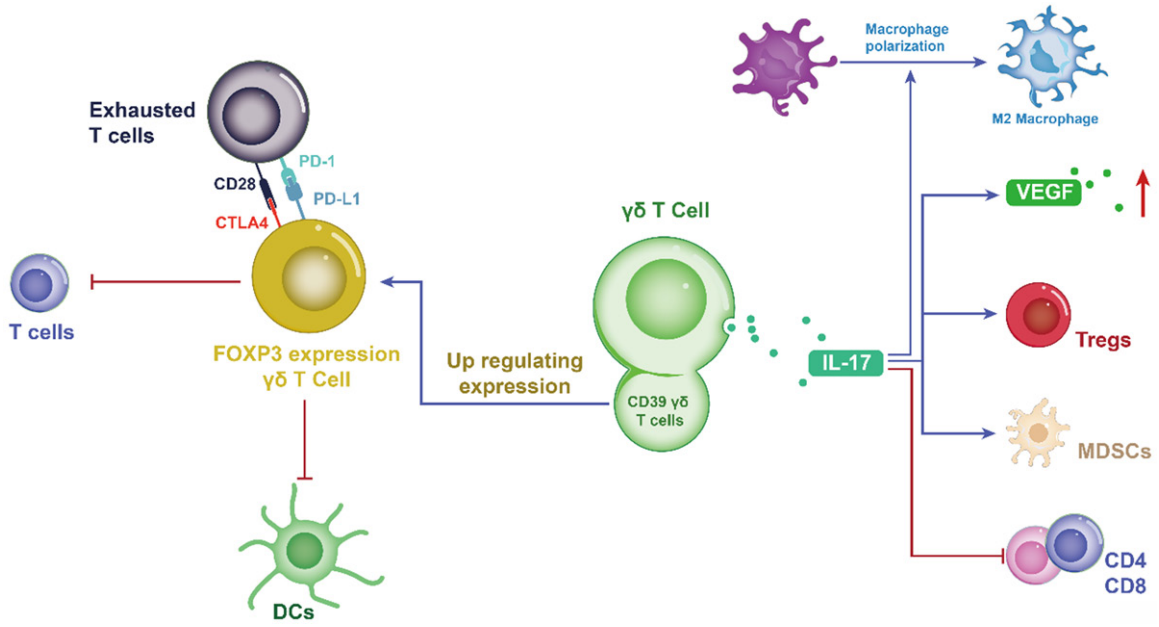

Figure 2. γδ T cells promote CRC cells.

γδ Treg lymphocytes induce cell cycle arrest in cytotoxic T lymphocytes and inhibit proliferation by inducing T cell senescence [101]. The polarized γδ T17 or γδ Treg cells generate high amounts of IL-17 and transforming growth factor-β (TGF-β), which trigger an inhibitory immune response and promote CRC advancement [102]. Wakita et al. [103] reported that γδ T17 cells induced angiogenesis and promoted tumor growth in CRC through the secretion of vascular endothelial growth factor. γδ T17 invasion is positively correlated with clinicopathological features, such as CRC stage, and tumor-induced disruption of the epithelial barrier may lead to invasion by commensal and bacterial products. Thus, dendritic cells activated by microbial products produce γδ T17 via *de novo* polarization [104]. Strong signaling on the CD27TCR along with γδ co-stimulation and lymphotoxin signaling directs progenitor cells into interferon γ-producing γδ T-cells, downregulating CD24 and CD25 [105].

Meanwhile, IL-10 and TGF-β, secreted by regulatory T cells or myeloid cells, induce the transformation of epithelial cells to mesenchymal cells, thereby increasing cancer aggressiveness [106]. Tumor-derived TGF-β induces differentiation of immunosuppressive CD39+ γδ Treg cells in CRC [107]. Rong et al. [14] described the disproportionate distribution of Vδ1+ and Vδ2+ T cells in rectal cancer tissues, with a higher proportion of Vδ1+ T cells and a lower proportion of Vδ2+ T cells in tumor tissues than adjacent normal tissues. Tumor-infiltrating Vδ1+ T cells had a strong suppressive function and positively correlated with the patient's tumor stage.

Although intestinal inflammatory responses persist, the Vδ1+ T cells underlie angiogenesis, the activation of survival signaling pathways, and the recruitment of MDSC, resulting in a chronic precancerous inflammatory environment [108]. A study of patients with CRC showed that tumor-infiltrating γδ T cell-produced IL-17A polarizes inflammatory macrophages, induces MDSC recruitment, and recruits neutrophils to the tumor location, thus promoting cancer metastasis [109]. CRC cell secretions also inhibit the growth of γδ T cells, which produce IFN-γ, and inhibit the production of CD4+ and CD8+ T cells [78]. Additionally, pro-inflammatory Vδ2 T cells may be involved in the pathogenesis of CRC through chronic inflammation [108] (**Figure 2**).

CD39+ inhibitory γδ T cells from human CRC increase GM-CSF and IL-17 levels in tumor tissues [107]. In CRC, CD39+ γδ T cells inhibit the proliferation of peripheral blood mononuclear cell (PBMC)-derived T cells by expressing FOXP3

while additionally inhibiting the immune response and recruiting monocyte-derived DCs through the adenosine pathway [107], suggesting that they are potential prognostic factors. The right CRC had significantly higher proportions of CD39+ γδ Tregs, and showed increased expression of IL-17A and Tim-3 and decreased expression of IFN-γ; whereas, the opposite was observed for the left CRC [110].

Treg-like Vδ2 T cells expressing FOXP3 alter the structure of the TME and inhibit the effector T cell activities by inducing the interaction of CD86/cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and PD-L1/PD-1 [111]. Due to the multifaceted and immunosuppressive nature of TME, the balance between pro- and anti-tumor effects of TILs often skews toward a tumor-supporting environment. Specifically, γδ T cells are often exhausted or impaired [112]. Activated Vδ1+ and Vδ2+ γδ T cells upregulate various C-C chemokine receptors (CCRs), such as CCR1 and CCR8, CCR2, CCR5, and CX-C chemokine receptor 3 (CXCR3), to mediate infiltration into the TME [113]. Notably, unlike Vδ2 T cells, Vδ1 T cells express CCR2 and exhibit a migratory response to CCL2. The expression of this chemokine is significantly altered in several human tumors, such as lung, prostate, liver, and breast cancer [114]. Due to the complex interactions between TME and tumor-infiltrating γδ T cells, activated γδ T cells can be functionally polarized to become anti-tumor Th1 and follicular Th (Tfh) cells or pro-tumor Th17 and T regulatory (Treg) cells [115].

Expansion of γδ T cells

γδ T cells can recognize malignant cells, infiltrate tumors, and exert potent cytotoxicity, thereby making them ideal candidates for CRC treatment. However, owing to the low numbers of γδ T cells in human peripheral blood, using them in cancer immunotherapy requires substantial *in vitro* expansion. Vδ2T cells represent the major mutant cells in the circulation, whereas Vδ1T cells are enriched in tissues. These cells exhibit potent anti-tumor cytotoxicity and are easily amplified from peripheral blood and ascites [116]. Moreover, Vδ1 T cells separated from the blood of patients with CRC and amplified *ex vivo* showed stronger cytotoxic activity than Vδ2 T cells [84]. Santos et al. [117] demonstrated that the amplified Vδ1 T cells effectively inhibited tumor proliferation and prevented tumor spread in xenograft models, thereby offering a preclinical rationale for their use in tumor-relay immunotherapy. In addition to being isolated from tissues and amplified *in vitro*, Vδ1 cells can also be separated from peripheral blood and amplified using the Vδ1+ T cell generation regimen that includes a 3-week clinical-level regimen of TCR and cytokine stimulation [118]. Human peripheral blood Vδ1 γδ T cells amplified *in vitro* can kill more CRC cells through the cytolytic pathway and receptor-ligand interactions [119]. Ang et al. [120] expanded Vγ9Vδ2 T cells with Zometa/IL-2 and implanted extracellular domain of NKG2D (NKG2DL)-based CARs in the cells using mRNA electroporation. The authors conducted a preclinical study in mice injected intraperitoneally with colon tumors and demonstrated that the NKG2DL CAR-γδ T cells led to tumor regression and prolonged the survival of mice.

Currently, there are two strategies for expanding γδ T cells. The first approach is to separate γδ T lymphocytes from PBMCs and stimulate them *in vitro* with synthetic PAGs or BPs. The second approach stimulates and amplifies γδ T cells *in vivo* through the systemic administration of PAG or nitrogen-containing bisphosphonates [83]. The clinical applications of γδ T cells started with the attempt to directly amplify *in vivo* autologous Vδ2 T cells and were based on their ability to penetrate solid tumors and readily expand [121]. Most early studies attempted to activate Vδ2T cells by direct application of nitrogen-containing bisphosphonates or synthetic PAG and low doses of IL-2. Several clinical studies have used this approach in solid tumors and hematological malignancies [122]. The addition of IL-15 to IL-2/ZOL also promotes γδ T cell expansion from PBMCs [123]. Xiao et al. [124] developed a method to increase γδ T cell proportion by more than 500,000-fold in just 17 days using K562 cells as artificial APCs, anti-CD3, ZOL, and IL-2. Bennouna et al. [125] treated patients with solid tumors via subcutaneous injection of BrHPP and low-dose IL-2. The outcomes showed that 1,500 mg BrHPP effectively increased the number of human Vδ2+ T cells in the peripheral blood of patients. Moreover, ZOL remarkably altered the phenotype of Vδ2T cells, transforming them into effector memory cells [126]. BPs stimulate the proliferation of Vδ2+ T cells *in vitro* and sensitize tumor

cells *in vivo*, thus exerting antitumor effects [127]. Vγ9Vδ2 T cells also recognize colon cancer stem cells that are sensitized by ZOL through TCR-PAG interactions and enhance the lethality of γδ T cells against these target cells using TRAIL and granzymes [128].

Furthermore, Zocchi et al. [129] found that zoledronate-stimulated local TME of CRC expresses BTN3A1/CD277, which stimulates and expands effector Vγ9Vδ2 T cells with antitumor activity. Therefore, systemic or topical ZOL may be envisaged to improve the therapeutic efficacy of metastatic Vδ2 T cells.

Following zoledronic acid stimulation *in vitro*, Vγ9Vδ2 T cells transmit co-stimulatory signals to DCs, thereby increasing the number of antigen-specific CD8+ αβ T cells while inhibiting the amplification of IL-2-dependent Tregs [130]. Additionally, Vγ9Vδ2 T cells reportedly expanded and survived well in patients with CRC in the absence of co-cytokines, with a promoting effect on the secretion of either endogenous IL-2 or IL-15 [131]. Cytomegalovirus-induced Vδ1 T cells inhibit not only the growth of primary colon cancer, but also the appearance of metastases [132]. Wu et al. [84] found that freshly isolated human peripheral blood Vδ1 T cells, especially *ex vivo* and *ex vivo*-expanded Vδ1 T cells, showed better cytotoxicity against adherent and spherical colon tumor cells when compared to Vδ2 T cells.

Preclinical research and clinical efficacy

The ability of γδ T cells to directly recognize and kill tumor cells makes the adoption and *in vivo* induction of γδ T cell expansion therapy a promising direction to explore in anti-cancer immunotherapy. Similar protective effects of γδ T cells have been validated in models of bladder cancer [133], malignant melanoma [134], B-cell lymphoma [135], and prostate cancer [136]. These important contributions to tissue homeostasis and cancer immunosurveillance have stimulated scientific interest in further exploring the biology of γδ T cells and their clinical translational potential. Generally, γδ T cells correlate positively with a better outcome in CRC [137]. Immunotherapy based on γδ T cells has demonstrated promising antitumor effects *in vitro* in transplantation tumor models and in phase I clinical trials [138]. Safety and tolerability of γδ T cell-based immunotherapies clinically approved for the treatment of non-small cell lung cancer and breast cancer have been reported [139]. Vδ2 T cells are highly appealing candidates for over-the-counter cellular immunotherapy, and the innate-like feature of MHC-independent activation readily permits the transfer of Vδ2 T cells between individuals in a syngeneic manner without incurring the risk of graft-versus-host disease [140]. Clinical studies have utilized aminobisphosphonates for inhibiting farnesyl pyrophosphate synthase activity in the mevalonate pathway, thereby promoting IPP buildup in cells, or synthetic phosphoantigen analogs for activating Vγ9Vδ2+ T cells in patients with malignancies [113]. Repeated infusions of allogeneic Vγ9Vδ2 T cells are safe and show favorable results, especially in treating liver and lung cancers [141]. Such therapies have been proven to be capable of killing cancer stem cells in colon cancer [141]. Moreover, tumor-infiltrating PD-1+ cells preferentially expressed IL-17, whereas PD-1- preferentially secreted IFN-γ. There was no overlap between IL-17 and IFN-γ-producing γδ cells [89].

Ang et al. [120] amplified Vγ9Vδ2 T cells with ZOL/IL2, and evaluated the *in vivo* tumor-killing effect of Vγ9Vδ2 T cells modified with *NKG2D* RNA chimeric antigen receptor (CAR) in a mouse model of CRC through multiple intraperitoneal injections of an invasive human CRC cell line. The modified Vγ9Vδ2 T cells substantially retarded tumor advancement, leading to a median survival time of 57 days in mice. Devaud et al. [132] reported that in a CRC model, treatment with human Vδ1 γδ T cells not only controlled the progression of the primary tumor, but also inhibited the migration of tumor cells. In a previous study, tumor-infiltrated Vδ1 γδ T cells from patients with CRC can lyse not only autologous tumor cells but also tumor cells, demonstrating the important role of Vδ1 γδ T cells in tumor monitoring [142]. Toussaint et al. [143] collected peripheral blood samples from 11 patients (6 CRC, 4 hepatocellular carcinomas (HCCs), 1 sarcoma) and 16 healthy donors for experiments. Treatment of tumor cells with the mevalonate pathway inhibitor ZOL enhanced the killing of HCC and CRC. The expansion index of Vγ9Vδ2 T cells was at similar levels in healthy donors or patients with cancer, and the total expansion was suitable for adoptive immunotherapy. The number of tumor

cells in ascites was significantly reduced and the level of IFN-γ increased after the transfer of activated Vγ9Vδ2 T cells. No major negative reactions were observed during the treatment period, and the proportion of ascites was markedly reduced [144]. ZOL can be used for the selective expansion of Vγ9Vδ2 T cells in blood specimens and for sensitizing tumor cells to kill Vγ9Vδ2 T cells. Thus, the addition of ZOL and IL-2 to PBMC cultures resulted in the selective expansion of Vγ9Vδ2 T cells, which in turn were highly efficient cancer cell killers after ZOL sensitization of cancer cells [145]. Izumi et al. [131] conducted a clinical trial using Vγ9Vδ2 T cell transfer therapy in six patients with CRC undergoing resection of lung metastases. Expansion with zoledronate and IL-2 increased the number of Vγ9Vδ2 T cells during treatment and remained stable long after the last infusion, expressing higher INF-γ and CD107a.

Nicol et al. [146] reported the results of a clinical study of autologous γδ T-cell immunotherapy for various metastatic solid tumors, including CRC. A total of 18 patients were recruited for the study. Among the 14 evaluable patients, 3 had stable disease and 11 had progressive disease. A phase I trial explored the treatment of 10 patients with metastatic CRC with adenosine polyphosphate-expanded γ9δ2 T cells. The most common adverse events included cold, runny nose, hypotension, and tachycardia. One patient developed disseminated intravascular coagulation, which lasted 25.7 weeks [147]. Immunotherapy with γδ T cells is a clinically safe method, and adverse reactions of severity greater than grade 2 are generally not associated with γδ T-cell therapy and can be managed properly [148].

Optimization of γδ T-cell immunotherapy regimens

Several novel tactics have been developed to increase the antitumor potency and reduce the adverse effects of γδ T cell-based immunotherapies. Additionally, aberrant effects of the combination of radiation and photodynamic or thermotherapy regimens may increase the expression of γδ T-cell ligands, leading to substantial clinical efficacy [83]. Previous studies have confirmed that CAR-γδ T cells targeting CD19 considerably increase the cytotoxicity of CD19+ tumor cells, suggesting that CAR-γδ T cells hold great promise in tumor immunotherapy [149].

The T cell activities of γδ are also modulated by immune checkpoint receptors, including PD-1, CTLA-4, lymphocyte activation gene 3, T cell immunoglobulin structural domain, and mucin structural domain 3 [113]. Integrating approaches aimed at pre-tumor γδ T cells with immune checkpoint blockade therapies considerably increase the response rate and long-term survival of patients with cancer [150]. Li et al. [151] found that there was a remarkable increase in the ratio of Tim-3+ γδ T cells in the peripheral blood and colon cancer tissues of the patients, and the stimulation of the Tim-3 signaling pathway remarkably suppressed the killing efficiency of Vγ9Vδ2 T cells against colon cancer cells. Additionally, activation of the Tim-3 signaling pathway decreased perforin and GZMB expression in Vγ9Vδ2 T cells and reduced the toxicity of Vγ9Vδ2 T cells against colon cancer cells, suggesting Tim-3 as a promising therapeutic target for improving the efficacy of the adoptive immunotherapy of Vγ9Vδ2 T cells for colon cancer. It was recently reported that PD-1+ γδ T cells aggregate in CRC tumor tissues with defective mismatch repair genes, and the PD-1+ γδ T cells were stimulated by PMA to produce IFN-γ, GZMB, and perforin, indicating the potential cytotoxic activity of completely activated PD-1+ γδ T cells in the antitumor immune response [100]. A research trial using the CTLA-4-targeting monoclonal antibody ipilimumab showed a significant correlation between the proportion of Vδ2 T cells and survival outcomes in patients who received the treatment [152].

To ameliorate the low persistence of Vγ9Vδ2 T cells in patients with advanced cancer, one strategy under clinical development is to transduce selected high-affinity Vγ9Vδ2 TCRs into αβ T cells, and this strategy is expected to produce durable memory responses [153]. In a colon cancer model, a nanoantibody was constructed to induce Vγ9Vδ2 T cell activation and tumor cell killing by targeting Vγ9Vδ2 T cells and epidermal growth factor receptors *in vitro* and *in vivo* [154]. Bispecific antibodies have been employed to promote γδ T cell activity and enhance tumor targeting. Transfer to Vγ9Vδ2 T cells with HER2/Vγ9 bispecific antibodies con-

siderably reduced colon cancer growth in a mouse xenograft model [155].

Simultaneous chemotherapy and radiotherapy, conventional treatments, enhance the toxicity of γδ T lymphocytes against malignant cells [156, 157]. Low doses of 5-fluorouracil, doxorubicin, and cisplatin sensitize colon cancer cells to Vγ9Vδ2 T cell killing [158]. NKG2D-targeted antibody inhibits Vγ9Vδ2 γδ T cell lysis in chemosensitive colon cancer-induced cells, whereas chemotherapeutic agents enhance TRAIL expression in colon cancer-induced cells [159]. Topical low-dose γ irradiation results in the normalization of abnormal blood vessels and effective recruitment of tumor-specific T cells, which could also be utilized to potentiate the translation of γδ T cells to the tumor [160]. While vitamin C is known to reduce the rate of apoptosis of γδ T cells during stimulation, the proliferation of γδ T cells is promoted by L ascorbic acid 2-phosphate. Thus, it has been used to increase the effectiveness of γδ T cells in antitumor immunotherapy [161].

Conclusions

As a common cancer and a serious human health problem, the incidence of CRC is progressively increasing among younger individuals; however, very few patients with CRC have benefited from conventional immunotherapy. In recent years, the potential of γδ T cells in anticancer immunotherapy has attracted considerable attention. An increasing number of studies have demonstrated that γδ T cells have antitumor activity against a variety of malignant tumors using their innate and adaptive immunity, and this has shown for the development of γδ T cell therapy for CRC. γδ T cells have received attention for their antitumor potential and MHC-independent recognition of antigens. γδ T cells help to regulate the balance between immune and immunosuppressive responses. γδ T cells may alternatively support tumorigenesis. Therefore, strategies must be developed to evade the suppressive activity of γδ T cells while augmenting their antitumor effects. Despite their promising applications, research on γδ T cells remains in its infancy, with unresolved challenges and obstacles. The main challenge in this area is the small number of γδ T cells and the absence of homology among animal models and humans, rendering the study of the biological features and functions of these cells challenging. Further evaluation of the clinical efficacy of γδ T-cell transfer therapy in prospective clinical trials is warranted, and the combination of this novel therapy with existing therapies is promising to enhance the survival of patients with CRC.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 82202989); the China Postdoctoral Science Foundation (No. 2022M722279, China); the Sichuan Science and Technology Program (No. 2023YFS0163, China); Postdoctoral Research Project of West China Hospital, Sichuan University, Chengdu, China (No. 2021HXBH045); Fundamental Research Funds for the Central Universities (No. 2022SCU1206); and Sichuan University Postdoctoral Interdisciplinary Innovation Fund (awarded to LZ, China); Science and Technology Foundation of Guizhou Provincial Health Commission (No: Gzwkj2023-373).

Disclosure of conflict of interest

None.

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References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[2] Van der Jeught K, Xu HC, Li YJ, Lu XB and Ji G. Drug resistance and new therapies in colorectal cancer. World J Gastroenterol 2018; 24: 3834-3848.

[3] Johdi NA and Sukor NF. Colorectal cancer immunotherapy: options and strategies. Front Immunol 2020; 11: 1624.

[4] Chen DS and Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017; 541: 321-330.

[5] Pei L, Liu Y, Liu L, Gao S, Gao X, Feng Y, Sun Z, Zhang Y and Wang C. Roles of cancer-associated fibroblasts (CAFs) in anti- PD-1/PD-L1 immunotherapy for solid cancers. Mol Cancer 2023; 22: 29.
[6] Giraldo NA, Sanchez-Salas R, Peske JD, Vano Y, Becht E, Petitprez F, Validire P, Ingels A, Cathelineau X, Fridman WH and Sautès-Fridman C. The clinical role of the TME in solid cancer. Br J Cancer 2019; 120: 45-53.
[7] Buhrmann C, Shayan P, Brockmueller A and Shakibaei M. Resveratrol suppresses crosstalk between colorectal cancer cells and stromal cells in multicellular tumor microenvironment: a bridge between in vitro and in vivo tumor microenvironment study. Molecules 2020; 25: 4292.
[8] Sawayama H, Miyamoto Y, Ogawa K, Yoshida N and Baba H. Investigation of colorectal cancer in accordance with consensus molecular subtype classification. Ann Gastroenterol Surg 2020; 4: 528-539.
[9] Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH and Diaz LA Jr. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nat Rev Gastroenterol Hepatol 2019; 16: 361-375.
[10] Fathi M, Pustokhina I, Kuznetsov SV, Khayrullin M, Hojjat-Farsangi M, Karpisheh V, Jalili A and Jadidi-Niaragh F. T-cell immunoglobulin and ITIM domain, as a potential immune checkpoint target for immunotherapy of colorectal cancer. IUBMB Life 2021; 73: 726-738.
[11] Lo Presti E, Dieli F, Fourniè JJ and Meraviglia S. Deciphering human γδ T cell response in cancer: lessons from tumor-infiltrating γδ T cells. Immunol Rev 2020; 298: 153-164.
[12] Chen H and He W. Human regulatory γδT cells and their functional plasticity in the tumor microenvironment. Cell Mol Immunol 2018; 15: 411-413.
[13] Le Floch AC, Rouvière MS, Salem N, Ben Amara A, Orlanducci F, Vey N, Gorvel L, Chretien AS and Olive D. Prognostic immune effector signature in adult acute lymphoblastic leukemia patients is dominated by γδ T cells. Cells 2023; 12: 1693.
[14] Rong L, Li K, Li R, Liu HM, Sun R and Liu XY. Analysis of tumor-infiltrating gamma delta T cells in rectal cancer. World J Gastroenterol 2016; 22: 3573-3580.
[15] Ma R, Yuan D, Guo Y, Yan R and Li K. Immune effects of γδ T cells in colorectal cancer: a review. Front Immunol 2020; 11: 1600.
[16] Mirzaei HR, Mirzaei H, Lee SY, Hadjati J and Till BG. Prospects for chimeric antigen receptor (CAR) γδ T cells: a potential game changer for adoptive T cell cancer immunotherapy. Cancer Lett 2016; 380: 413-423.
[17] Guerra-Maupome M, Slate JR and McGill JL. Gamma delta T cell function in ruminants. Vet Clin North Am Food Anim Pract 2019; 35: 453-469.
[18] Park SG, Mathur R, Long M, Hosh N, Hao L, Hayden MS and Ghosh S. T regulatory cells maintain intestinal homeostasis by suppressing γδ T cells. Immunity 2010; 33: 791-803.
[19] Lo Presti E, Di Mitri R, Mocciaro F, Di Stefano AB, Scibetta N, Unti E, Cicero G, Pecoraro G, Conte E, Dieli F and Meraviglia S. Characterization of γδ T cells in intestinal mucosa from patients with early-onset or long-standing inflammatory bowel disease and their correlation with clinical status. J Crohns Colitis 2019; 13: 873-883.
[20] Wu P, Wu D, Ni C, Ye J, Chen W, Hu G, Wang Z, Wang C, Zhang Z, Xia W, Chen Z, Wang K, Zhang T, Xu J, Han Y, Zhang T, Wu X, Wang J, Gong W, Zheng S, Qiu F, Yan J and Huang J. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. Immunity 2014; 40: 785-800.
[21] Bilate AM, London M, Castro TBR, Mesin L, Bortolatto J, Kongthong S, Harnagel A, Victora GD and Mucida D. T cell receptor is required for differentiation, but not maintenance, of intestinal CD4(+) intraepithelial lymphocytes. Immunity 2020; 53: 1001-1014, e1020.
[22] Matsuzawa-Ishimoto Y, Yao X, Koide A, Ueberheide BM, Axelrad JE, Reis BS, Parsa R, Neil JA, Devlin JC, Rudensky E, Dewan MZ, Cammer M, Blumberg RS, Ding Y, Ruggles KV, Mucida D, Koide S and Cadwell K. The γδ IEL effector API5 masks genetic susceptibility to Paneth cell death. Nature 2022; 610: 547-554.
[23] Mikulak J, Oriolo F, Bruni E, Roberto A, Colombo FS, Villa A, Bosticardo M, Bortolomai I, Lo Presti E, Meraviglia S, Dieli F, Vetrano S, Danese S, Della Bella S, Carvello MM, Sacchi M, Cugini G, Colombo G, Klinger M, Spaggiari P, Roncalli M, Prinz I, Ravens S, di Lorenzo B, Marcenaro E, Silva-Santos B, Spinelli A and Mavilio D. NKp46-expressing human gut-resident intraepithelial Vδ1 T cell subpopulation exhibits high antitumor activity against colorectal cancer. JCI Insight 2019; 4: e125884.
[24] Sullivan ZA, Khoury-Hanold W, Lim J, Smillie C, Biton M, Reis BS, Zwick RK, Pope SD, Israni-Winger K, Parsa R, Philip NH, Rashed S, Palm N, Wang A, Mucida D, Regev A and Medzhitov R. γδ T cells regulate the intestinal response to nutrient sensing. Science 2021; 371: eaba8310.
[25] Ismail AS, Behrendt CL and Hooper LV. Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lympho-

cytes during mucosal injury. J Immunol 2009; 182: 3047-3054.
[26] Sun D, Ko MK, Shao H and Kaplan HJ. Augmented Th17-stimulating activity of BMDCs as a result of reciprocal interaction between γδ and dendritic cells. Mol Immunol 2021; 134: 13-24.
[27] Lopes N, McIntyre C, Martin S, Raverdeau M, Sumaria N, Kohlgruber AC, Fiala GJ, Agudelo LZ, Dyck L, Kane H, Douglas A, Cunningham S, Prendeville H, Loftus R, Carmody C, Pierre P, Kellis M, Brenner M, Argüello RJ, Silva-Santos B, Pennington DJ and Lynch L. Distinct metabolic programs established in the thymus control effector functions of γδ T cell subsets in tumor microenvironments. Nat Immunol 2021; 22: 179-192.
[28] Matsuda S, Kudoh S and Katayama S. Enhanced formation of azoxymethane-induced colorectal adenocarcinoma in gammadelta T lymphocyte-deficient mice. Jpn J Cancer Res 2001; 92: 880-885.
[29] Tyler CJ, McCarthy NE, Lindsay JO, Stagg AJ, Moser B and Eberl M. Antigen-presenting human γδ T cells promote intestinal CD4(+) T cell expression of IL-22 and mucosal release of calprotectin. J Immunol 2017; 198: 3417-3425.
[30] Brandes M, Willimann K and Moser B. Professional antigen-presentation function by human gammadelta T cells. Science 2005; 309: 264-268.
[31] Alnaggar M, Xu Y, Li J, He J, Chen J, Li M, Wu Q, Lin L, Liang Y, Wang X, Li J, Hu Y, Chen Y, Xu K, Wu Y and Yin Z. Allogenic Vγ9Vδ2 T cell as new potential immunotherapy drug for solid tumor: a case study for cholangiocarcinoma. J Immunother Cancer 2019; 7: 36.
[32] Rampal R, Wari N, Singh AK, Das U, Bopanna S, Gupta V, Nayak B, Velapandian T, Kedia S, Kumar D, Awasthi A and Ahuja V. Retinoic acid is elevated in the mucosa of patients with active ulcerative colitis and displays a proinflammatory role by augmenting IL-17 and IFNγ production. Inflamm Bowel Dis 2021; 27: 74-83.
[33] Lo Presti E, Corsale AM, Di Simone M, Dieli F and Meraviglia S. Characterisation of γδ T cells infiltrating colorectal cancer. Gut 2021; 70: 1001-1003.
[34] Wu Y, Kyle-Cezar F, Woolf RT, Naceur-Lombardelli C, Owen J, Biswas D, Lorenc A, Vantourout P, Gazinska P, Grigoriadis A, Tutt A and Hayday A. An innate-like Vδ1(+) γδ T cell compartment in the human breast is associated with remission in triple-negative breast cancer. Sci Transl Med 2019; 11: eaax9364.
[35] Yu L, Wang Z, Hu Y, Wang Y, Lu N and Zhang C. Tumor-infiltrating gamma delta T-cells reveal exhausted subsets with remarkable heterogeneity in colorectal cancer. Int J Cancer 2023; 153: 1684-1697.
[36] Mangan BA, Dunne MR, O'Reilly VP, Dunne PJ, Exley MA, O'Shea D, Scotet E, Hogan AE and Doherty DG. Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human Vδ3 T cells. J Immunol 2013; 191: 30-34.
[37] Giri S and Lal G. Differentiation and functional plasticity of gamma-delta (γδ) T cells under homeostatic and disease conditions. Mol Immunol 2021; 136: 138-149.
[38] Lu H, Dai W, Guo J, Wang D, Wen S, Yang L, Lin D, Xie W, Wen L, Fang J and Wang Z. High abundance of intratumoral γδ T cells favors a better prognosis in head and neck squamous cell carcinoma: a bioinformatic analysis. Front Immunol 2020; 11: 573920.
[39] Born WK, Kemal Aydintug M and O'Brien RL. Diversity of γδ T-cell antigens. Cell Mol Immunol 2013; 10: 13-20.
[40] La Gruta NL, Gras S, Daley SR, Thomas PG and Rossjohn J. Understanding the drivers of MHC restriction of T cell receptors. Nat Rev Immunol 2018; 18: 467-478.
[41] Yazdanifar M, Barbarito G, Bertaina A and Airoldi I. γδ T cells: the ideal tool for cancer immunotherapy. Cells 2020; 9: 1305.
[42] Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, McWilliam HEG, Hudson C, Tutuka C, Wheatley AK, Kent SJ, Villadangos JA, Pal B, Kurts C, Simmonds J, Pelzing M, Nash AD, Hammet A, Verhagen AM, Vairo G, Maraskovsky E, Panousis C, Gherardin NA, Cebon J, Godfrey DI, Behren A and Uldrich AP. Butyrophilin 2A1 is essential for phosphoantigen reactivity by γδ T cells. Science 2020; 367: eaay5516.
[43] Xu B, Pizarro JC, Holmes MA, McBeth C, Groh V, Spies T and Strong RK. Crystal structure of a gammadelta T-cell receptor specific for the human MHC class I homolog MICA. Proc Natl Acad Sci U S A 2011; 108: 2414-2419.
[44] Minculescu L, Sengelov H, Marquart HV, Ryder LP, Fischer-Nielsen A and Haastrup E. Granulocyte colony-stimulating factor effectively mobilizes TCR γδ and NK cells providing an allograft potentially enhanced for the graft-versus-leukemia effect for allogeneic stem cell transplantation. Front Immunol 2021; 12: 625165.
[45] Wang X, Mou W, Han W, Xi Y, Chen X, Zhang H, Qin H, Wang H, Ma X and Gui J. Diminished cytolytic activity of γδ T cells with reduced DNAM-1 expression in neuroblastoma patients. Clin Immunol 2019; 203: 63-71.
[46] Niu C, Jin H, Li M, Xu J, Xu D, Hu J, He H, Li W and Cui J. In vitro analysis of the proliferative capacity and cytotoxic effects of ex vivo induced natural killer cells, cytokine-induced killer cells, and gamma-delta T cells. BMC Immunol 2015; 16: 61.
[47] Silva-Santos B, Mensurado S and Coffelt SB. γδ T cells: pleiotropic immune effectors with

therapeutic potential in cancer. Nat Rev Cancer 2019; 19: 392-404.
[48] Lameris R, Ruben JM, Iglesias-Guimarais V, de Jong M, Veth M, van de Bovenkamp FS, de Weerdt I, Kater AP, Zweegman S, Horbach S, Riedl T, Winograd B, Roovers RC, Adang AEP, de Gruijl TD, Parren PWHI and van der Vliet HJ. A bispecific T cell engager recruits both type 1 NKT and Vγ9Vδ2-T cells for the treatment of CD1d-expressing hematological malignancies. Cell Rep Med 2023; 4: 100961.
[49] Lo Presti E, Pizzolato G, Gulotta E, Cocorullo G, Gulotta G, Dieli F and Meraviglia S. Current advances in γδ T cell-based tumor immunotherapy. Front Immunol 2017; 8: 1401.
[50] Moulin M, Alguacil J, Gu S, Mehtougui A, Adams EJ, Peyrottes S and Champagne E. Vγ9Vδ2 T cell activation by strongly agonistic nucleotidic phosphoantigens. Cell Mol Life Sci 2017; 74: 4353-4367.
[51] Rodrigues NV, Correia DV, Mensurado S, Nóbrega-Pereira S, deBarros A, Kyle-Cezar F, Tutt A, Hayday AC, Norell H, Silva-Santos B and Dias S. Low-density lipoprotein uptake inhibits the activation and antitumor functions of human Vγ9Vδ2 T cells. Cancer Immunol Res 2018; 6: 448-457.
[52] Chitadze G, Lettau M, Luecke S, Wang T, Janssen O, Fürst D, Mytilineos J, Wesch D, Oberg HH, Held-Feindt J and Kabelitz D. NKG2D- and T-cell receptor-dependent lysis of malignant glioma cell lines by human γδ T cells: Modulation by temozolomide and A disintegrin and metalloproteases 10 and 17 inhibitors. Oncoimmunology 2016; 5: e1093276.
[53] Alice AF, Kramer G, Bambina S, Bahjat KS, Gough MJ and Crittenden MR. Listeria monocytogenes-infected human monocytic derived dendritic cells activate Vγ9Vδ2 T cells independently of HMBPP production. Sci Rep 2021; 11: 16347.
[54] Corvaisier M, Moreau-Aubry A, Diez E, Bennouna J, Mosnier JF, Scotet E, Bonneville M and Jotereau F. V gamma 9V delta 2 T cell response to colon carcinoma cells. J Immunol 2005; 175: 5481-5488.
[55] Dar AA, Patil RS and Chiplunkar SV. Insights into the relationship between toll like receptors and gamma delta T cell responses. Front Immunol 2014; 5: 366.
[56] Dokouhaki P, Han M, Joe B, Li M, Johnston MR, Tsao MS and Zhang L. Adoptive immunotherapy of cancer using ex vivo expanded human gammadelta T cells: a new approach. Cancer Lett 2010; 297: 126-136.
[57] Morandi F, Yazdanifar M, Cocco C, Bertaina A and Airoldi I. Engineering the bridge between innate and adaptive immunity for cancer immunotherapy: focus on γδ T and NK cells. Cells 2020; 9: 1757.
[58] Tosolini M, Pont F, Poupot M, Vergez F, Nicolau-Travers ML, Vermijlen D, Sarry JE, Dieli F and Fournié JJ. Assessment of tumor-infiltrating TCRVγ9Vδ2 γδ lymphocyte abundance by deconvolution of human cancers microarrays. Oncoimmunology 2017; 6: e1284723.
[59] Hudspeth K, Silva-Santos B and Mavilio D. Natural cytotoxicity receptors: broader expression patterns and functions in innate and adaptive immune cells. Front Immunol 2013; 4: 69.
[60] Schilbach K, Welker C, Krickeberg N, Kaißer C, Schleicher S and Hashimoto H. In the absence of a TCR signal IL-2/IL-12/18-stimulated γδ T cells demonstrate potent anti-tumoral function through direct killing and senescence induction in cancer cells. Cancers (Basel) 2020; 12: 130.
[61] Zhao Y, Niu C and Cui J. Gamma-delta (γδ) T cells: friend or foe in cancer development? J Transl Med 2018; 16: 3.
[62] Feng N, Luo JM and Guo X. The immune influence of a parabiosis model on tumour-bearing mice. Swiss Med Wkly 2018; 148: w14678.
[63] Xu Y, Zhang X, Wang Y, Pan M, Wang M and Zhang J. A VEGFR2-MICA bispecific antibody activates tumor-infiltrating lymphocytes and exhibits potent anti-tumor efficacy in mice. Cancer Immunol Immunother 2019; 68: 1429-1441.
[64] Hudspeth K, Fogli M, Correia DV, Mikulak J, Roberto A, Della Bella S, Silva-Santos B and Mavilio D. Engagement of NKp30 on Vδ1 T cells induces the production of CCL3, CCL4, and CCL5 and suppresses HIV-1 replication. Blood 2012; 119: 4013-4016.
[65] Lee HW, Chung YS and Kim TJ. Heterogeneity of human γδ T cells and their role in cancer immunity. Immune Netw 2020; 20: e5.
[66] Ghadially H, Brown L, Lloyd C, Lewis L, Lewis A, Dillon J, Sainson R, Jovanovic J, Tigue NJ, Bannister D, Bamber L, Valge-Archer V and Wilkinson RW. MHC class I chain-related protein A and B (MICA and MICB) are predominantly expressed intracellularly in tumour and normal tissue. Br J Cancer 2017; 116: 1208-1217.
[67] de Vries NL, van de Haar J, Veninga V, Chalabi M, Ijsselsteijn ME, van der Ploeg M, van den Bulk J, Ruano D, van den Berg JG, Haanen JB, Zeverijn LJ, Geurts BS, de Wit GF, Battaglia TW, Gelderblom H, Verheul HMW, Schumacher TN, Wessels LFA, Koning F, de Miranda NFCC and Voest EE. γδ T cells are effectors of immunotherapy in cancers with HLA class I defects. Nature 2023; 613: 743-750.
[68] Glatzel A, Wesch D, Schiemann F, Brandt E, Janssen O and Kabelitz D. Patterns of chemokine receptor expression on peripheral blood gamma delta T lymphocytes: strong expression of CCR5 is a selective feature of V delta 2/V

gamma 9 gamma delta T cells. J Immunol 2002; 168: 4920-4929.
[69] Laplagne C, Meddour S, Figarol S, Michelas M, Calvayrac O, Favre G, Laurent C, Fournié JJ, Cabantous S and Poupot M. Vγ9Vδ2 T cells activation through phosphoantigens can be impaired by a RHOB rerouting in lung cancer. Front Immunol 2020; 11: 1396.
[70] Herrmann T, Fichtner AS and Karunakaran MM. An update on the molecular basis of phosphoantigen recognition by Vγ9Vδ2 T cells. Cells 2020; 9: 1433.
[71] Kang I, Kim Y and Lee HK. Double-edged sword: γδ T cells in mucosal homeostasis and disease. Exp Mol Med 2023; 55: 1895-1904.
[72] Murayama M, Tanaka Y, Yagi J, Uchiyama T and Ogawa K. Antitumor activity and some immunological properties of gammadelta T-cells from patients with gastrointestinal carcinomas. Anticancer Res 2008; 28: 2921-2931.
[73] Thompson K, Roelofs AJ, Jauhiainen M, Mönkkönen H, Mönkkönen J and Rogers MJ. Activation of γδ T cells by bisphosphonates. Adv Exp Med Biol 2010; 658: 11-20.
[74] Abdalhai MH, Bashari M, Lagnika C, He Q and Sun X. Effect of ultrasound treatment prior to vacuum and modified atmosphere packaging on microbial and physical characteristics of fresh beef. J Food Nutr Res 2014; 2: 312-320.
[75] Lo Presti E, Dieli F and Meraviglia S. Tumor-infiltrating γδ T lymphocytes: pathogenic role, clinical significance, and differential programing in the tumor microenvironment. Front Immunol 2014; 5: 607.
[76] Voskoboinik I, Whisstock JC and Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol 2015; 15: 388-400.
[77] Varesano S, Zocchi MR and Poggi A. Zoledronate triggers Vδ2 T cells to destroy and kill spheroids of colon carcinoma: quantitative image analysis of three-dimensional cultures. Front Immunol 2018; 9: 998.
[78] Meraviglia S, Lo Presti E, Tosolini M, La Mendola C, Orlando V, Todaro M, Catalano V, Stassi G, Cicero G, Vieni S, Fourniè JJ and Dieli F. Distinctive features of tumor-infiltrating γδ T lymphocytes in human colorectal cancer. Oncoimmunology 2017; 6: e1347742.
[79] Zou C, Zhao P, Xiao Z, Han X, Fu F and Fu L. γδ T cells in cancer immunotherapy. Oncotarget 2017; 8: 8900-8909.
[80] Giuliani A, Tentori L, Pepponi R, Porcelli SA, Aquino A, Orlando L, Sugita M, Brenner MB, Bonmassar E and Graziani G. Cytokine-induced expression of CD1b molecules by peripheral blood monocytes: influence of 3'-azido-3'-deoxythymidine. Pharmacol Res 1997; 35: 135-140.
[81] Li Y, Li G, Zhang J, Wu X and Chen X. The dual roles of human γδ T cells: anti-tumor or tumor-promoting. Front Immunol 2021; 11: 619954.
[82] Poggi A and Zocchi MR. γδ T lymphocytes as a first line of immune defense: old and new ways of antigen recognition and implications for cancer immunotherapy. Front Immunol 2014; 5: 575.
[83] Kabelitz D, Serrano R, Kouakanou L, Peters C and Kalyan S. Cancer immunotherapy with γδ T cells: many paths ahead of us. Cell Mol Immunol 2020; 17: 925-939.
[84] Wu D, Wu P, Wu X, Ye J, Wang Z, Zhao S, Ni C, Hu G, Xu J, Han Y, Zhang T, Qiu F, Yan J and Huang J. Ex vivo expanded human circulating Vδ1 γδT cells exhibit favorable therapeutic potential for colon cancer. Oncoimmunology 2015; 4: e992749.
[85] Pistoia V, Tumino N, Vacca P, Veneziani I, Moretta A, Locatelli F and Moretta L. Human γδ T-cells: from surface receptors to the therapy of high-risk leukemias. Front Immunol 2018; 9: 984.
[86] Gronke K, Hernández PP, Zimmermann J, Klose CSN, Kofoed-Branzk M, Guendel F, Witkowski M, Tizian C, Amann L, Schumacher F, Glatt H, Triantafyllopoulou A and Diefenbach A. Interleukin-22 protects intestinal stem cells against genotoxic stress. Nature 2019; 566: 249-253.
[87] Bruni E, Cimino MM, Donadon M, Carriero R, Terzoli S, Piazza R, Ravens S, Prinz I, Cazzetta V, Marzano P, Kunderfranco P, Peano C, Soldani C, Franceschini B, Colombo FS, Garlanda C, Mantovani A, Torzilli G, Mikulak J and Mavilio D. Intrahepatic CD69(+)Vδ1 T cells re-circulate in the blood of patients with metastatic colorectal cancer and limit tumor progression. J Immunother Cancer 2022; 10: e004579.
[88] Guzik TJ, Nosalski R, Maffia P and Drummond GR. Immune and inflammatory mechanisms in hypertension. Nat Rev Cardiol 2024; [Epub ahead of print].
[89] Reis BS, Darcy PW, Khan IZ, Moon CS, Kornberg AE, Schneider VS, Alvarez Y, Eleso O, Zhu C, Schernthanner M, Lockhart A, Reed A, Bortolatto J, Castro TBR, Bilate AM, Grivennikov S, Han AS and Mucida D. TCR-Vγδ usage distinguishes protumor from antitumor intestinal γδ T cell subsets. Science 2022; 377: 276-284.
[90] Van Hede D, Polese B, Humblet C, Wilharm A, Renoux V, Dortu E, de Leval L, Delvenne P, Desmet CJ, Bureau F, Vermijlen D and Jacobs N. Human papillomavirus oncoproteins induce a reorganization of epithelial-associated γδ T cells promoting tumor formation. Proc Natl Acad Sci U S A 2017; 114: E9056-E9065.
[91] Fleming C, Morrissey S, Cai Y and Yan J. γδ T cells: unexpected regulators of cancer devel-

opment and progression. Trends Cancer 2017; 3: 561-570.
[92] Sanaei MJ, Baghery Saghchy Khorasani A, Pourbagheri-Sigaroodi A, Shahrokh S, Zali MR and Bashash D. The PI3K/Akt/mTOR axis in colorectal cancer: oncogenic alterations, noncoding RNAs, therapeutic opportunities, and the emerging role of nanoparticles. J Cell Physiol 2022; 237: 1720-1752.
[93] Zheng Q, Diao S, Wang Q, Zhu C, Sun X, Yin B, Zhang X, Meng X and Wang B. IL-17A promotes cell migration and invasion of glioblastoma cells via activation of PI3K/AKT signalling pathway. J Cell Mol Med 2019; 23: 357-369.
[94] Wang K, Kim MK, Di Caro G, Wong J, Shalapour S, Wan J, Zhang W, Zhong Z, Sanchez-Lopez E, Wu LW, Taniguchi K, Feng Y, Fearon E, Grivennikov SI and Karin M. Interleukin-17 receptor a signaling in transformed enterocytes promotes early colorectal tumorigenesis. Immunity 2014; 41: 1052-1063.
[95] Silva-Santos B, Serre K and Norell H. γδ T cells in cancer. Nat Rev Immunol 2015; 15: 683-691.
[96] Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM and Sears CL. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009; 15: 1016-1022.
[97] Miller JE, Ahn SH, Marks RM, Monsanto SP, Fazleabas AT, Koti M and Tayade C. IL-17A modulates peritoneal macrophage recruitment and M2 polarization in endometriosis. Front Immunol 2020; 11: 108.
[98] Schönefeldt S, Wais T, Herling M, Mustjoki S, Bekiaris V, Moriggl R and Neubauer HA. The diverse roles of γδ T cells in cancer: from rapid immunity to aggressive lymphoma. Cancers (Basel) 2021; 13: 6212.
[99] Ye J, Ma C, Hsueh EC, Eickhoff CS, Zhang Y, Varvares MA, Hoft DF and Peng G. Tumor-derived γδ regulatory T cells suppress innate and adaptive immunity through the induction of immunosenescence. J Immunol 2013; 190: 2403-2414.
[100] Chen D, Guo Y, Jiang J, Wu P, Zhang T, Wei Q, Huang J and Wu D. γδ T cell exhaustion: opportunities for intervention. J Leukoc Biol 2022; 112: 1669-1676.
[101] Morrow ES, Roseweir A and Edwards J. The role of gamma delta T lymphocytes in breast cancer: a review. Transl Res 2019; 203: 88-96.
[102] Xiang Z and Tu W. Dual face of Vγ9Vδ2-T cells in tumor immunology: anti- versus pro-tumoral activities. Front Immunol 2017; 8: 1041.
[103] Wakita D, Sumida K, Iwakura Y, Nishikawa H, Ohkuri T, Chamoto K, Kitamura H and Nishimura T. Tumor-infiltrating IL-17-producing gammadelta T cells support the progression of tumor by promoting angiogenesis. Eur J Immunol 2010; 40: 1927-1937.
[104] Andreu-Ballester JC, Galindo-Regal L, Hidalgo-Coloma J, Cuéllar C, García-Ballesteros C, Hurtado C, Uribe N, Del Carmen Martín M, Jiménez AI, López-Chuliá F and Llombart-Cussac A. Differences in circulating γδ T cells in patients with primary colon cancer and relation with prognostic factors. PLoS One 2020; 15: e0243545.
[105] Sumaria N, Grandjean CL, Silva-Santos B and Pennington DJ. Strong TCRγδ signaling prohibits thymic development of IL-17A-secreting γδ T cells. Cell Rep 2017; 19: 2469-2476.
[106] Yi Y, He HW, Wang JX, Cai XY, Li YW, Zhou J, Cheng YF, Jin JJ, Fan J and Qiu SJ. The functional impairment of HCC-infiltrating γδ T cells, partially mediated by regulatory T cells in a TGFβ- and IL-10-dependent manner. J Hepatol 2013; 58: 977-983.
[107] Hu G, Wu P, Cheng P, Zhang Z, Wang Z, Yu X, Shao X, Wu D, Ye J, Zhang T, Wang X, Qiu F, Yan J and Huang J. Tumor-infiltrating CD39(+)γδ Tregs are novel immunosuppressive T cells in human colorectal cancer. Oncoimmunology 2017; 6: e1277305.
[108] Lo Presti E, Mocciaro F, Mitri RD, Corsale AM, Di Simone M, Vieni S, Scibetta N, Unti E, Dieli F and Meraviglia S. Analysis of colon-infiltrating γδ T cells in chronic inflammatory bowel disease and in colitis-associated cancer. J Leukoc Biol 2020; 108: 749-760.
[109] Chen X, Shang W, Xu R, Wu M, Zhang X, Huang P, Wang F and Pan S. Distribution and functions of γδ T cells infiltrated in the ovarian cancer microenvironment. J Transl Med 2019; 17: 144.
[110] Zhan Y, Zheng L, Liu J, Hu D, Wang J, Liu K, Guo J, Zhang T and Kong D. PLA2G4A promotes right-sided colorectal cancer progression by inducing CD39+γδ Treg polarization. JCI Insight 2021; 6: e148028.
[111] Peters C, Oberg HH, Kabelitz D and Wesch D. Phenotype and regulation of immunosuppressive Vδ2-expressing γδ T cells. Cell Mol Life Sci 2014; 71: 1943-1960.
[112] Lo Presti E, Pizzolato G, Corsale AM, Caccamo N, Sireci G, Dieli F and Meraviglia S. γδ T cells and tumor microenvironment: from immunosurveillance to tumor evasion. Front Immunol 2018; 9: 1395.
[113] Chan KF, Duarte JDG, Ostrouska S and Behren A. γδ T cells in the tumor microenvironment-interactions with other immune cells. Front Immunol 2022; 13: 894315.
[114] Lança T, Costa MF, Gonçalves-Sousa N, Rei M, Grosso AR, Penido C and Silva-Santos B. Pro-

tective role of the inflammatory CCR2/CCL2 chemokine pathway through recruitment of type 1 cytotoxic γδ T lymphocytes to tumor beds. J Immunol 2013; 190: 6673-6680.
[115] Imbert C and Olive D. γδ T cells in tumor microenvironment. Adv Exp Med Biol 2020; 1273: 91-104.
[116] Peters C, Kouakanou L, Oberg HH, Wesch D and Kabelitz D. In vitro expansion of Vγ9Vδ2 T cells for immunotherapy. Methods Enzymol 2020; 631: 223-237.
[117] Almeida AR, Correia DV, Fernandes-Platzgummer A, da Silva CL, da Silva MG, Anjos DR and Silva-Santos B. Delta one T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept. Clin Cancer Res 2016; 22: 5795-5804.
[118] Di Lorenzo B, Simões AE, Caiado F, Tieppo P, Correia DV, Carvalho T, da Silva MG, Déchanet-Merville J, Schumacher TN, Prinz I, Norell H, Ravens S, Vermijlen D and Silva-Santos B. Broad cytotoxic targeting of acute myeloid leukemia by polyclonal delta one T cells. Cancer Immunol Res 2019; 7: 552-558.
[119] Wu D, Wu P, Qiu F, Wei Q and Huang J. Human γδT-cell subsets and their involvement in tumor immunity. Cell Mol Immunol 2017; 14: 245-253.
[120] Ang WX, Ng YY, Xiao L, Chen C, Li Z, Chi Z, Tay JC, Tan WK, Zeng J, Toh HC and Wang S. Electroporation of NKG2D RNA CAR improves Vγ9Vδ2 T cell responses against human solid tumor xenografts. Mol Ther Oncolytics 2020; 17: 421-430.
[121] Cazzetta V, Depierreux D, Colucci F, Mikulak J and Mavilio D. NKG2A immune checkpoint in Vδ2 T cells: emerging application in cancer immunotherapy. Cancers (Basel) 2023; 15: 1264.
[122] Pressey JG, Adams J, Harkins L, Kelly D, You Z and Lamb LS Jr. In vivo expansion and activation of γδ T cells as immunotherapy for refractory neuroblastoma: a phase 1 study. Medicine (Baltimore) 2016; 95: e4909.
[123] Van Acker HH, Campillo-Davo D, Roex G, Versteven M, Smits EL and Van Tendeloo VF. The role of the common gamma-chain family cytokines in γδ T cell-based anti-cancer immunotherapy. Cytokine Growth Factor Rev 2018; 41: 54-64.
[124] Xiao L, Chen C, Li Z, Zhu S, Tay JC, Zhang X, Zha S, Zeng J, Tan WK, Liu X, Chng WJ and Wang S. Large-scale expansion of Vγ9Vδ2 T cells with engineered K562 feeder cells in G-Rex vessels and their use as chimeric antigen receptor-modified effector cells. Cytotherapy 2018; 20: 420-435.
[125] Bennouna J, Levy V, Sicard H, Senellart H, Audrain M, Hiret S, Rolland F, Bruzzoni-Giovanelli H, Rimbert M, Galéa C, Tiollier J and Calvo F. Phase I study of bromohydrin pyrophosphate (BrHPP, IPH 1101), a Vgamma9Vdelta2 T lymphocyte agonist in patients with solid tumors. Cancer Immunol Immunother 2010; 59: 1521-1530.
[126] Meraviglia S, Eberl M, Vermijlen D, Todaro M, Buccheri S, Cicero G, La Mendola C, Guggino G, D'Asaro M, Orlando V, Scarpa F, Roberts A, Caccamo N, Stassi G, Dieli F and Hayday AC. 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: 290-297.
[127] Okuno D, Sugiura Y, Sakamoto N, Tagod MSO, Iwasaki M, Noda S, Tamura A, Senju H, Umeyama Y, Yamaguchi H, Suematsu M, Morita CT, Tanaka Y and Mukae H. Comparison of a novel bisphosphonate prodrug and zoledronic acid in the induction of cytotoxicity in human Vgamma2Vdelta2 T cells. Front Immunol 2020; 11: 1405.
[128] Todaro M, D'Asaro M, Caccamo N, Iovino F, Francipane MG, Meraviglia S, Orlando V, La Mendola C, Gulotta G, Salerno A, Dieli F and Stassi G. Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. J Immunol 2009; 182: 7287-7296.
[129] Zocchi MR, Costa D, Venè R, Tosetti F, Ferrari N, Minghelli S, Benelli R, Scabini S, Romairone E, Catellani S, Profumo A and Poggi A. Zoledronate can induce colorectal cancer microenvironment expressing BTN3A1 to stimulate effector γδ T cells with antitumor activity. Oncoimmunology 2017; 6: e1278099.
[130] Altvater B, Pscherer S, Landmeier S, Kailayangiri S, Savoldo B, Juergens H and Rossig C. Activated human γδ T cells induce peptide-specific CD8+ T-cell responses to tumor-associated self-antigens. Cancer Immunol Immunother 2012; 61: 385-396.
[131] Izumi T, Kondo M, Takahashi T, Fujieda N, Kondo A, Tamura N, Murakawa T, Nakajima J, Matsushita H and Kakimi K. Ex vivo characterization of γδ T-cell repertoire in patients after adoptive transfer of Vγ9Vδ2 T cells expressing the interleukin-2 receptor β-chain and the common γ-chain. Cytotherapy 2013; 15: 481-491.
[132] Devaud C, Rousseau B, Netzer S, Pitard V, Paroissin C, Khairallah C, Costet P, Moreau JF, Couillaud F, Dechanet-Merville J and Capone M. Anti-metastatic potential of human Vδ1(+) γδ T cells in an orthotopic mouse xenograft model of colon carcinoma. Cancer Immunol Immunother 2013; 62: 1199-1210.
[133] Nguyen S, Chevalier MF, Benmerzoug S, Cesson V, Schneider AK, Rodrigues-Dias SC, Dartiguenave F, Lucca I, Jichlinski P, Roth B, Nardelli-Haefliger D and Derré L. Vδ2 T cells

are associated with favorable clinical outcomes in patients with bladder cancer and their tumor reactivity can be boosted by BCG and zoledronate treatments. J Immunother Cancer 2022; 10: e004880.
[134] Hajdara A, Çakır U, Érsek B, Silló P, Széky B, Barna G, Faqi S, Gyöngy M, Kárpáti S, Németh K and Mayer B. Targeting melanoma-associated fibroblasts (MAFs) with activated γδ (Vδ2) T cells: an in vitro cytotoxicity model. Int J Mol Sci 2023; 24: 12893.
[135] Bold A, Gaertner J, Bott A, Mordstein V, Schaefer-Eckart K and Wilhelm M. Haploidentical γδ T cells induce complete remission in chemorefractory B-cell non-Hodgkin lymphoma. J Immunother 2023; 46: 56-58.
[136] Frieling JS, Tordesillas L, Bustos XE, Ramello MC, Bishop RT, Cianne JE, Snedal SA, Li T, Lo CH, de la Iglesia J, Roselli E, Benzaïd I, Wang X, Kim Y, Lynch CC and Abate-Daga D. γδ-enriched CAR-T cell therapy for bone metastatic castrate-resistant prostate cancer. Sci Adv 2023; 9: eadf0108.
[137] Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang CD, Diehn M, West RB, Plevritis SK and Alizadeh AA. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 2015; 21: 938-945.
[138] Xu Y, Xiang Z, Alnaggar M, Kouakanou L, Li J, He J, Yang J, Hu Y, Chen Y, Lin L, Hao J, Li J, Chen J, Li M, Wu Q, Peters C, Zhou Q, Li J, Liang Y, Wang X, Han B, Ma M, Kabelitz D, Xu K, Tu W, Wu Y and Yin Z. Allogeneic Vγ9Vδ2 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: 427-439.
[139] Sebestyen Z, Prinz I, Déchanet-Merville J, Silva-Santos B and Kuball J. Translating gammadelta (γδ) T cells and their receptors into cancer cell therapies. Nat Rev Drug Discov 2020; 19: 169-184.
[140] Minculescu L, Marquart HV, Ryder LP, Andersen NS, Schjoedt I, Friis LS, Kornblit BT, Petersen SL, Haastrup E, Fischer-Nielsen A, Reekie J and Sengelov H. Improved overall survival, relapse-free-survival, and less graft-vs.-host-disease in patients with high immune reconstitution of TCR gamma delta cells 2 months after allogeneic stem cell transplantation. Front Immunol 2019; 10: 1997.
[141] Lai D, Wang F, Chen Y, Wang C, Liu S, Lu B, Ge X and Guo L. Human ovarian cancer stem-like cells can be efficiently killed by γδ T lymphocytes. Cancer Immunol Immunother 2012; 61: 979-989.
[142] Kuroda H, Saito H and Ikeguchi M. Decreased number and reduced NKG2D expression of Vδ1 γδ T cells are involved in the impaired function of Vδ1 γδ T cells in the tissue of gastric cancer. Gastric Cancer 2012; 15: 433-439.
[143] Bouet-Toussaint F, Cabillic F, Toutirais O, Le Gallo M, Thomas de la Pintière C, Daniel P, Genetet N, Meunier B, Dupont-Bierre E, Boudjema K and Catros V. Vgamma9Vdelta2 T cell-mediated recognition of human solid tumors. Potential for immunotherapy of hepatocellular and colorectal carcinomas. Cancer Immunol Immunother 2008; 57: 531-539.
[144] Wada I, Matsushita H, Noji S, Mori K, Yamashita H, Nomura S, Shimizu N, Seto Y and Kakimi K. Intraperitoneal injection of in vitro expanded Vγ9Vδ2 T cells together with zoledronate for the treatment of malignant ascites due to gastric cancer. Cancer Med 2014; 3: 362-375.
[145] Lança T, Correia DV, Moita CF, Raquel H, Neves-Costa A, Ferreira C, Ramalho JS, Barata JT, Moita LF, Gomes AQ and Silva-Santos B. The MHC class Ib protein ULBP1 is a nonredundant determinant of leukemia/lymphoma susceptibility to gammadelta T-cell cytotoxicity. Blood 2010; 115: 2407-2411.
[146] Nicol AJ, Tokuyama H, Mattarollo SR, Hagi T, Suzuki K, Yokokawa K and Nieda M. Clinical evaluation of autologous gamma delta T cell-based immunotherapy for metastatic solid tumours. Br J Cancer 2011; 105: 778-786.
[147] Bennouna J, Bompas E, Neidhardt EM, Rolland F, Philip I, Galéa C, Salot S, Saiagh S, Audrain M, Rimbert M, Lafaye-de Micheaux S, Tiollier J and Négrier S. 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: 1599-1609.
[148] Lamb LS Jr, Bowersock J, Dasgupta A, Gillespie GY, Su Y, Johnson A and Spencer HT. Engineered drug resistant γδ T cells kill glioblastoma cell lines during a chemotherapy challenge: a strategy for combining chemo- and immunotherapy. PLoS One 2013; 8: e51805.
[149] Deniger DC, Switzer K, Mi T, Maiti S, Hurton L, Singh H, Huls H, Olivares S, Lee DA, Champlin RE and Cooper LJ. Bispecific T-cells expressing polyclonal repertoire of endogenous γδ T-cell receptors and introduced CD19-specific chimeric antigen receptor. Mol Ther 2013; 21: 638-647.
[150] Iwasaki M, Tanaka Y, Kobayashi H, Murata-Hirai K, Miyabe H, Sugie T, Toi M and Minato N. Expression and function of PD-1 in human γδ T cells that recognize phosphoantigens. Eur J Immunol 2011; 41: 345-355.
[151] Li X, Lu H, Gu Y, Zhang X, Zhang G, Shi T and Chen W. Tim-3 suppresses the killing effect of

Vγ9Vδ2 T cells on colon cancer cells by reducing perforin and granzyme B expression. Exp Cell Res 2020; 386: 111719.
[152] Wistuba-Hamprecht K, Martens A, Haehnel K, Geukes Foppen M, Yuan J, Postow MA, Wong P, Romano E, Khammari A, Dreno B, Capone M, Ascierto PA, Demuth I, Steinhagen-Thiessen E, Larbi A, Schilling B, Schadendorf D, Wolchok JD, Blank CU, Pawelec G, Garbe C and Weide B. Proportions of blood-borne Vδ1+ and Vδ2+ T-cells are associated with overall survival of melanoma patients treated with ipilimumab. Eur J Cancer 2016; 64: 116-126.
[153] Gründer C, van Dorp S, Hol S, Drent E, Straetemans T, Heijhuurs S, Scholten K, Scheper W, Sebestyen Z, Martens A, Strong R and Kuball J. γ9 and δ2CDR3 domains regulate functional avidity of T cells harboring γ9δ2TCRs. Blood 2012; 120: 5153-5162.
[154] de Bruin RCG, Veluchamy JP, Lougheed SM, Schneiders FL, Lopez-Lastra S, Lameris R, Stam AG, Sebestyen Z, Kuball J, Molthoff CFM, Hooijberg E, Roovers RC, Santo JPD, van Bergen En Henegouwen PMP, Verheul HMW, de Gruijl TD and van der Vliet HJ. A bispecific nanobody approach to leverage the potent and widely applicable tumor cytolytic capacity of Vγ9Vδ2-T cells. Oncoimmunology 2017; 7: e1375641.
[155] Oberg HH, Peipp M, Kellner C, Sebens S, Krause S, Petrick D, Adam-Klages S, Röcken C, Becker T, Vogel I, Weisner D, Freitag-Wolf S, Gramatzki M, Kabelitz D and Wesch D. Novel bispecific antibodies increase γδ T-cell cytotoxicity against pancreatic cancer cells. Cancer Res 2014; 74: 1349-1360.
[156] Formenti SC, Rudqvist NP, Golden E, Cooper B, Wennerberg E, Lhuillier C, Vanpouille-Box C, Friedman K, Ferrari de Andrade L, Wucherpfennig KW, Heguy A, Imai N, Gnjatic S, Emerson RO, Zhou XK, Zhang T, Chachoua A and Demaria S. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med 2018; 24: 1845-1851.
[157] Shimizu T, Tomogane M, Miyashita M, Ukimura O and Ashihara E. Low dose gemcitabine increases the cytotoxicity of human Vγ9Vδ2 T cells in bladder cancer cells in vitro and in an orthotopic xenograft model. Oncoimmunology 2018; 7: e1424671.
[158] Todaro M, Meraviglia S, Caccamo N, Stassi G and Dieli F. Combining conventional chemotherapy and γδ T cell-based immunotherapy to target cancer-initiating cells. Oncoimmunology 2013; 2: e25821.
[159] Todaro M, Orlando V, Cicero G, Caccamo N, Meraviglia S, Stassi G and Dieli F. Chemotherapy sensitizes colon cancer initiating cells to Vγ9Vδ2 T cell-mediated cytotoxicity. PLoS One 2013; 8: e65145.
[160] Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, Pfirschke C, Voss RH, Timke C, Umansky L, Klapproth K, Schäkel K, Garbi N, Jäger D, Weitz J, Schmitz-Winnenthal H, Hämmerling GJ and Beckhove P. Low-dose irradiation programs macrophage differentiation to an iNOS$^+$/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 2013; 24: 589-602.
[161] Kouakanou L, Xu Y, Peters C, He J, Wu Y, Yin Z and Kabelitz D. Vitamin C promotes the proliferation and effector functions of human γδ T cells. Cell Mol Immunol 2020; 17: 462-473.