REVIEW

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γδ T cell exhaustion: Opportunities for intervention

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Abstract

T lymphocytes are the key protective contributors in chronic infection and tumor, but experience exhaustion by persistent antigen stimulation. As an unconventional lineage of T cells, γδ T cells can rapidly response to varied infectious and tumor challenges in a non-MHC-restricted manner and play key roles in immune surveillance via pleiotropic effector functions, showing promising as candidates for cellular tumor immunotherapy. Activated γδ T cells can also acquire exhaustion signature with elevated expression of immune checkpoints, such as PD-1, decreased cytokine production, and functional impairment. However, the exhaustion features of γδ T cells are distinct from conventional αβ T cells. Here, we review the researches regarding the characteristics, heterogeneity, and mechanisms of γδ T cell exhaustion. These studies provide insights into the combined strategies to overcome the exhaustion of γδ T cells and enhance antitumor immunity.

Summary sentence: Review of the characteristics, heterogeneity, and mechanisms of γδ T cell exhaustion provides insights into the combined strategies to enhance γδ T cell-based antitumor immunotherapy.

KEYWORDS
exhaustion, immune checkpoints, immunotherapy, PD-1, γδ T cells

1 | INTRODUCTION

Continuously activated T cells displayed decreased capability of cytokine production and this functional state of T cells is defined as exhaustion, which was originally described during chronic lymphocytic choriomeningitis virus infection in mice.[1] Subsequently, T cell exhaustion has been widely demonstrated during chronic infection and tumor microenvironment both in various animal and human research models.[2] Exhausted T cells are functionally distinct from effector and memory T cells. The hallmarks of T cell exhaustion are progressive loss of effector function and proliferative ability, sustained high expression of inhibitory receptors (IRs), reduced responsiveness to homeostatic cytokines, altered epigenetic and transcriptional landscape, and specific metabolic program.[3] Among these features, the up-regulation of programmed cell death-1 (PD-1) has emerged as a major marker of T cell exhaustion.[4] Current insights into the mechanisms of exhaustion suggests that T cell exhaustion is driven by continuous viral or tumor antigen stimulation, the negative regulatory signals of IRs and chronic inflammation.[5] Coexpression of multiple distinct IRs was associated with T cell exhaustion severity.[6] Growing evidence demonstrates that T cell exhaustion primarily contributes to immune imbalance during

TABLE 1 The distribution and repertoire of $\gamma\delta$ T cells

Species	Distribution	Predominant V gene segment usage	Paired V gene segment usage	Refs
Mouse	Liver, lung, intestine	Vγ1	Vδ2/4/5/6	11–13,40
	Lung	Vγ2	Vδ5	12,13
	Epidermis	Vγ3	Vδ1	12,13
	Liver, lung, intestine, dermis and lymph nodes	Vγ4	Vδ1/2/4/5	11–13,40
	Epidermis, lung	Vγ5	Vδ1	11–14
	Tongue, dermis, lung, intestine, uterus, testis, peritoneal Cavity, adipose tissue and brain meninges	Vγ6	Vδ1	11–13,40
	Intestine	Vγ7	Vδ2/4/5/6	11–14,40
Human	PB, skin, gut, spleen, liver	Vδ1	Vγ2/3/4/5/8/9	12,17
	PB	Vδ2	Vγ9	12,16
	PB, liver	Vδ3	Vγ2/3/8	12,15,18
	PB	Vδ5	Vγ4	12,19
	PB	Vδ4/6/7/8	Unknown	12,20

chronic infection and tumor progression.[2] Therefore, reversing T cell exhaustion is paramount to antitumor immunity. Blockade of PD-1 or its ligand PD-L1 to inhibit the PD-1/PD-L1 axis in T cells has been recently shown to be effective for tumor therapy.[2] While most extensive studies focus on T cell exhaustion, an unconventional lineage of T cells expressing the $\gamma\delta$ TCR sharing certain cellular characteristics with $\alpha\beta$ T cells is reported to exhibit exhaustion feature.[7] This review will focus on recent investigation regarding $\gamma\delta$ T cell exhaustion and the clinical implications for tumor therapy involving the reinvigoration of $\gamma\delta$ T cell exhaustion.

2 | $\gamma\delta$ T LYMPHOCYTES

2.1 | The distribution and repertoire of $\gamma\delta$ T cells

$\gamma\delta$ T cells, characterized by TCRs composed of γ and δ chains, display tropism for mucosal epithelial tissues, providing a first line of defense against foreign pathogens.[8,9] $\gamma\delta$ T cells arise early in the thymus during fetal thymic ontogeny and make up a minor fraction of rodent and human thymocytes.[8] Similar to $\alpha\beta$ TCR, $\gamma\delta$ TCR is also formed by the rearrangement of V (variable), D (diversity), and J (joining) gene segments. Structural diversity of $\gamma\delta$ TCR is less than that of traditional $\alpha\beta$ TCR due to the Vγ and Vδ chain pairing requirements.[8] In mouse, structural diversity of $\gamma\delta$ T cells in particular tissue locations depends on the biased use of certain TCR γ chain.[10] Seven distinct Vγ subsets (Vγ1–7) derived from early "waves" of fetal $\gamma\delta$ thymocytes that vary in localization, paired Vδ chain, effector function, and contribution to homeostasis and disease.[11,12,13] For instance, the well-studied innate-like $\gamma\delta$ T subsets in mouse were skin epidermal Vγ5+ and intestinal Vγ7+ cells, which coincides with their tissue localization and function.[14] Individual $\gamma\delta$ T cell subsets in particular tissue locations are summarized in Table 1.

No obvious homologies between mouse and human $\gamma\delta$ TCR genes was observed. In human, $\gamma\delta$ T cells are primarily divided into Vδ1, Vδ2, and Vδ3 subsets according to their TCR δ chain usage.[15] Vδ2 subsets are predominant in human peripheral blood (PB) with almost exclusively paired with Vγ9 chain (also termed Vγ9Vδ2 $\gamma\delta$ T cell), while Vδ1 and Vδ3subsets constitute less than 30% of $\gamma\delta$T cells in PB and are enriched in mucosal epithelial tissues and liver, respectively, with diverse paired Vγ chains.[16] Novel structural subsets and the Vγ and Vδ chain pairing are gradually discovered (Table 2). Recently, Vδ1 T cells were reported to pair with Vγ9 chain, displaying $\gamma\delta$ TCR-dependent adaptive immune surveillance.[17] Another novel PB Vδ3Vγ8 subset was demonstrated to recognize MHC class I-related protein (MR1) independent of the presented antigen.[18] A rare Vγ4Vδ5 clone in human PB was reported to directly bind endothelial protein C receptor (EPCR).[19] In addition, PB Vδ4, Vδ6, Vδ7, and Vδ8 subsets have been detected in lymphoma patients.[20] The tissue distribution and $\gamma\delta$ TCR repertoire of different $\gamma\delta$ T cell subsets may determine their strikingly different activation modes and response to varied infectious and tumor challenges.

2.2 | The activation and function of $\gamma\delta$ T cells

$\gamma\delta$ T cells rapidly recognize conserved peptide and nonpeptide antigens that are up-regulated by stressed cells in a MHC-unrestricted manner, which distinguishes them from of $\alpha\beta$ T cells.[18,21] Phosphoantigens (PAgs) produced by microbes and transformed cells are known to uniquely activate human Vδ2 T cells via TCR-dependent manner, enabling them to rapidly respond to extracellular and intracellular pathogens.[22] $\gamma\delta$ T cells can also be activated following recognition of distress signals by TLRs and NK receptors (NKRs).[23,24] Activated $\gamma\delta$ T cells exhibit varied effector functions, including lysis of infected or stressed cells, cytokine and chemokine production, B cell help and IgE production, priming of $\alpha\beta$ T cells via antigen presentation, dendritic cell maturation, and regulation of stromal cell function via growth factor production, maintaining the functional integrity of epithelial barriers

TABLE 2 The exhaustion phenotype and dysfunction of $\gamma\delta$ T cells

Species	Disease model	Subset	Exhaustion phenotype	Dysfunction	Refs
Mouse	Plasmodium infection	Vγ1	TIM-3, LAG-3, and PD-1	Decreased IFN-γ-producing	32
	Colon cancer	Vγ6	PD-1	Elevated IL-17 expression and decreased cytotoxicity	40
Human	Plasmodium vivax	No report	PD-1, CTLA-4, Tim-3, and LAG-3	No report	34
	HIV infection	Vδ1	CD95 and PD1	Decreased IFN-γ response	35
	HIV infection	CD3ε^{lo} Vδ1	PD-1 but not LAG-3	Unable to produce IL-17	36
	HIV infection	Vδ1	TIGIT	Impairment of cytokine production	47
	Tuberculosis	Vδ2	PD-1	Decreased response to IL-23	37
	Common variable immunodeficiency (CVID)	Vδ2	PD-1	Decrease of Vδ2 frequency	39
	Acute dengue infection	Vδ2	High TIM-3 but not PD-1	Impairment of IFN-γ production	46
	Acute myeloid leukemia (AML)	Vδ2	PD-1	Decreased IFN-γ secretion and increased IL-17 secretion	41
	Acute myeloid leukemia (AML)	Vδ2	PD-1 and Tim-3	Decreased TNF-α and IFN-γ expression	50
	Non-M3 AML	Vδ2	TIGIT	Associated with poor prognosis	51
	Breast cancer	Vδ2	PD-1	Associated with tumor-draining lymph node invasion	42
	Ovarian cancer	Vδ1	Coexpression of PD-1, Tim-3, CD39 with TIGIT	Increased TEMRA (terminally differentiated effector memory) differentiation	43

and provide immunosurveillance by modulation of innate and adaptive immune responses.[25] Functionally pleiotropic $\gamma\delta$ T cells play nonredundant roles in various physiopathologic processes including infection, allergy, autoimmunity, and cancer.[26,27,28] $\gamma\delta$ T cells can fight against common pathogen infections, such as *Mycobacterium tuberculosis* (Mtb), *Listeria monocytogenes*, influenza viruses, HIV, EBV, and HBV.[29]

A large number of studies have proved that $\gamma\delta$ T cells are widely involved in the immune response of a variety of malignant tumors and are one of the most effective early antitumor effector cells.[25] Tumor-infiltrating $\gamma\delta$ T cells have been demonstrated to be the most significant predictor of favorable prognosis in a variety of tumors.[30] In our previous study, we have found that high cytotoxic human PB Vδ1 T cells can directly kill colon cancer cells via cytolytic receptor–ligand interactions.[31] Taken together, $\gamma\delta$ T cells are activated in a non-MHC-restricted manner and play key roles in immune surveillance via pleiotropic effector function, making them to be the promising candidates for cellular tumor immunotherapy.

3 | ACTIVATED $\gamma\delta$ T CELLS EXHIBIT EXHAUSTION SIGNATURE

3.1 | The exhaustion phenotype of $\gamma\delta$ T cells in infection

Similar to CD8+ T cells, persistent antigen stimulation affects the composition of $\gamma\delta$ T cell subsets and immune exhaustion.[7] Pathogen infection is the major driver of peripheral $\gamma\delta$ T cell activation. In

a mouse model of Plasmodium infection, Vγ1$^+$ $\gamma\delta$ T cells highly expressed markers of T-cell exhaustion (TIM-3, LAG-3, and PD-1) and the IFN-γ-producing ability of Vγ1$^+$ $\gamma\delta$ T cells is reduced in late-phase due to $\gamma\delta$ T-cell dysfunction.[32] The number of $\gamma\delta$ T cells dramatically increased in the spleen of metformin treated mice during the later phase of Plasmodium infection with high expression of IRs and severe defects in cytokine production, suggesting a state of exhaustion.[33]

In human, continuous exposure to Plasmodium vivax induces upregulation of the exhaustion markers including PD-1, CTLA-4, Tim-3, and LAG-3 on $\gamma\delta$ T cells.[34] HIV infection is associated with a rapid and sustained inversion of the Vδ1:Vδ2 T-cell ratio in PB. Activated Vδ1 subset exhibited significant expression of exhaustion markers CD95 and PD1 in HIV patients, suggesting persistent activation and altered function of Vδ1 T cells.[35] In another study, CD3ε^{lo} Vδ1 T cells were reported to frequently express terminally differentiated phenotypes and the immune checkpoint PD$-$1 but not LAG-3, suggesting these cells are in a state of exhaustion and are unable to produce IL-17 in HIV infection.[36] CD3ε can be transiently down-regulated by Vδ1 T cell activation and is restored in the presence of exogenous IL-2, indicating that the exhaustion phenotype of Vδ1 T cells is transient and could be reversed by in vitro conditional culture.[36] Tuberculosis destroy the effects of IL-2 and IL-23 signaling and induce the HMBPP-specific Vδ2 T-cell subpopulation at the cytokine level.[37] Recently, single-cell RNAseq profiling of human $\gamma\delta$ T lymphocytes showed that the exhaustion signature was up-regulated on $\gamma\delta$ T cells in lung lesions from acute COVID-19-infected patients.[38] Elevated PD-1 expression on Vδ2 T

cells and was also reported to be associated with immune activation and exhaustion in common variable immunodeficiency (CVID).[39]

3.2 | The exhaustion phenotype of $\gamma\delta$ T cells in tumor

Although widely characterized in chronic infection, the exhaustion of $\gamma\delta$ T cells has been investigated in tumor in recent years. In a colon cancer mouse model, tumor-infiltrating CD8$\alpha\alpha^-$ PD-1$^+$ $\gamma\delta$ T cells showed decreased expression of cytotoxic-related genes, whereas displayed increased expression of genes associated with IL-17 and protumor activity.[40] Analogous to the observations in mice, PD-1 expression was elevated on $\gamma\delta$ T cells with decreased IFN-γ secretion and increased IL-17 secretion in acute myeloid leukemia (AML), while TNF-α and IL-2 secretion level was similar to their PD-1$^-$ $\gamma\delta$ T counterparts.[41] Furthermore, PD-1 expression on the surface of $\gamma\delta$ T cells was down-regulated in patients with complete remission after chemotherapy. These findings suggest PD-1$^+$ $\gamma\delta$ T cells were highly activated or immune exhausted with unique cytokine secretion profile distinct from $\alpha\beta$ T cells.[41] Peripheral terminally differentiated Vδ2 T cells of breast cancer patients were demonstrated to display exhaustion phenotype with elevated PD-1 expression, which was significantly associated with tumor-draining lymph node invasion.[42] Recently, coexpression of PD-1, Tim-3, CD39 with TIGIT was reported on tumor-infiltrating Vδ1 T cells in ovarian cancer, implying an increased state of exhaustion.[43] A majority of tumor-infiltrating Vγ9$^-$ $\gamma\delta$ T cells were detected in both lymphomas and solid tumors, which is quantitatively correlated with tissue residency and exhaustion and response to immune checkpoint therapy.[44] However, tissue resident memory $\gamma\delta$ T cells with PD-1 high expression were reported to maintain the capacity to produce IFN-γ upon stimulation,[45] suggesting the expression of IRs does not always mark exhausted $\gamma\delta$ T cells and can also be tightly linked to $\gamma\delta$ T cell activation and differentiation. Recently, we have identified a $\gamma\delta$ T subset characterized by high expression of PD-1 that were significantly increased in colorectal cancer tissues. These tumor-infiltrating PD-1$^+$$\gamma\delta$ T cells express tissue resident, activation, and exhaustion markers, and maintain the capacity of a certain level of GzmB and Perforin secretion (unpublished data). The above evidence has demonstrated that activated $\gamma\delta$ T cells can also exhibit exhaustion features both in chronic infection and tumor as described in CD8$^+$ T cells, characterized but not restricted by simultaneous and progressive high expression of immune checkpoints.

3.3 | The heterogeneity of exhausted $\gamma\delta$ T cells

Distinct from $\alpha\beta$ T cells, high PD-1 expression is not necessary for defining $\gamma\delta$ T cell exhaustion. In acute dengue infection, high TIM-3 but not PD-1 expression contributes to the impairment of IFN-γ production by circulating Vδ2 T cells.[46] Healthy aging and HIV infection independently drive TIGIT and multi-IR expression on $\gamma\delta$ T cells. CD160$^+$ $\gamma\delta$ T cells are a potential resting/precursor population to

TIGIT$^+$, TIGIT$^+$CD160$^+$, and PD-1$^+$TIGIT$^+$CD160$^+$ subsets and such IR expression is suggestive of an activated or exhausted state.[47]

In addition to the role in infection, the capability to provide an early source of TNF-α and IFN-γ early is critical to $\gamma\delta$ T cell-mediated anti-tumor response.[48,49] In AML patients, the PD-1$^+$TIM-3$^-$ Vδ2 subset presented higher TNF-α and IFN-γ expression than the PD-1$^+$TIM-3$^+$ subset, implicating the up-regulation of PD-1 alone was insufficient to indicate functional impairment of $\gamma\delta$ T cells.[50] $\gamma\delta$ T cells display an exhaustion phenotype defined by increased TIGIT expression and TIGIT$^+$CD226$^-$ $\gamma\delta$ T cells may predict poor prognosis in non-M3 AML patients.[51] These evidence demonstrates that $\gamma\delta$ T cells display a vast heterogeneity according to the IR expression and functional impairment (Figure 1). The existence of progenitor cells and whether the effector function of $\gamma\delta$ T cells could be enhanced by stimulating progenitor cell proliferation and differentiation to terminal exhaustion state needs further study.

3.4 | Molecular insights into $\gamma\delta$ T cell exhaustion

The transcription factors including TOX, PTPN2, TCF-1, and Eomes are involved in the regulation of T cell exhaustion.[2,52,53,54] EOMES^{hi}PD1^{hi} T cells represent a terminal progeny subsets of exhausted T cells with higher coexpression of other IRs and limited proliferative capacity.[55] Similarly, mouse Eomes^{hi} $\gamma\delta$ T cells coexpressed Th1 lineage-related factors such as CD27, T-bet, and Ly6C, displayed an exhausted phenotype with high levels of PD-1 and CD160, and were less capable of IFN-γ production, highlighting Eomes as a marker for the differentiation exhaustion of Th1-like effector $\gamma\delta$ T cells.[56]

In human, it has been reported that the impairment of IL-23-induced expansion of Vδ2 T cells driven by tuberculosis appears to be different from T-cell exhaustion linked to PD-1 signaling.[37] Tuberculosis might inhibit the STAT3/JAK2 signaling pathway in Vδ2 T cells, leading to $\gamma\delta$ T cell exhaustion in response to IL-23/HMBPP costimulation. While the expansion of Vδ2 T cells induced by IL-23 cannot be restored via blockade of the PD-1 signaling.[37] The molecular mechanisms of $\gamma\delta$ T cell exhaustion are poorly explored, and a clearer molecular understanding of $\gamma\delta$ T cell exhaustion could help reveal new therapeutic targets for persistent infection and cancer.

3.5 | The reinvigoration potential of exhausted $\gamma\delta$ T cells

The functional activity of $\gamma\delta$ T cells is strikingly modulated by their activation level and activation pathway.[57] The functional remodeling of exhausted $\gamma\delta$ T cells is rarely studied, and most studies focus on PB $\gamma\delta$ T cells. Zoledronic acid (Zol)+IL-2 activates human PB $\gamma\delta$ T cells in vitro to induce up-regulation of PD-1 expression, and anti-PD-1 monoclonal antibody can enhance IFN-γ secretion of PD-1$^+$ $\gamma\delta$ T cells.[58] Blockage of PD-1 can enhance the secretion of GzmB and lysosomal-associated membrane protein from PB PD-1$^+$ $\gamma\delta$ T cells induced by histone deacetylase inhibitors, thus enhancing the antitumor effect of
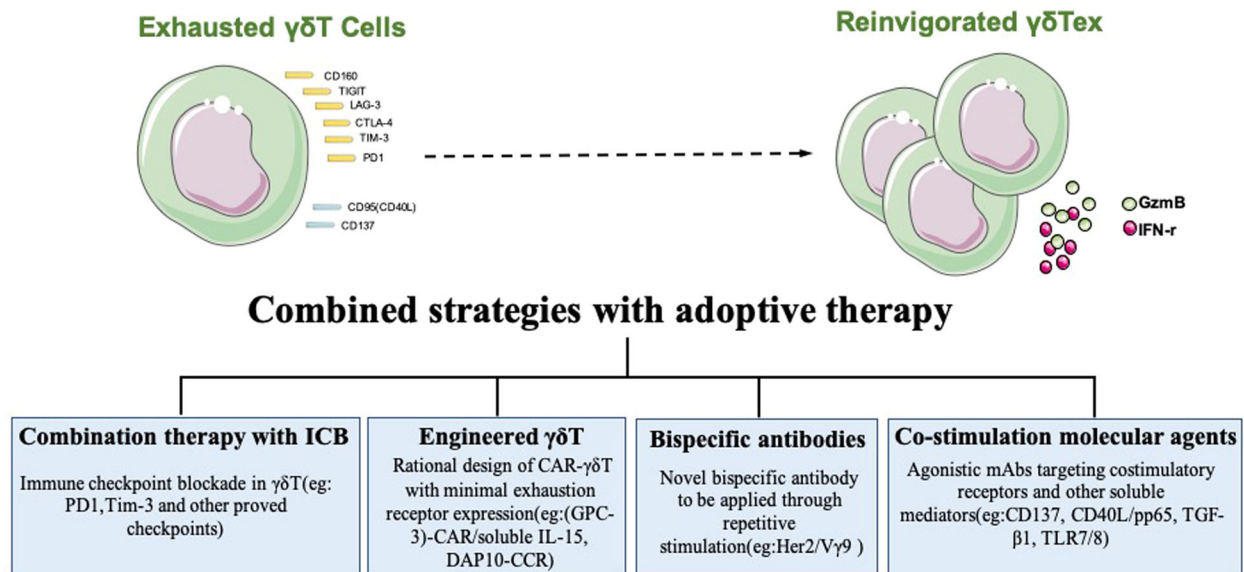
FIGURE 1 Expression of immune checkpoints on exhausted $\gamma\delta$ T cells and strategies to improve $\gamma\delta$ T cell-based immunotherapy.

PD-1$^+$ $\gamma\delta$ T cells.[59] Anti-PD-L1 monoclonal antibodies reversed the antigen-activated killing activity of PB PD-1$^+$ $\gamma\delta$ T cells against PD-L1$^+$ lymphoma cells,[60] demonstrating that PD-1 delivered a coinhibitory signal and blocking the PD-1 signal enhanced $\gamma\delta$ T cell function. TGF-β1 potentiates Vγ9Vδ2 T cell adoptive immunotherapy of cancer.[61] TGF-β has been shown to repress mammalian target of mTOR signaling to promote a less exhausted T cell metabolic state, indicating that TGF-β1 may contribute to rescue Vδ2 T cell exhaustion.[61] TLR7/8 activation decreased the potential exhaustion of Vδ2 T cells in the Zol+IL-2 culture.[62] The only study on $\gamma\delta$ T cells in tumor tissues has shown that blocking PD-1 enhances the antitumor cytotoxic activity of follicular lymphoma infiltrating PD-1$^+$CD16$^+$ $\gamma\delta$ T cells and the antibody-dependent cellular cytotoxicity.[63] Recently, PD-1$^+$ $\gamma\delta$ T cells were reported to accumulate in tumor tissue of colorectal cancer with mismatch repair gene defects, and highly expressed activation-related markers such as CD69, CD38, and HLA-DR. These PD-1$^+$ $\gamma\delta$ T cells can produce IFN-γ, GzmB, and perforin stimulated by PMA, implicating that fully activated PD-1$^+$ $\gamma\delta$ T cells displayed potentially cytotoxic activity in the antitumor immune response and are the potential target cells treated by immunocheckpoint blockade (ICB).[64] These evidence demonstrates that exhausted $\gamma\delta$ T cells have the reinvigoration potential, providing opportunities for antitumor intervention.

4 | THE STRATEGIES TO OVERCOME EXHAUSTION AND IMPROVE $\gamma\delta$ T CELL-BASED IMMUNOTHERAPY

In view of the direct recognition characteristics, cytolytic activity, and interaction with other immune cells, $\gamma\delta$ T cells have irreplaceable advantages in immunotherapy. Many clinical trials have been con-

ducted to evaluate the antitumor role of $\gamma\delta$ T cells via in vivo activation, adoptive cell transfer, and genetic engineering for cancer treatment.[65] The MHC-independent antitumor activity endows $\gamma\delta$ T cells potent promising for allogeneic adoptive immunotherapy.

Recently, a phase I clinical trial in 132 late-stage lung or liver cancer patients validated the clinical safety and survival benefit of allogeneic Vγ9Vδ2 T-cell immunotherapy.[66] Despite of the therapeutic efficiency, the loss of the $\gamma\delta$ T cell persistent response is likely due to activation-induced exhaustion and cell death due to repeated treatments with PAg. In avoid of Vδ2 T cell exhaustion through repetitive PAg stimulation, novel bispecific antibody Her2/Vγ9 were designed and applied after initially activated by PAg and IL-2 in vivo.[67] This attempt provides a tool to further increase $\gamma\delta$ T cytotoxicity when PAg failed because of exhaustion. Therefore, it is urgent and necessary to develop combination strategies for $\gamma\delta$ T cell-based cancer treatment.

Different strategies have been developed to improve the antitumor effect of $\gamma\delta$ T cell immunotherapy for clinical application (Figure 1).[68] Activated $\gamma\delta$ T cells highly expressed PD-1, suggesting the potential for a combination therapy harnessing adoptive $\gamma\delta$ T cell therapy and ICB.[69] PD-1 checkpoint blockade has been demonstrated to enhance the effectiveness of adoptive immunotherapy with human $\gamma\delta$ T cells in treating prostate tumors in a preclinical model.[70] Expanded and activated polyclonal Vδ1 cells costimulated by CD40L and CMV antigen-pp65 maintains the memory phenotype without inducing overexpression of exhaustion markers, representing an attractive antitumor therapeutic option.[71] In influenza virus infection, the expression of CD137 was inducible in Vγ9Vδ2 T cells following continuous antigen stimulation. CD137$^+$ Vγ9Vδ2 T cells displayed more potent antiviral activity than their CD137$^-$ counterparts both in vitro and in vivo.[72] In this infectious model, the efficiency of CD137 costimulation for Vγ9Vδ2 T cell activation, proliferation, survival, and effector

function, provides a novel strategy of combination targeting CD137 with $\gamma\delta$ T cell-based immunotherapy to improve the antitumor therapeutic efficacy.[72]

The expression of a second-generation CD19-CAR (chimeric antigen receptor) on Vδ2 T cells was reported to be associated with significant increase in exhaustion markers.[73] While DAP10-CCR (chimeric costimulatory receptor) was designed based on the important role of NKG2D in $\gamma\delta$ T cell activation, which had no effect on $\gamma\delta$ T cell exhaustion profile.[73] Expanded glypican-3 (GPC-3)-CAR/soluble IL-15 Vδ1 T cells were reported to display robust antitumor efficacy against hepatocellular carcinoma (HCC) with minimal exhaustion receptor expression.[74] These studies demonstrated that rational design of CAR-$\gamma\delta$ T could overcome functional exhaustion. Further clinical studies examining the combination therapy with ICB, costimulation molecular agents, bispecific antibodies, and engineered $\gamma\delta$ T cell immunotherapy are needed to ensure successful clinical application of $\gamma\delta$ T cell-based antitumor immunotherapy.

5 | CONCLUDING REMARKS

T cell exhaustion is the core of immune imbalance and $\gamma\delta$ T cells are the critical participants in this process but are rarely studied compared to $\alpha\beta$ T cells. $\gamma\delta$ T cell exhaustion is characterized by the high expression of IRs and decreased cytokine production distinct from conventional $\alpha\beta$ T cells. High expression of PD-1 is not always associated with functional impairment and is even not necessary for defining $\gamma\delta$ T cell exhaustion. $\gamma\delta$ T cell exhaustion is more complex and unique, and there might be more accurate molecules in defining exhausted $\gamma\delta$ T cells. In summary, $\gamma\delta$ T cells show promising therapeutic potential in antitumor treatment, but the study of $\gamma\delta$ T cell exhaustion is still in its infancy. Therefore, fully deciphering the characteristics, heterogeneity, and mechanisms of $\gamma\delta$ T cell exhaustion will pave the way for combined strategies to overcome exhaustion and enhance antitumor immunity.

AUTHORSHIP

Di Chen, Yinglu Guo, and Jiahuan Jiang contributed in literature collection and manuscript writing. Pin Wu and Ting Zhang contributed in review discussion and language editing. Dang Wu, Jian Huang, and Qichun Wei participated in the design and review of the manuscript. Di Chen, Yinglu Guo, and Jiahuan Jiang contributed equally to this work.

DISCLOSURE

None of the authors have any conflict of interests.

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