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# Dual Face of V $\gamma$ 9V $\delta$ 2-T Cells in Tumor Immunology: Anti- versus Pro-Tumoral Activities

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V $\gamma$ 9V $\delta$ 2-T cells are considered as potent effector cells for tumor immunotherapy through directly killing tumor cells and indirectly regulating other innate and adaptive immune cells to establish antitumoral immunity. The antitumoral activity of V $\gamma$ 9V $\delta$ 2-T cells is governed by a complicated set of activating and inhibitory cell receptors. In addition, cytokine milieu in tumor microenvironment can also induce the pro-tumoral activities and functional plasticity of V $\gamma$ 9V $\delta$ 2-T cells. Here, we review the anti- versus pro-tumoral activities of V $\gamma$ 9V $\delta$ 2-T cells and discuss the mechanisms underlying the recognition, activation, differentiation and regulation of V $\gamma$ 9V $\delta$ 2-T cells in tumor immunosurveillance. The comprehensive understanding of the dual face of V $\gamma$ 9V $\delta$ 2-T cells in tumor immunology may improve the therapeutic efficacy and clinical outcomes of V $\gamma$ 9V $\delta$ 2-T cell-based tumor immunotherapy.

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## INTRODUCTION

Human  $\gamma\delta$ -T cells can be classified into two main subsets depending on the expression of T cell receptor (TCR)  $\delta$  chain (1). V $\delta$ 1  $\gamma\delta$ -T cells with different V $\gamma$  elements account for the majority of mucosal-associated lymphoid tissue  $\gamma\delta$ -T cells, and they mediate the immune responses to *Listeria monocytogenes*, Cytomegalovirus, and certain hematological malignancies (2, 3). In contrast,  $\gamma\delta$ -T cells bearing the V $\delta$ 2 gene with the co-expression of the V $\gamma$ 9 chain (V $\gamma$ 9V $\delta$ 2-T cells) are abundant in the peripheral blood and lymphoid organs of most healthy individuals, and they are involved in the first line of the immune responses to mycobacteria, Epstein-Barr virus (EBV), and some solid tumors (1, 2, 4–10).

The V $\gamma$ 9V $\delta$ 2-T cell is an important component of immune effector cells that contribute to tumor immunosurveillance against many types of tumors, such as lymphoma, myeloma (11, 12), hepatocellular, and colorectal carcinoma (13), and prostate (14), lung (15), colon (16), breast (17), and ovary cancers (18). Recently, our group discovered a novel strategy to treat EBV-induced B-cell lymphoma by boosting V $\gamma$ 9V $\delta$ 2-T cell immunity (19, 20). V $\gamma$ 9V $\delta$ 2-T cells can directly kill tumor cells through the secretion of cytolytic molecules or indirectly prime and modulate immunological functions of other innate and adaptive immune cells to develop and establish profound antitumor immunity (21, 22). Unlike the conventional  $\alpha\beta$ -T cells, the V $\gamma$ 9V $\delta$ 2-T cell is a member of the non-conventional lymphocyte family (23), and the antigen recognition of V $\gamma$ 9V $\delta$ 2-T cells is major histocompatibility complex (MHC)-unrestricted (24, 25). Recent growing evidence has suggested that the V $\gamma$ 9V $\delta$ 2-T cell is one of the most attractive candidates for antitumor immunotherapy. In this review, we will discuss recent advances in the basic V $\gamma$ 9V $\delta$ 2-T cell research and evidence from clinical applications of V $\gamma$ 9V $\delta$ 2-T cells. Most importantly, this review will provide an overview of the current knowledge about mechanisms of V $\gamma$ 9V $\delta$ 2-T cell mediated antitumor immunity and the potential limitations of V $\gamma$ 9V $\delta$ 2-T cell-based immunotherapy.

## PLASTICITY OF V $\gamma$ 9V $\delta$ 2-T CELLS IN TUMOR IMMUNITY

The antitumor activity of V $\gamma$ 9V $\delta$ 2-T cells is influenced by their functional plasticity that is driven by environmental factors (26). Similar to  $\alpha\beta$ -T cells, V $\gamma$ 9V $\delta$ 2-T cells also display the plasticity that contributes to their functional specialization (27). Accumulating evidence indicates that V $\gamma$ 9V $\delta$ 2-T cells can differentiate into the cells with various characteristics associated with Th1-like, Th2-like, Th17-like, follicular T helper cells (Tfh)-like, or regulatory T cells (Treg)-like characteristics (26).

Upon phosphoantigen stimulation, V $\gamma$ 9V $\delta$ 2-T cells preferentially differentiate into Th1-like cells with profound IFN- $\gamma$  and TNF- $\alpha$  responses (28, 29). Th1-like V $\gamma$ 9V $\delta$ 2-T cells can be induced through isopentenyl pyrophosphate (IPP) activation with IL-12 and anti-IL-4 antibody, and even the addition of IL-21 (30–32). Phosphoantigens and IL-2 can promote their cytolytic activity by upregulating CD56 expression and increasing granule secretion (32, 33). Interestingly, V $\gamma$ 9V $\delta$ 2-T cells can also be polarized into Th2-like cells, which are characterized by increased secretion of IL-4 upon stimulation with IPP, IL-4, and anti-IL-12 antibody (30).

V $\gamma$ 9V $\delta$ 2-T cells with Tfh-like functions can be induced by IL-21 and phosphoantigens stimulation (34, 35). These Tfh-like V $\gamma$ 9V $\delta$ 2-T cells also have the capability to migrate into the lymph node germinal center (35). Similar to Tfh CD4<sup>+</sup> T-cells, cell-to-cell contact is necessary for the B cell helper activity of the Tfh-like V $\gamma$ 9V $\delta$ 2-T cells.

IL-17-producing  $\gamma\delta$ -T cells have been extensively discussed in the murine model (36). Recent findings also suggested that human  $\gamma\delta$ -T cells can produce IL-17 (37, 38). Some groups reported that naïve V $\gamma$ 9V $\delta$ 2-T cells can be induced into the Th17-like phenotype or mixed Th1/Th17-like phenotype (39–41). V $\gamma$ 9V $\delta$ 2-T cells require IL-1 $\beta$ , IL-23, and TGF- $\beta$ , but not IL-6, for differentiation into Th17-like cells (41). In human colorectal cancer (CRC), activated inflammatory dendritic cells (DCs) polarize V $\gamma$ 9V $\delta$ 2-T cells into IL-17-producing  $\gamma\delta$ -T cells, which can secrete high levels of IL-17 in an IL-23-dependent manner (42).

Upon stimulation of IPP in the presence of IL-15 and TGF- $\beta$ , the V $\gamma$ 9V $\delta$ 2-T cells can be induced into transcription factor forkhead box P3 (Foxp3)-expressing Treg-like  $\gamma\delta$ -T cells with regulatory/immunosuppressive function (43). When combined with IL-15, IL-2, TGF- $\beta$ , and phosphoantigen stimulation, decitabine can also induce the immunoregulatory activity of V $\gamma$ 9V $\delta$ 2-T cells (44).

Therefore, V $\gamma$ 9V $\delta$ 2-T cells can be induced into different functional subsets depending on the cytokine milieu in the tumor microenvironment.

## ANTITUMORAL RESPONSE OF V $\gamma$ 9V $\delta$ 2-T CELLS

The antitumoral activity of V $\gamma$ 9V $\delta$ 2-T cells has been well studied (45, 46). Their antitumoral activity mostly relies on the recognition of phosphoantigens and stressed molecules by TCR (47) and

other cellular receptors (48), like NKG2D (49). These receptors can respond to perturbations in the endogenous isoprenoid biosynthesis and the presence of “danger signals” that occur during cell stress and malignant transformation. Other molecules can act as co-stimulatory signals to regulate the antitumoral activity of V $\gamma$ 9V $\delta$ 2-T cells (48). Apart from the direct cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells, these cells can also stimulate and regulate other immune components to establish the antitumoral activity (7, 50). Last, but not least, the homing receptors expressed on V $\gamma$ 9V $\delta$ 2-T cells lead cell migration to tumor sites where they display broad and potent antitumoral activity (51).

## Mechanisms of Tumor Cell Recognition

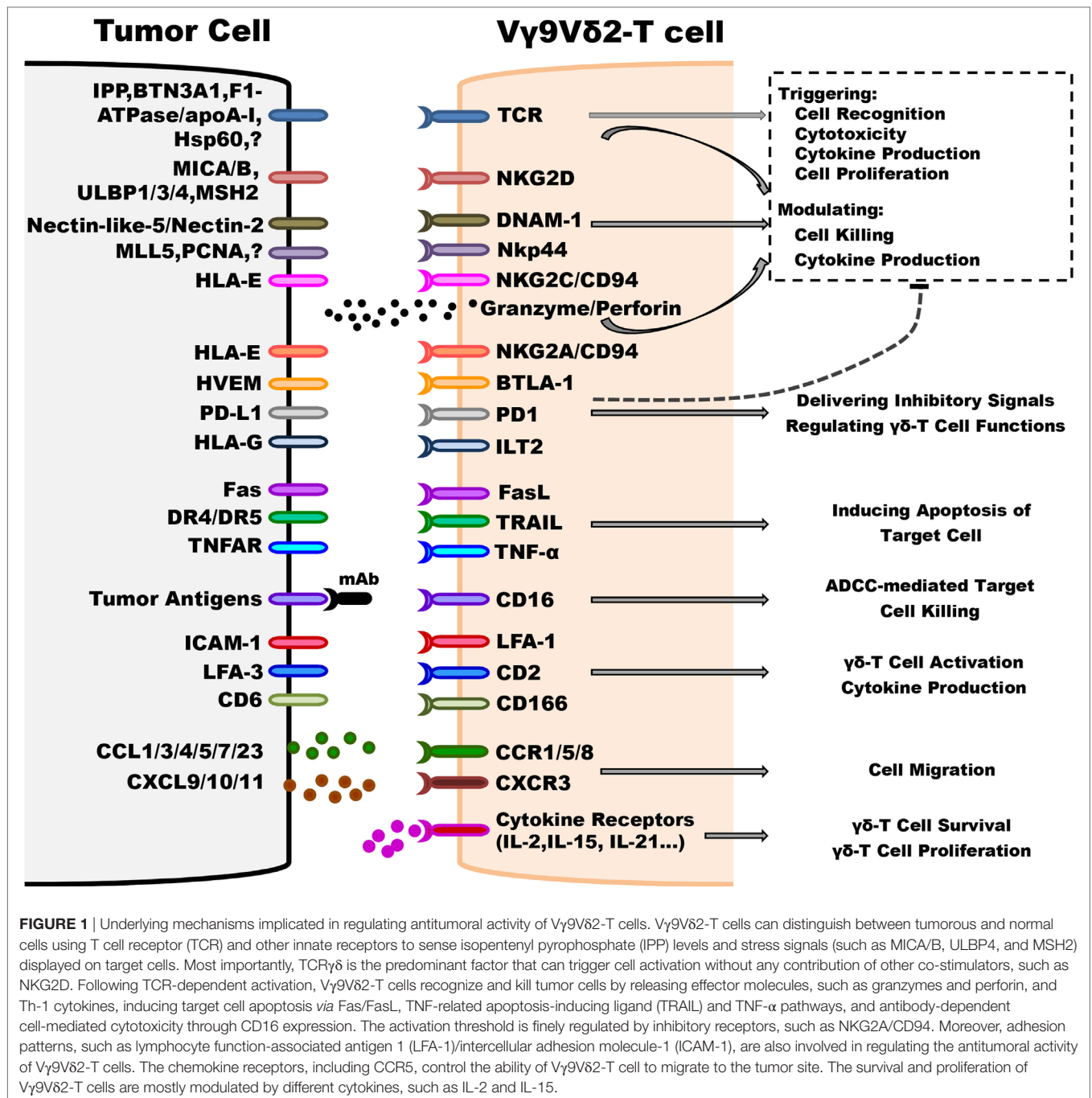
### TCR $\gamma\delta$ Recognition

#### *Non-Peptide Ligands*

In tumor immunosurveillance, although V $\gamma$ 9V $\delta$ 2-T cells share some features with other immune cells, such as NK cells and  $\alpha\beta$ -T cells (21, 52), a distinctive characteristic of V $\gamma$ 9V $\delta$ 2-T cells is the TCR-dependent recognition of non-peptidic phosphorylated antigens (also called phosphoantigens). V $\gamma$ 9V $\delta$ 2-TCR recognizes the molecules that are normally expressed in specific conditions, such as when cells undergo stressful conditions. For example, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and IPP are the products of the prokaryotic isoprenoid pathway and the mevalonate isoprenoid pathway, respectively (53–56). All of these products can be recognized by V $\gamma$ 9V $\delta$ 2-T cells and lead to subsequent activation of V $\gamma$ 9V $\delta$ 2-T cells. The levels of these naturally occurring metabolites are too low to be detected as a dangerous signal by V $\gamma$ 9V $\delta$ 2-T cells in normal cells. The dysfunctional metabolism of malignant tumor cells can result in the accumulation of endogenous phosphoantigens that are recognized by V $\gamma$ 9V $\delta$ 2-T cells. Furthermore, bisphosphonates (such as pamidronate and zoledronate) can cause tumor cells to be more sensitive to the cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells *via* inhibition of the farnesyl pyrophosphate synthase enzyme in the isoprenoid pathway, which leads to IPP accumulation (57). Meanwhile, current findings have indicated that several molecules, such as F1-ATPase (combined with apolipoprotein A-I, called Apo A-I) (58, 59) and butyrophilin 3A1 (BTN3A1, CD277), might be involved with phosphoantigens to mediate V $\gamma$ 9V $\delta$ 2-T cells activation (60, 61) (**Figure 1**).

#### *Peptide Ligands*

- (1) Self ligands: in addition to non-peptide ligands, V $\gamma$ 9V $\delta$ 2-T cells can also recognize some molecules of cellular origin, which could be capable of indicating cellular stress or malignant transformation (49, 62). Several self-antigens have been confirmed to bind to V $\gamma$ 9V $\delta$ 2-TCR, including heat shock protein-60 (HSP 60) (63), U16-binding protein 4 (ULBP-4) (64), human MutS homolog 2 (hMSH2) (63, 65), and F1-ATP synthase (F1-ATPase) (59, 66). The expressions of these proteins are shown to be upregulated on the surface of different tumor cells and they can promote recognition by V $\gamma$ 9V $\delta$ 2-T cells. It is intriguing that ULBP-4 and hMSH2 can also bind to NKG2D to induce the cytotoxicity



of V $\gamma$ 9V $\delta$ 2-T cells against tumor cells through TCR and NKG2D engagement (63–65) (Figure 1).

(2) Non-self ligands: tetanus toxoid (67), Ig  $\lambda$  light chain (68), and viral proteins, such as glycoprotein I from *Herpes simplex* (69) and staphylococcal enterotoxin A (70), are antigens that were reported to be capable of stimulating V $\gamma$ 9V $\delta$ 2-T cell responses.

### Cell Receptor Engagement

Besides the V $\gamma$ 9V $\delta$ 2-TCR engagement, some other cellular receptors, especially the NK receptors (NKR), are involved

in the effective triggering of antitumoral responses of V $\gamma$ 9V $\delta$ 2-T cells (49) (Figure 1). Together with previous studies, we reported that NKG2D can bind to its ligands (71), such as MICA, MICB, and ULBP-1, -2, -3, and -4, which are expressed in different tumors, including leukemia, lymphoma, ovarian, and colon carcinoma (72–74). In particular, the high expression level of ULBP1 indicates the susceptibility of lymphoma to V $\gamma$ 9V $\delta$ 2-T cell-mediated cytotoxicity (74). Furthermore, ULBP-4 expression is detected on the cell surface of EBV-transformed lymphoid cells lines as well as on colon, ovarian, and liver cancer cells (64).

Another NKR implicated in tumor recognition by V $\gamma$ 9V $\delta$ 2-T cells is the DNAX accessory molecule-1 (DNAM-1) (75, 76). Nectin-like-5 and Nectin-2, ligands of DNAM-1, are expressed on most hepatocellular carcinoma (HCC) cell lines (75). In addition, some V $\gamma$ 9V $\delta$ 2-T cells also express Nkp44, which can mediate their cytotoxic activity against multiple myeloma (MM) cell lines (77, 78).

Similar to NK cells, V $\gamma$ 9V $\delta$ 2-T cells also express high levels of CD16 (Fc $\gamma$ R III) upon phosphoantigen stimulation (79), and thus leading to antibody-dependent cell-mediated cytotoxicity (ADCC) against tumor cells (80–83).

## $\gamma\delta$ -T CELLS ACT AS EFFECTOR CELLS

### V $\gamma$ 9V $\delta$ 2-T Cells with Killer Functions

Interaction of TCR and/or NKG2D with their respective ligands can stimulate the activation of V $\gamma$ 9V $\delta$ 2-T cells. Once activated, V $\gamma$ 9V $\delta$ 2-T cells secrete IFN- $\gamma$  and TNF- $\alpha$ , and increase the release of antitumor effector molecules, such as perforin and granzymes. The DNAM-1 signaling pathway can positively regulate the cytotoxic activity and IFN- $\gamma$  secretion of V $\gamma$ 9V $\delta$ 2-T cells against a broad range of tumors.

Antibody-dependent cell-mediated cytotoxicity mediated by V $\gamma$ 9V $\delta$ 2-T cells can be activated *via* the binding of CD16 to antibodies, such as rituximab, trastuzumab, atumumab, and alemtuzumab, coated on the certain tumor cells (80–83).

In addition, activated V $\gamma$ 9V $\delta$ 2-T cells can also induce tumor cell apoptosis *via* TNF-related apoptosis-inducing ligand and Fas/FasL pathways (84–86).

### V $\gamma$ 9V $\delta$ 2-T Cells with Helper Functions

Activated V $\gamma$ 9V $\delta$ 2-T cells may secrete chemokines, such as C-C motif chemokine ligand 3 (CCL3), CCL4, C-X-C motif chemokine 10 (CXCL10), and CXCL13, to recruit  $\alpha\beta$ -T cells, B cells, NK cells, and macrophages/DCs to the tumor site (22, 31, 87). Activated V $\gamma$ 9V $\delta$ 2-T cells not only stimulate DC maturation and macrophage activation (88, 89), but also induce CD4<sup>+</sup> and CD8<sup>+</sup>  $\alpha\beta$ -T cell differentiation for enhancing antitumoral activity through secretion of IFN- $\gamma$  and TNF- $\alpha$  and upregulation of CD40L expression (88, 90, 91). Moreover, activated V $\gamma$ 9V $\delta$ 2-T cells can mimic antigen presentation cell (APC) functions by stimulating the antitumoral activity of  $\alpha\beta$ -T cells through the upregulation of several surface molecules, such as MHC I and II, CD40, CD83, and CD86 (92, 93). Activated V $\gamma$ 9V $\delta$ 2-T cells can also present glycolipid antigens to iNKT cells through the uptake of CD-1d by trogocytosis. Subsequently, activated iNKT cells can trigger downstream reactions to boost antitumoral immunity (94). Furthermore, activated CD137L<sup>+</sup> V $\gamma$ 9V $\delta$ 2-T cells can stimulate the antitumoral activity of NK cells by acting as a co-stimulator through interacting with CD137 expressed on NK cells. Thus, the costimulatory signals can upregulate the cytotoxic activity of NK cells to kill the solid tumor cells, which usually show resistance to NK cells (95). Although the contribution of IL-17 in tumor surveillance is still controversial (96–99), some *in vitro* and *in vivo* evidence have indicated that IL-17 secreted by V $\gamma$ 9V $\delta$ 2-T cells might be involved in antitumoral immune responses *via* indirect mechanisms (96) (Figure 2).

### V $\gamma$ 9V $\delta$ 2-T Cells with Homing Functions

CC and CXC chemokines are produced by most tumor cells, such as breast, cervix, pancreatic, and ovarian tumor cells (57). To provide optimal protection against tumor cells, the cytotoxic V $\gamma$ 9V $\delta$ 2-T cells must migrate from the bloodstream to tumor site (33, 100, 101) (Figure 2). Unlike V $\delta$ 1  $\gamma\delta$ -T cells which express lymph node homing chemokine receptor C-C chemokine receptor 7 (CCR7), the circulating V $\gamma$ 9V $\delta$ 2-T cells preferentially express inflammatory homing chemokine receptor CCR5, which can mediate the migration of V $\gamma$ 9V $\delta$ 2-T cells to CCR5 ligands that are expressed in tumor cells (20, 102). Apart from the predominantly expressed CCR5, V $\gamma$ 9V $\delta$ 2-T cells also express other chemokine receptors including CCR1, CCR8, and C-X-C motif chemokine receptor 3, which are involved in regulating the homing ability of V $\gamma$ 9V $\delta$ 2-T cells (13, 87, 103).

In addition, some of the adhesion molecules, such as lymphocyte function-associated antigen 1 (LFA-1), L-selectin, and CD44v6, are also involved in the migration of V $\gamma$ 9V $\delta$ 2-T cells to the tumor site (104) (Figure 1).

## REGULATION MECHANISMS OF $\gamma\delta$ -T CELL ACTIVATION

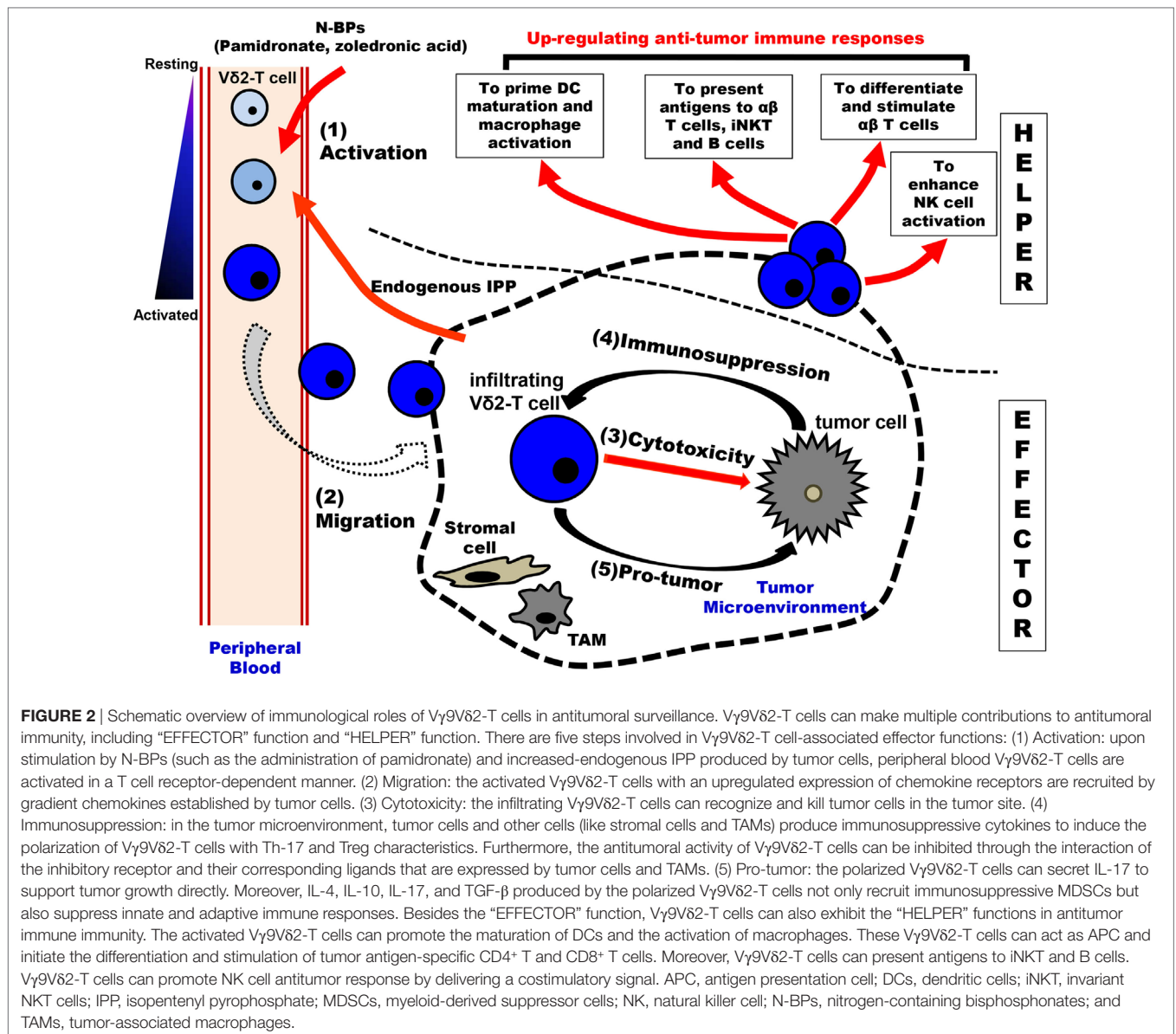
### V $\gamma$ 9V $\delta$ 2-T Cell Activation

In tumor immunosurveillance, the antitumoral activities of V $\gamma$ 9V $\delta$ 2-T cells is composed of several steps (23): (1) detecting and sensing any types of stress signals in a non-MHC-restricted manner; (2) producing a huge quantity of effector molecules that can kill tumor cells through direct and indirect mechanisms; and (3) exhibiting potent cytotoxic and cytolytic activities against a broad panel of tumors. In order to tightly monitor the initiation and development of tumorigenesis, the activation of V $\gamma$ 9V $\delta$ 2-T cells is triggered by TCR-mediated recognition and precisely regulated by various innate immune cells and cytokine receptors. Furthermore, the activation and differentiation of V $\gamma$ 9V $\delta$ 2-T cells are molecularly controlled by surface receptors that capture key extracellular cues and convey downstream intracellular signals (105). More detailed information can be found in Figure 1.

### Regulation of V $\gamma$ 9V $\delta$ 2-T Cell Activation Regulation by TCR

V $\gamma$ 9V $\delta$ 2-T cells mainly recognize the non-peptide ligands, phosphoantigens, which are shown to act as a trigger signal to activate V $\gamma$ 9V $\delta$ 2-T cells (105). Cipriani et al. concluded that IPP induces rapid and persistent PKC-dependent phosphorylation of ERK 1/2, p38 MAPK, and JNK, which can lead to the activation of NF- $\kappa$ B and AP-1 and the secretion of IFN- $\gamma$  and TNF- $\alpha$  (106). Some studies have shown that crosslinking of CD3 and phosphoantigens stimulation can induce highly sustained calcium signaling in V $\gamma$ 9V $\delta$ 2-T cells *via* phosphorylation of Zap70, Pi3K, LAT, ERK 1/2, and p38 MAPK (105, 107, 108).

Due to upregulated self-antigen expressions in the transformed tumor cells, V $\gamma$ 9V $\delta$ 2-T cells discriminate these malignant cells by directly binding to these self-antigens (49), such as HSP 60,



ULBP-4, hMSH2, and F1-ATPase, which can enhance the activation and cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells.

### Regulation by NK-Associated Receptors

The expression of NK-associated receptors is a distinguishing feature of V $\gamma$ 9V $\delta$ 2-T cells that mediates the recognition of stress ligands expressed by normal cells during infection and cell transformation by different pathogens. NKG2D and its ligands, such as MICA/B and ULBPs (64, 74, 109), are well-defined NK-associated receptors and ligands for V $\gamma$ 9V $\delta$ 2-T cells. For example, expressions of these ligands are normally induced during cellular stress, such as DNA damage that occurs in tumor cells.

Whether NKG2D plays a primary stimulatory or a co-stimulatory role in V $\gamma$ 9V $\delta$ 2-T cells is still being debated (49). Some evidence have indicated that an additive effect of the NKG2D pathway on TCR-mediated activation through increasing

cytokine production (110), and upregulating intracellular calcium mobilization and enhancing cytotoxic activity (105). On the other hand, other studies have reported that the NKG2D pathway alone can activate V $\gamma$ 9V $\delta$ 2-T cells without TCR engagement (111), and the blockade of the NKG2D pathway, but not the blockade of  $\gamma\delta$ -TCR, can inhibit the cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells against some hematological tumors (74).

DNAX accessory molecule-1 is another NK-associated receptor that regulates V $\gamma$ 9V $\delta$ 2-T cells, whereby blocking the interaction between DNAM-1 and its ligands, such as Nectin-like-5 and Nectin-2, could impair the cytotoxic capacity and IFN- $\gamma$  production of V $\gamma$ 9V $\delta$ 2-T cells against HCC cells (75).

A recent study demonstrated that NKp44, a natural cytotoxicity receptor, is involved in the cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells against MM cell lines that lack the expression of NKG2D ligands (77). Because V $\gamma$ 9V $\delta$ 2-T cells are potentially highly self-reactive,

TCR-mediated activity needs to be tightly controlled by a close interplay between activating and inhibitory NKR (105). Like NK cells, most human circulating V $\gamma$ 9V $\delta$ 2-T cells express several inhibitory NKRs belonging to the lectin-like receptor family (such as the NKG2A/CD94 heterodimer), or the immunoglobulin (Ig) family [such as Ig-like transcript 2 (ILT-2)]. Upon interaction with the classical and/or non-classical MHC I molecules, the inhibitory signals are delivered by these receptors to V $\gamma$ 9V $\delta$ 2-T cells. This mechanism allows TCR-activated V $\gamma$ 9V $\delta$ 2-T cells to target the tumor cells rather than the normal cells depending on the expression level of the MHC I molecules, and thus preventing self-reactivity while enhancing antitumoral activity (105). In addition, NKG2C/CD94 also can regulate V $\gamma$ 9V $\delta$ 2-T cell effector functions, including cytokine secretion, cell proliferation, and cytotoxic activity (112).

### Regulation by Co-Stimulatory Receptors

There are two main types of costimulatory receptors that are expressed on V $\gamma$ 9V $\delta$ 2-T cells: the Ig and tumor necrosis factor receptor (TNFR) superfamilies. Ribot et al. suggested that CD28, which is an important member of the Ig superfamily, is constitutively expressed on lymphoid V $\gamma$ 9V $\delta$ 2-T cells. Co-stimulation with CD28 promotes the survival and proliferation of V $\gamma$ 9V $\delta$ 2-T cells by enhancing IL-2 production, whereas blocking antibodies target its B7 ligands (CD80 and CD86) can inhibit cell survival and proliferation (113). CD27 is a member of the TNFR superfamily that is expressed in 80% of V $\gamma$ 9V $\delta$ 2-T cells (114). CD27 pathway has been confirmed to play critical roles in co-stimulating V $\gamma$ 9V $\delta$ 2-T cell activation. Furthermore, CD27 is also involved in promoting cell proliferation, upregulating the antiapoptotic gene *Bcl2a1* to maintain cell survival and enhancing IFN- $\gamma$  production and cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells (105, 114). Upon activation, CD30, another member of the TNFR superfamily, is also expressed on V $\gamma$ 9V $\delta$ 2-T cells and it can increase pro-inflammatory cytokine production and promote TCR-induced calcium fluxes (115). A high level of CD137L that is expressed on activated V $\gamma$ 9V $\delta$ 2-T cells can transmit a reversal signal to regulate V $\gamma$ 9V $\delta$ 2-T cell activation (95, 116).

### Regulation by Adhesion Molecules

In addition to TCR and NKR, other surface receptors, such as adhesion molecules, are also important for regulating V $\gamma$ 9V $\delta$ 2-T cell function. To date, several adhesion molecules, including LFA-1/intercellular adhesion molecule-1 (ICAM-1) (117), CD2/LFA-1/3 (118), and CD6/CD166 (119), have been identified as regulators in modulating V $\gamma$ 9V $\delta$ 2-T cell activation.

High expressions of LFA-1 and its ligand, ICAM-1, have been detected on the surface of V $\gamma$ 9V $\delta$ 2-T cells and most of the tumor cell lines, respectively. LFA-1/ICAM-1 adhesive interaction is necessary but not sufficient for IFN- $\gamma$  production and the cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells (117). The interaction between LFA-3 on V $\gamma$ 9V $\delta$ 2-T cells and CD2 on lymphoid cells can stimulate TNF- $\alpha$  secretion by V $\gamma$ 9V $\delta$ 2-T cells (118). Additionally, CD6 is a member of the scavenger receptor family, which is also expressed on the surface of V $\gamma$ 9V $\delta$ 2-T cells. CD6 binds to its ligand, CD166,

which is associated with the capability of tumor cells to activate V $\gamma$ 9V $\delta$ 2-T cells upon phosphoantigen induction (119).

Most importantly, the interactions between LFA-1/ICAM-1 and CD2/LFA-3 can stabilize the immunological synapses after V $\gamma$ 9V $\delta$ 2-TCR/phosphoantigen triggers the formation of immunological synapses (120, 121).

### Regulation by Toll-Like Receptors (TLRs)

The roles of TLRs in regulating the activation of V $\gamma$ 9V $\delta$ 2-T cells are not completely understood. After ligation of TLR2, TLR3, and TLR5, the V $\gamma$ 9V $\delta$ 2-T cells can produce IFN- $\gamma$ , TNF- $\alpha$ , granulocyte-macrophage colony-stimulating factor, CCL3, and CCL5 (122). The triggering of TLR3, 4, 5, and 9 can also induce the early activation of V $\gamma$ 9V $\delta$ 2-T cell and the production of IFN- $\gamma$  (105). In addition, TLR3 and TLR7 agonists can enhance the antitumoral activity of V $\gamma$ 9V $\delta$ 2-T cells against adenocarcinoma cells (123). Nevertheless, these responses require simultaneous stimulation of V $\gamma$ 9V $\delta$ 2-TCR (105).

### Regulation by Cytokine Receptors

The interleukin receptors are essential for the development and homeostasis of V $\gamma$ 9V $\delta$ 2-T cells due to the pivotal effect of interleukins on cell proliferation, differentiation, and survival, and the regulation of the immunological functions of the V $\gamma$ 9V $\delta$ 2-T cells. IL-2 and IL-15 can be used to stimulate V $\gamma$ 9V $\delta$ 2-T cell expansion, but they cannot fully induce effector functions without  $\gamma\delta$ -TCR-mediated activation and downstream signals, such as the ERK and AKT pathway (124, 125). IL-12 and IL-18 are reported to be beneficial for differentiation into IFN- $\gamma$ <sup>+</sup> V $\gamma$ 9V $\delta$ 2-T cells (126). Recent studies also indicated that other interleukins, such as IL-23, IL-1 $\beta$  (39, 40, 127), and IL-21 (34, 35), are also involved in the induction of V $\gamma$ 9V $\delta$ 2-T cell plasticity.

### Regulation by Inhibitory Receptors

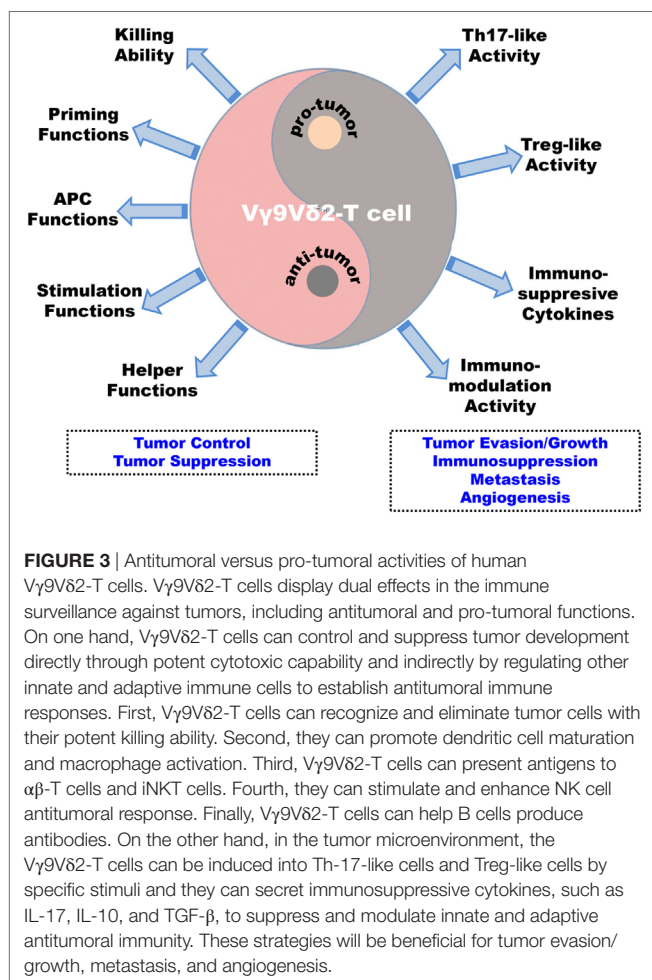
Like inhibitory NKRs, some other inhibitory molecules, such as programmed cell death protein-1 (PD-1) and B- and T-lymphocyte attenuator (BTLA), act in a similar manner in V $\gamma$ 9V $\delta$ 2-T cells. PD-1 and BTLA can negatively regulate V $\gamma$ 9V $\delta$ 2-T cell responses. V $\gamma$ 9V $\delta$ 2-T cells do not normally express detectable PD-1; however, once activated, the expression of PD-1 is upregulated in V $\gamma$ 9V $\delta$ 2-T cells (128). Iwasaki et al. found that the interaction of PD-1 and PD-L1 can attenuate the antitumoral activity of V $\gamma$ 9V $\delta$ 2-T cells by downregulating IFN- $\gamma$  production and lowering cytotoxicity (128). BTLA engagement can downregulate phosphoantigen/ $\gamma\delta$ -TCR-mediated signaling and inhibit V $\gamma$ 9V $\delta$ 2-T cell proliferation, including the response to lymphoma cells (129). Moreover, the HLA-G expressed on the surface of tumor cells can bind to the ILT-2 on V $\gamma$ 9V $\delta$ 2-T cells. This interaction could impair cytokine production and the cytotoxicity of V $\gamma$ 9V $\delta$ 2-T cells (130).

## PRO-TUMORAL RESPONSE OF V $\gamma$ 9V $\delta$ 2-T CELLS

Recently, more evidence indicates that V $\gamma$ 9V $\delta$ 2-T cells can display the functions for promoting tumor development

through direct or indirect strategies (27). As mentioned above, the differentiation of unique subpopulations of V $\gamma$ 9V $\delta$ 2-T cells with immunosuppressive features can be induced in the presence of specific stimuli, such as in the tumor-established microenvironment (Figure 2). For example, V $\gamma$ 9V $\delta$ 2-T cells may display Th2-, Th17-, or Treg-like profile and produce IL-4, IL-17, IL-10, and TGF- $\beta$ , respectively. IL-10 and TGF- $\beta$  are the cytokines with immunosuppressive activity, while IL-10 can impair APC function of DCs, and induce the pro-tumoral functions of V $\gamma$ 9V $\delta$ 2-T cells (131, 132). Some evidence indicates that IL-17 produced by Th17-like V $\gamma$ 9V $\delta$ 2-T cells can directly promote the proliferation and dissemination of tumor cells in breast cancer (133). Most importantly, in the tumor microenvironment, IL-17 plays a critical regulatory role to other immune components, such as MDSCs and macrophage, and thus indirectly influences tumor immunosurveillance (42). Moreover, similar to classical Treg cells, the suppressive effect of regulatory-like V $\gamma$ 9V $\delta$ 2-T cells also depends on cell-to-cell contact *via* CD86/CTLA-4 and PD-L1/PD-1 interactions between activated V $\gamma$ 9V $\delta$ 2-T cells and their responder cells (134) (Figure 3).

More recently, Peters et al. demonstrated that V $\gamma$ 9V $\delta$ 2-T cell is a major source of IL-9 *in vitro* (135). According to this study,



V $\gamma$ 9V $\delta$ 2-T cells can secrete IL-9 when culturing with TGF- $\beta$  and IL-15 and in the absence of IL-4. IL-9-producing CD8<sup>+</sup> T cells and Th9 cells are always considered as new players in antitumoral immune responses (136–138); however, in some studies, IL-9 also displays potential pro-tumoral activity. Qiu et al. found that in ALK<sup>+</sup> anaplastic large-cell lymphoma, IL-9 can promote lymphoma cell proliferation and colony formation by regulating Jak3 activation in an autocrine manner (139). Furthermore, IL-9 can abolish the establishment of immunological memory to tumor re-challenge and exhibits an immunomodulatory role in pro-tumoral immunity (140). Indeed, elevated serum IL-9 was found in the patients with metastatic breast cancer or diffuse large B-cell lymphoma (DLBCL) (141, 142). Meantime, Lv et al. found that IL-9 can support the survival of DLBCL cells and enhance the resistance of these tumor cells to chemotherapeutic drugs by upregulating p21CIP1 genes (141). Thus, in tumor immunity, the exact immunological roles of IL-9-producing V $\gamma$ 9V $\delta$ 2-T cells are still unknown. So far, the evidence for pro-tumoral activity of V $\gamma$ 9V $\delta$ 2-T cells mostly comes from *ex vivo* experiments. More clinical evidence is needed to evaluate the potential pro-tumoral ability of V $\gamma$ 9V $\delta$ 2-T cells *in vivo*.

## THERAPEUTIC EFFECTS OF V $\gamma$ 9V $\delta$ 2-T CELLS IN CLINICAL TRIALS

To date, two main therapeutic strategies based on V $\gamma$ 9V $\delta$ 2-T cells have been proposed for tumor immunotherapy: the *in vivo* expansion of V $\gamma$ 9V $\delta$ 2-T cells by aminobisphosphonates, and the adoptive transfer of *ex vivo*-expanded V $\gamma$ 9V $\delta$ 2-T cells (143). Several clinical trials have been initiated to evaluate the safety and therapeutic efficacy of V $\gamma$ 9V $\delta$ 2-T cell immunotherapy based on *in vivo* and *ex vivo* expansion. The clinical outcomes of these strategies have been confirmed in patients with different types of tumors, including leukemia/lymphoma, melanoma, RCC, hormone-refractory prostate cancer, breast cancer, NSCLC, CRC, MM, gastrointestinal tumors, ovarian cancer, cervical cancer, and bone cancer (45, 57). For instance, Wilhelm et al. first reported that administration of pamidronate and low-dose of IL-2 to expand V $\gamma$ 9V $\delta$ 2-T cells *in vivo* was well tolerated and could induce objective tumor response in patients with low-grade non-Hodgkin lymphoma or MM (18). Lang and coworkers conducted a pilot trial to determine the therapeutic effects of zoledronate with low-dose of IL-2 in patients with RCC (144). Furthermore, zoledronate with low dose of IL-2 have also been tested in treating prostate cancer and advanced breast cancer where partial remissions have been reported (14, 17). Bennouna et al. conducted a phase I study that, after treatment of bromohydrin pyrophosphate (BrHPP) and IL-2, in total of 28 patients, 12 patients had stable disease and 16 had progressive disease after three cycles of administration (145).

Adoptive transfer of zoledronate-expanded V $\gamma$ 9V $\delta$ 2-T cells was reported to induce a complete remission of RCC patient with lung metastasis. (146). Kobayashi et al. also reported that adoptive transfer of V $\gamma$ 9V $\delta$ 2-T cells in combination with zoledronate and IL-2 was well tolerated and the objective clinical responses could



be achieved in some patients with advanced RCC (147). More detailed information about clinical studies of V $\gamma$ 9V $\delta$ 2-T cells had been systematically reviewed in recent articles summarized by Deniger et al. and Kobayashi and Tanaka (45, 148).

Although these two strategies have yielded clinical success, there are still some limitations. For the *in vivo* expansion and activation of V $\gamma$ 9V $\delta$ 2-T cells, one of the limitations is that the sustained proliferative activity is impaired, probably due to an energy or exhaustion of V $\gamma$ 9V $\delta$ 2-T cells induced by the successive infusions of BrHPP and IL-2 (45, 149). Additionally, Kalyan et al. demonstrated that neutrophils in human peripheral blood could uptake of zoledronate and cause the suppression of V $\gamma$ 9V $\delta$ 2-T cells (150). Fowler et al. suggested that systemic use of zoledronate reduced the tumor homing ability of V $\gamma$ 9V $\delta$ 2-T cells (151). Indeed, repeated administration of zoledronate and IL-2 in the patients with breast cancer and RCC could inhibit the proliferative capacity of V $\gamma$ 9V $\delta$ 2-T cells and reduced the responsiveness of V $\gamma$ 9V $\delta$ 2-T cells to re-stimulation (144, 152). For the adoptive cell transfer therapy, the main problem is difficulty to expand V $\gamma$ 9V $\delta$ 2-T cells *ex vivo* from the advanced cancer patients with limited initial number of V $\gamma$ 9V $\delta$ 2-T cells, especially after radiotherapy and chemotherapy (45). Moreover, the effect of immunosuppressive tumor microenvironment on adoptively transferred V $\gamma$ 9V $\delta$ 2-T cells is still not clear.

Up to now, aminobisphosphonates have only been found to target and stimulate V $\gamma$ 9V $\delta$ 2-T cell subsets, but they do not target other subpopulations of  $\gamma\delta$ -T cells, such as V $\delta$ 1-T cells. Some synthetic phosphoantigens, such as BrHPP, HMBPP, and 2-methyl-3-butenyl-1-pyrophosphate (2M3B1PP), are also found to activate and expand V $\gamma$ 9V $\delta$ 2-T cells by mimicking the effects of aminobisphosphonates (153). Upon stimulation by these compounds, V $\gamma$ 9V $\delta$ 2-T cells can enhance their antitumoral activities by release of IFN- $\gamma$  and TNF- $\alpha$  (57). The administration of BrHPP was also tested in a Phase I study; the data showed that the BrHPP treatment was well tolerated and expansion of V $\gamma$ 9V $\delta$ 2-T cells was successfully induced in patients with solid tumors (145). In addition to synthetic phosphoantigens, some monoclonal antibodies targeting  $\gamma\delta$ -TCR are also good candidates for tumor immunotherapy. Starick et al. demonstrated that BTN3A (CD277)-specific monoclonal antibody 20.1 induced

TCR-mediated activation of V $\gamma$ 9V $\delta$ 2-T cells (154). This study not only provided the novel mechanisms involved in V $\gamma$ 9V $\delta$ 2-T cell activation but also showed the potential for using BTN3A-specific antibodies to manipulate V $\gamma$ 9V $\delta$ 2-T cell immunity in tumor immunotherapy (154, 155).

Some recent studies have focused on the development of novel protocols to expand V $\gamma$ 9V $\delta$ 2-T cells using immobilized antigens (156), agonistic monoclonal antibodies (15, 101), and tumor-driven artificial antigen-presenting cells (157, 158). Meanwhile, V $\gamma$ 9V $\delta$ 2-T cells are also amenable to genetic modifications *via* the introduction of  $\alpha\beta$  TCRs (159), and chimeric antigen receptors (160). Furthermore, V $\gamma$ 9V $\delta$ 2-T cells are suggested to act as a novel cellular vaccine to treat cancer patients (161).

## CONCLUSION

Recent advances in V $\gamma$ 9V $\delta$ 2-T cell research have paved a way for developing innovative therapeutic strategies for tumor immunotherapy. As discussed in this review, V $\gamma$ 9V $\delta$ 2-T cells can recognize tumor cells through TCR and other cell surface receptors, and their antitumoral activity is strictly regulated by activating and inhibitory receptors and their ligands. In addition, stimuli/cytokine milieu in tumor microenvironment can also induce pro-tumoral activity and functional plasticity of V $\gamma$ 9V $\delta$ 2-T cells. Further study on the dual face of V $\gamma$ 9V $\delta$ 2-T cells in tumor immunology could optimize current therapeutic protocols and improve the therapeutic efficacy and clinical outcomes of V $\gamma$ 9V $\delta$ 2-T cell-based tumor immunotherapy.

## AUTHOR CONTRIBUTIONS

ZX and WT wrote the manuscript. WT revised the manuscript.

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