OnLine Journal of Biological Sciences

Literature review

Fundamental Questions about γδT Cells

[1]Moulay Yassine Belghali, [2]Saadia Ba-M'hamed, [3]Mouna Khouchani and [1,4]Brahim Admou

[1]Laboratory of Immunology, Center of Clinical Research, University Hospital Mohamed VI, Morocco
[2]Laboratory of Pharmacology, Neurobiology and Behavior, Cadi Ayyad University, Marrakech, Morocco
[3]Group of Morphology and Biology of Cancers,
Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakech, Morocco
[4]B2S Research Laboratory, FMPM, Cadi Ayyad University, Marrakech, Morocco

Article history
Received: 18-07-2020
Revised: 12-12-2020
Accepted: 14-12-2020

Corresponding Author:
Belghali Moulay Yassine
Laboratory of Immunology,
Center of Clinical Research,
University Hospital Mohamed VI, Morocco
Email: myassine.belghali@gmail.com

Abstract: γδT cells is a minor subgroup of T lymphocytes expressing γδ-T Cell Receptor (TCR), with many subsets, dominated by Vδ2 and Vδ1. γδT cells recognize antigens independently of the context of MHC class I, MHC class II, or CD1 presenting molecules. However, this recognition requires the expression of the transmembrane Butyrophilin proteins by the presenting cells. Their activation is controlled by many surface receptors, namely, co-stimulatory receptors, cytokines receptors, NK receptors and inhibitory receptors. Once activated, γδT cells polarize into Th1, Th2, Th17, follicular T helper or Treg cells. They can play direct anti infectious and antitumor roles through perforin-granzyme molecules, FasL and Tumor-necrosis-factor Related Apoptosis Inducing Ligand (TRAIL), antibody-dependent cellular cytotoxicity and by IFN-γ and TNF-α cytokine's release. They also exert an indirect antitumor activity by cooperating with B cells, dendritic cells, αβT cells and NK cells. Additionally, γδT cells can infiltrate solid cancers and display a selective cytolytic activity. Conversely, γδT cells might promote cancer progression either directly through IL17 and/or VEGF, or indirectly by impairing other antitumor immune cell activities. Given its complex functions, γδT cell-based immunotherapy seems efficient and well tolerated, yet needs to overcome many obstacles including those related to the tumor environment.

Keywords : γδT Cells, Antigens, Chemokines, Cytokines, Immunotherapy

Introduction

In 1984, a third chain (γ) of the TCR was accidentally discovered and in 1987 a new subgroup of T cells expressing TCR that contains γ and δ chains termed γδ T cell subsets were officially described (Zhao *et al*., 2018; Born *et al*., 1987). Along the last three decades, a plethora of studies has led to promising findings about these cells (Kabelitz, 2016). However, many aspects of γδT cells remain not clearly elucidated, especially their receptors, the antigens they can recognize, the antigen recognition process, their products, the functions of their various subsets and their involvement in infections and malignancies, as well as their utility as an immunotherapy modality. In this review, we attempt to answer some fundamental questions by highlighting the phenotypic characteristics of the main subsets of γδT cells, their main functions and their role in the pathomechanism of some diseases.

Methods

This review intended establishing through the available literature, the finest answers to fundamental questions about γδT cells. The search strategy aimed to access all accessible studies focusing on these cells and published by peer reviewing indexed journals. The initial search terms was "γδT cells ", "γδT cells + antigens", "γδT cells + chemokines", "γδT cells + cytokines", "γδT cells + immunotherapy" "γδ T infiltrating lymphocytes" and "γδT cells + cancer". We explored articles in English, preferentially indexed in

the following databases: Science Citation Index, Web of Science, Medline Cochrane Library Web search and Scopus. The choice emphasis on articles published in the last 10 years. When not found, we considered articles published earlier. In sum, we elected 177 articles containing 128 original research and 49 reviews, all published in 78 journals. Dates of publication correspond on four intervals [2010; 2018], [2000; 2009], [1990; 1999] and [1987; 1989], including 69, 70, 35 and 3 articles respectively.

Are γδT Cells Rare?

γδT cells correspond to a minority of T subpopulations (Zhao *et al*., 2018; Born *et al*., 1987). Like αβ T cells, they derive from multipotent CD4-CD8- precursors in the thymus (Porritt *et al*., 2004), but they typically range from 1 to 4% of all CD3+ T cells in the peripheral blood of healthy adults). Yet, their proportion largely varies according to age (Table 1) (Schatorjé *et al*., 2012).

The proportion of γδT cells relative to total number of T cells also varies according to the anatomic localization. For example, γδT cells are abundant in the gut mucosa and other epithelial surfaces of the intestine and the skin where they represent one of the first lines of defence (Vantourout and Hayday, 2013; De Rosa *et al*., 2004). Additionally, they respond prior to αβ T cells and may prime pro-inflammatory or anti-inflammatory response (Chien *et al*., 2014). The fluctuations of these cells in blood may reflect their changes and/or their altered circulation between different peripheral sites. However, the proportion of naïve cells in peripheral localizations is quit lower compared to the blood, due to a selective homing of memory and activated cells to those sites. γδT cells are also characterized by an earlier activation and conversion to memory cells compared to αβ T lineages (De Rosa *et al*., 2004).

How γδ-TCR is Assembled?

The human TCR γ and δ loci are localized in the short arm of chromosome 7 and the long arm of chromosome 14 respectively. During the maturation process of lymphocytes, the rearrangement of the encoding gene segments (V,D,J) generates a large spectre of receptors resulting on various amino-acid sequences and thus distinct molecular surfaces at their Complementarity-Determining Regions (CDRs) (Fig. 1) (Kabelitz, 2016; Kazen and Adams, 2011; Allison and Garboczi, 2002). The δ locus is embedded inside the α-locus and only three Vδ genes are usually expressed (Vδ1, Vδ2 and Vδ3), but other Vα genes are rarely used in δ-chain rearrangement, specifically Vδ4 (Vα14), Vδ5 (Vα29), Vδ6 (Vα23), Vδ7 (Vα36) and Vδ8 (Vα38) (Thedrez *et al*., 2007). The Vγ repertoire is also small, with 12 Vγ genes; seven of them are functional: Vγ2, Vγ3, Vγ4, Vγ5, Vγ8, Vγ9 and Vγ11while Vγ1, Vγ5P, Vγ6, Vγ7 and Vγ10 are pseudogenes (Thedrez *et al*., 2007; Kazen an Adams, 2011). The number of expressed Vγ and Vδ genes is smaller than αβ TCR (Kabelitz, 2016) and the limited number of J gene segments encoding for both δ and γ (four and five, respectively) reflects their low diversity (Kazen and Adams, 2011).

The γ and δ genes rearrange like the Immunoglobulin (Ig) genes to form models for numerous TCR protein molecules (Kazen and Adams, 2011; Haas *et al*., 1993). Despite the low number of Vγ and Vδ genes compared to IgV and TCRαβ V genes, the recombinatorial probabilities for producing γδ-TCRs are almost limitless, widely due to the unique capacity of the δ genes to rearrange D segments in tandem and to use all reading frames (Davis and Bjorkman, 1988). This rearrangement provides different γδ T cell subsets (Wu *et al*., 2014). Vδ2, Vγ8 and Vγ9 segments are the first rearranged subsets in the foetal liver and thymus (McVay and Carding, 1996). At birth, the range of γδT cells in cord blood is wide, with no preferred Vγ/Vδ tandem (Morita *et al*., 1994). Four to six months after birth, a shift in the TCR δ and TCR γ loci conducts to changes during the rearrangement and recombination processes of genes encoding Vγ2, Vγ3, Vγ4, Vγ5, Vγ8 and Vγ11 (Adams *et al*., 2015). Vδ1 and Vδ3 γδT cells have minor subsets of δ and γ TCR chains (Wu *et al*., 2014). Additionally, experimental studies focusing on the structure of CDRs showed a diversity of γδ-TCR that varies considerably over γ and δ loci and some gene segments show signatures of strong selection at the CDR1 and CDR2 loops encoding regions. The large diversity of the CDR3δ loop conditions the type of ligands recognized by the TCRs (Kazen and Adams, 2011; Allison and Garboczi, 2002; Adams *et al*., 2008).

Table 1: Percentage of γδT cells among total peripheral lymphocyte population according to age (Schatorjé *et al*., 2012)

Population	Cord blood	1 week-2 months	2-5 months	5-9 months	9-15 months	15-24 months	2-5 years	5-10 years	10-16 years	>16 years
% of γδT cells among total peripheral lymphocyte population.	2 (0.58-5)	2 (0.27-15)	3 (1-7)	3 (0.82-10)	3 (1-10)	4 (1-13)	6 (0.92-38)	7 (2-24)	6 (2-17)	3 (0.83-11)

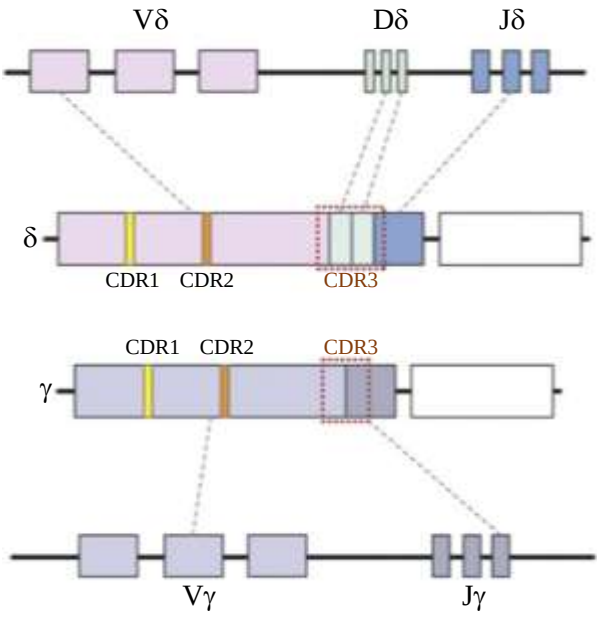

Fig. 1: Mechanism of the genetic rearrangement of TCR (V: variable; D: Diversity and J: Joining encoding genes) (Kazen and Adams, 2011); Top: V-D-J recombination resulting on the δ-chain; Bottom: V-J recombination to resulting on the γ-chain. Regions encoding the CDR1, CDR2 and CDR3 loops are mentioned in yellow, orange and red respectively

Cells expressing these different Vγ chains colonize different peripheral tissues (Khairallah *et al*., 2018). The Vγ9Vδ2 subset (or Vδ2) represents 50 to 95% of γδT cells population in the peripheral blood (Hinz *et al*., 1997); meanwhile Vδ1 subset is present in normal epithelia, spleen, liver, dermis, small intestine and colon. In patients suffering of viral infections and patients with leukaemia, this subset is present in peripheral blood (Almeida *et al*., 2016; Holtmeier *et al*., 1997). The distribution of Vδ3 subset is mainly observed in gut epithelium and liver tissue (Ramirez *et al*., 2015). This distribution usually allows γδT cells to interact directly or indirectly with microbiota or the pathogens that invade these locations, which promotes suitable proinflammatory actions. They also represent long-lived memory populations upon local infection (Khairallah *et al*., 2018). Regarding the diversity of γδ T cell localization, the γδ-TCR repertoire remains oligoclonal, allowing the recognition of different but limited sets of ligands (Holtmeier *et al*., 1995). Of note, in healthy adults, there are significant variations in γδT cell numbers according to ethnic groups. For example, in peripheral blood of healthy adults from West Africa, Vδ1 cells are more prevalent than Vδ2 (Esin *et al*., 1996; Hviid *et al*., 2000). The structure and the subsets of γδT cells, as well as their distribution, may determine aspects of their function.

Which Receptors Determine γδT Cell's Functions?

The efficacy of γδT cells depends on their activation, expansion and differentiation into effectors that can eliminate infected or mutated cells. This process depends on γδT cells expressed receptors including γδ TCR, costimulatory, cytokine, NK and inhibitory receptors (Table 2) (Ribeiro *et al*., 2015).

T cell Receptor

The stimulation via γδ-TCR is crucial for γδT cell function (Bonneville *et al*., 2010). The structure of γδ-TCR corresponds to a complex containing γδ-TCR and many CD3 chains (Siegers *et al*., 2007). γδT cell development is directly determined by the assembly of a γδ-TCR complex in thymic progenitors. Depending on the strength of TCR signal, γδT cells likely adopt an IL-17-producing effector profile when there is a relatively weak TCR signal, whereas an intense TCR signal seems to promote an IFN-γ γδ-T cell profile (Jensen *et al*., 2008).

Co-Stimulatory Receptors

Co-stimulatory receptors reduce the activation thresholds of T cells and enhance their functions. They include two subsets according to their structure. The first subset is the super family of Immunoglobulin (Ig), namely, CD28 which is constitutively expressed on γδT cells and stimulates their own survival and proliferation through IL-2 production (Ribot *et al*., 2011). The second one is the Tumor Necrosis Factor Receptor (TNFR) super families, such CD27. In naïve mice, it was demonstrated that these receptors are selectively involved in the production of IFN-γ. According to CD27 phenotype, γδT cells polarize to either IFN-γ+ (CD27+) or IL-17+ (CD27−) profiles. Besides, the development of IFNγ producing γδT cells requires strong TCR signalling along with CD27 co-stimulation in the thymus (Ribot *et al*., 2009).

Cytokine Receptors

As demonstrated in murine model, the development and the homeostasis of γδT cells depend on IL-7, IL-15 and IL-2 cytokines (Malissen *et al*., 1997; Baccala *et al*., 2005). In the dermis, IL-7 supports the development and the survival of resident γδT cells and promotes the expansion of human and murine IL-17-producing γδT cells (Zhao *et al*., 2005). Concerning IL-15, it plays an important role in sustaining the intraepithelial γδ T cell group present in the gut (Casetti *et al*., 2005). In a study on human, γδT cells where Vδ1 represented more that 80% it was reported that IL-15 and IL-2 induce IFN-γ production in functionally immature γδ thymocytes. The same result was not reported with IL-7 (Yamaguchi *et al*., 1998).

Table 2: Co-receptors of γδT cells – extracellular ligands and intracellular signaling pathways (Ribeiro *et al*., 2015)

Receptor	Ligands	Intracellular signaling initiators/adaptors	Downstream signaling pathway	Target molecules
CD28	B7.1 (CD80), B7.2 (CD86)	PI3K, ITK, Grb2	PI3K/AKT, Grb2/MEK/ERK	IL-2, NF-κB, AP-1, Bcl-x_L, NFAT
CD27	CD70	TRAF2 TRAF5 Siva	IKK/NF-κB, JNK	NF-κB, Ca^{2+}, cyclinD2, Bcl2a1, Bcl-x_L
IL-2R IL-15R	IL-2 IL-15	Jak1, Jak3	PI3K/AKT, Jak/STAT4/STAT5, MEK/ERK STAT1	IFNγ, TNF-α, T-bet, eomesodermin
NKG2D	MIC (A–B), ULBP (1–6), H60, MULT1, RAE1	DAP10	PI3K/AKT, Grb2/VAV1/SOS1, PKCθ/Ca^{2+}	NF-κB, RelB, Bcl-xL, Bcl-2
PD-1	PD-L1 (B7-H1) PD-L2 (B7-DC)	SHP-1, SHP-2	CK2/PTEN/PI3K/AKT, MEK/ERK	GSK-3, Bcl-xLSmad3, Cdc25A, IFNγ, IL-2
BTLA	HVEM	SHP-1, SHP-2	Zap70/ERK	IL-17, TNF, IL-2

Similar findings were observed for peripheral γδT cells extracted from patients with cancer (Yamaguchi *et al*., 1998). It was also shown that IL-2 and IL-15 are highly required for the expansion of Vδ2 T cells in response to microbial phospho-Antigens (pAg) and Nitrogen-containing bisphosphonates (N-bis) (Casetti *et al*., 2005). Inflammatory cytokines, namely IL-12 and IL-18 are also significantly promoting effector γδ T-cell differentiation which stimulates the production of the IFN-γ, while, IL-1β and IL-23 induce production of IL-17 cytokine (Yin *et al*., 2000). On the other hand, the production of antibodies with high affinity against microbial infections is the result of the B-cell helper activity displaying a T follicular helper cell-like phenotype provided to human Vδ2 subset by IL-21 (Bansal *et al*., 2012).

Natural Killer Receptors (NKR)

γδT cell express NKR, which are essential for the recognition of some ligands. NKG2D is the most studied concerning γδT cells. Actually, this receptor modulates their antiviral and antitumor activity, benefitting from the overexpression of various extracellular ligands upon cellular stress induced by such circumstances (Hayday, 2000; Eagle and Trowsdale, 2007; Champsaur and Lanier, 2010) specifically MHC class I chain-related protein A (MICA) and B (MICB) and HCMV UL-16-Binding Protein (ULBP) families. Inside the cell, NKG2D binds to DNAX-Activating Protein (DAP10). The complex of NKG2D/DAP10 may deliver T cells co-stimulatory signals that synergize with the ITAM based TCR/CD3 molecules (Long, 2002). The co-stimulatory action of NKG2D on Vδ2 T cells is possible by additional effects on TCR-mediated activation. However, it is still controversial whether NKG2D plays a primary stimulatory or co-stimulatory role (Correia *et al*., 2013; Das *et al*., 2001). Conversely, it was suggested that γδT cells could be activated by NKG2D signals without the TCR engagement (Rincon-Orozco *et al*., 2005). In addition, when blocked, NKG2D inhibits Vδ2 T-cell cytotoxicity against various types of blood cancers, which is not the same with TCR (Lanca *et al*., 2010).

Inhibitory Receptors

The return to the homeostasis is necessary after the activation of γδT cells. PD-1 or CTLA-4 as inhibitory receptors are necessary for this. Normally, PD-1 is absent or expressed only at low levels on circulating Vδ2 T cells, but it becomes rapidly overexpressed after activation (Iwasaki *et al*., 2011).

Once phosphorylated, Immunoreceptor Tyrosine based Inhibitory Motif (ITIM) and Immunoreceptor Tyrosine Switch Motif (ITSM) existing on the cytoplasmic tail of PD-1 engage inhibitors of Lck activity downstream the TCR complex (Kulpa *et al*., 2013). Furthermore, PD-1 captation impairs survival and proliferation of γδT cells, as well as IL-2 production through increasing the activity of the Protein Phosphatase and Tensin Homolog (PTEN) which inhibits PI3K/AKT signalling (Pedoeem *et al*., 2014). The engagement of PD-1 with Herpes Virus Entry Mediator (HVEM) inhibits Vδ2 T-cell proliferation in response to lymphoma cells (Gertner-Dardenne *et al*., 2013). It was also reported that the expression of PD-L1 on tumor cells inhibits Vδ2 T-cell cytotoxicity and the production of IFNγ (Iwasaki *et al*., 2011). γδT cells expresses another inhibitory receptor, namely B- and T-Lymphocyte Attenuator (BTLA), structurally related to PD-1 and CTLA-4 (Ribeiro *et al*., 2015).

What Antigens can γδT Cells Recognize?

Upon different stress signals, γδT cells recognize a diversity of antigens and ligands (Ribeiro *et al*., 2015; Witherden and Havran, 2012). But in contrast to conventional αβ T cells, they recognize also lipid antigens presented by CD1 molecules (CD1a, b, c) mainly expressed on the surface of professional Antigen Presenting Cells (APCs) and for which Vδ1 subset has a prominent reactivity, particularly to CD1c (Rincon-Orozco *et al*., 2005). Besides, γδT cells use multiple other pathways to recognize and clear tumor cells. Supporting this fact, MHC class I-related molecules (MICA and MICB) are recognized by intestinal derived human γδT cells . In addition, during infection, malignancy and other environmental

challenges, Vγ4Vδ5 subset can recognize subtle quantitative and qualitative changes of self-molecules like endothelial protein C receptor that appear on cytomegalovirus-infected cells and tumor cells (Witherden and Havran, 2012; Willcox *et al*., 2012). Independently of TCR, the NKG2D receptor intervene in the recognition of MHC class I related molecules, like MICA/B and UL16 Binding Protein (ULBP), frequently expressed by malignant cells, can trigger γδT cells cytotoxicity (Rincon-Orozco *et al*., 2005). Peripheral γδT cells may recognize molecules expressed by epithelial cells via Vδ1 subset. For instance, γδ infiltrating T cells in lung cancers can recognize the Monomeric Laminin Receptor (MLR) expressed on tumor cells through Vδ1 (Ferrarini *et al*., 1996).

Additionally, Vδ2 TCR may recognize other surface proteins, for example F1-ATPase-related structure expressed by Daudi Burkitt's lymphoma cells line (Scotet *et al*., 2005). Some soluble proteins are also considered as γδT cell ligands such as Tetanus toxoid, ESAT-6 (The 6 kDa early secretory antigenic target produced by *Mycobacterium tuberculosis*) and HSV glycoprotein-1 (Kozbor *et al*., 1990; Bitter *et al*., 2009; Johnson *et al*., 1992). Smaller peptides can also be recognized by γδT cells, e.g., the mycobacterial derived Heat Shock Protein-65 (HSP-65), which preferentially stimulates Vγ1 T cells (O'Brien *et al*., 1992). Along the same line of thought, a correlation has been reported between lung tumor cells' HSP-72 expression and the presence of γδT Infiltrating Lymphocytes (TILs) belonging to the Vδ1 subset (Ferrarini *et al*., 1996). γδT cells also recognize natural non-peptidic antigens belonging to several mycobacteria such as TUBag4 (Tanaka *et al*., 1994), Isopentenyl Pyrophosphate (IPP) (Tanaka *et al*., 1995) and the HMBPP (Hintz *et al*., 2001) a metabolite in the 2-C-methylD-erythritol-4 pathway for isoprenoid synthesis (Fox and Poulter, 2002). Other synthetic antigens like bisphosphonates and alkylamines (Kunzmann *et al*., 1999; Bukowski *et al*., 1999) can block Farnesyl Pyrophosphate Synthase (FPS) in the mevalonate pathway, thereby increasing cellular isopentenyl pyrophosphate IPP levels and indirectly stimulate γδT cells (Gober *et al*., 2003). IPP represents a eukaryotic homologue of microbial pAg that is accumulated during the mevalonate process activation and is specifically recognized by the Vδ2 TCR (Gober *et al*., 2003). For note, n-BP inhibits FPS, leading to the upstream accumulation of IPP (Li *et al*., 2009). Phospholipids represent another category of ligands that γδT cells can recognize, namely, cardiolipin that is detected by γδ-TCR in the context of CD1d (Dieudé *et al*., 2011). In addition, it has been reported that peripheral or nasal mucosa γδT cells derived from cypress pollen-sensitive patients was reactive to Phosphatidyl Ethanolamine (PE) after in vitro incubation with pollen. This immune reaction was dependent on CD1d molecule (Russano *et al*., 2006).

How γδT Cells Recognize Antigens?

As already discussed, γδT cells can recognize antigens independently of the context of MHC class I, MHC class II, or even CD1 presenting molecules (Morita *et al*., 1995) (Fig. 2). In presence of microbial or endogenous pAg, the activation of human γδT cells involves Butyrophilin (BTN) proteins (Harly *et al*., 2012). The type-1 transmembrane BTN proteins belong to the Immunoglobulin (Ig) superfamily. It is composed of an extracellular Ig-like domain, a transmembrane domain and an intracellular B30.2 signaling domain in some cases (Fig. 3) (Rhodes *et al*., 2016).

Immune cells and epithelial cells widely express BTN proteins that can elicit several immune-regulatory activities (Rhodes *et al*., 2016). On the other hand, the immune response against infections can be altered consecutively to genetic mutations in BTN genes (Ampuero *et al*., 2015). It was demonstrated that BTN3A (CD277) expressed on tumor cells could exert positive or negative co-stimulatory signals. Other authors showed that it could exert inhibitory effects on T-cell proliferation and cytokine production when over expressed on antigen-presenting cells (Cubillos-Ruiz *et al*., 2010). Facing microbial or tumor derived pAg, BTN3A or CD277 has a major role in driving Vδ2 subset activity. In fact, the presence of CD277 monoclonal Antibody (mAb) 20.1 promotes Vδ2 cells anti-tumoral cytotoxicity (Harly *et al*., 2012) while, in the presence of CD277 mAb 103.2 these cells are inhibited (Palakodeti *et al*., 2012). Other BTN family molecules have been described in murine intestinal epithelial cells such as BTNL1, BTNL6 and BTN3A1. The two first molecules enhance the proliferative activity of intraepithelial Vγ7Vδ4 cells and the third one induces the maturation of mouse thymic Vγ5Vδ1 cells (Lebrero-Fernández and Bas-Forsberg, 2016; Boyden *et al*., 2008).

Two models are proposed to explain the molecular mechanism of BTN3A for the activation of human γδT cell by pyrophosphates (Fig. 4). The first is the 'presenting mechanism' where BTN3A1 extracellular domain serves as an antigen-presenting molecule of pAg that the human Vδ2 TCR can recognize (Fig. 4A) (De Libero *et al*., 2015). However, this mechanism has not been confirmed by other studies (Sandstrom *et al*., 2014). The second is the 'pyrophosphate sensing mechanism' where the cytosolic domain (B30.2) is involved. The latter can directly bind numerous γδT cell-stimulating pAg via a positively charged surface pocket, which, furthermore, would affect TCR engagement indirectly by changing membrane mobility and/or the structure of extracellular BTN3A1 domains (Sandstrom *et al*., 2014; Silva-Santos *et al*., 2015).

Actually, existing data confirm the pyrophosphate-sensing function of the cytosolic B30.2 domain. In this mechanism the liaison of pyrophosphate antigens to the BTN3A1's cytosolic domain, which is in contact with periplakin and RhoB molecules, provokes spatial rearrangement of BTN3A1 and leads to TCR-dependent selective activation of γδT cell (Gu *et al*., 2015; Sebestyen *et al*., 2016) (Fig. 4B). The mystery of how signals are transmitted to T cells via BTN3A1 is still unsettled. In fact, BTN3A2 and BTN3A3 are other isoforms that are implicated in pAg-mediated γδT cells activation (Rhodes *et al*., 2015). This suggests the existence of other actors that might explain the whole mechanism of cell surface-rearranged BTN3A molecules and how pAg particularly activate human γδT cells (Kabelitz *et al*., 2017).

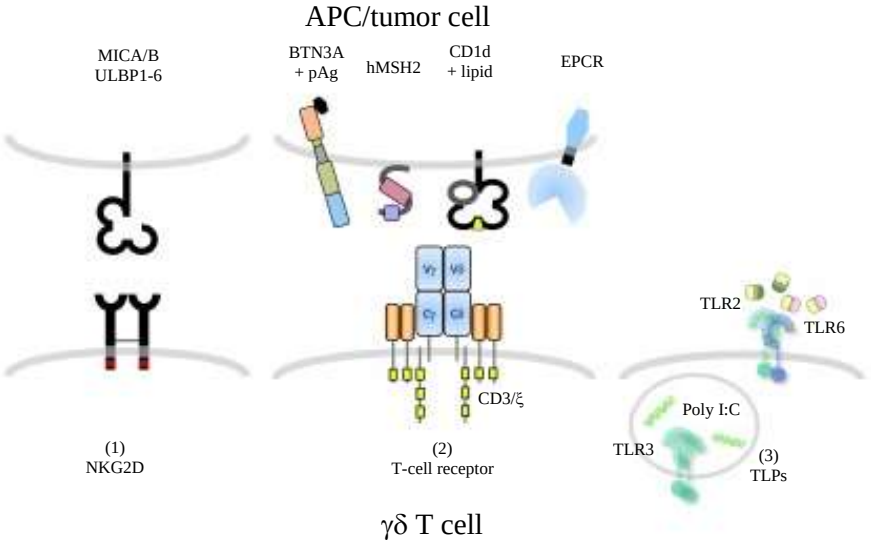

Fig. 2: Categories of receptors regulating the activation of human γδT cells (Kabelitz *et al*., 2017) NKG2D recognizes stress-inducible ligands, (ex. MICA-B and ULBP 1-6), inducing cytotoxic activity and cytokine production via the PI3K pathway. γδ TCR recognizes pAg in a BTN3A-dependent way. Human MutS Homolog 2 (hMSH2) or lipids bound to CD1d and endothelial protein C receptor (EPCR). TLR2 recognizes conserved microbial ligands such as acetylated lipids heterodimer and activate γδT cell via the NF-κB B cell pathway

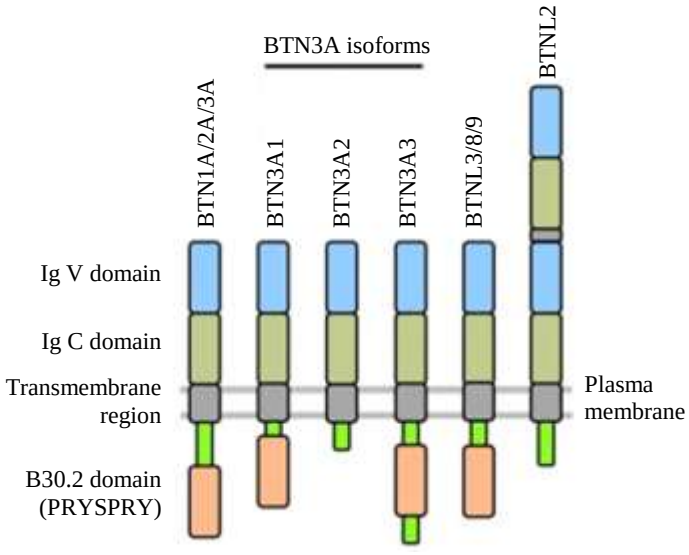

Fig. 3: Structure of BTN proteins (Kabelitz *et al*., 2017) The structure of BTN proteins corresponding to extracellular (immunoglobulin V (IgV) and IgC-like), transmembrane and a cytosolic (B30.2) domains. B30.2 domain and additional amino acids determine three BTN3A isoforms

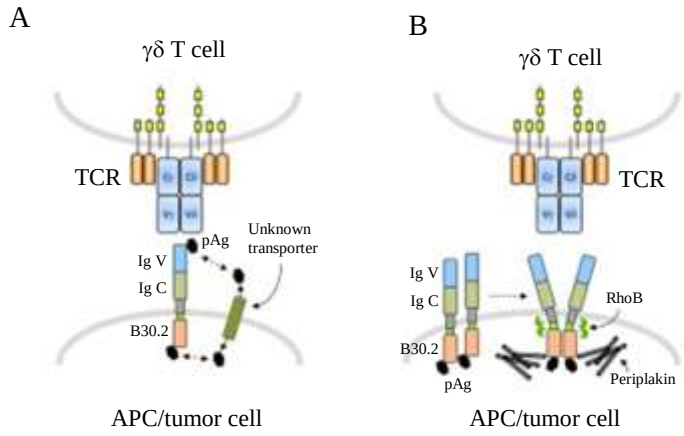

Fig. 4: Alternative roles of BTN3A molecules in pAg-mediated γδ T cell activation (Kabelitz *et al*., 2017); A: Binding of pAg to IgV domain and their direct recognition by γδ-TCR. Transport of pAg from the cytosol to the extracellular compartment by unknown molecules, to be presented by BTN3A1; B: pAg bind to the B30.2 domain, which recruit linker proteins such as periplakin and activate the RhoB GTPase, leading to a conformation change of BTN3A1 that γδ-TCR could recognize

How do γδT Cells Reach the Tumor Site?

In certain pathophysiological conditions, such as infection, inflammation and tumors, both circulating and resident γδT cells express various patterns of chemokines receptors. This allows their extravasation and migration to reach specific destinations (Kabelitz and Wesch, 2003). For example, the migration of γδT cells to the small intestine is guided by CCR9 and the local expression of the corresponding ligand CCL25 (Kabelitz and Wesch, 2003). Vδ2 T cells express multiple chemokines receptors, including CCR1, CCR5 and CXCR4 (Cipriani *et al*., 2000; Brandes *et al*., 2003). The expression of CXCR4 transiently increases following pAg stimulation (Brandes *et al*., 2003). On the other hand, a high production of CXCL12, ligand of CXCR4 by tumor-associated fibroblasts also stimulates Vδ2 T cells infiltration (Orimo *et al*., 2005). This chemokine is additionally involved in the migration of renal carcinoma-infiltrating Vδ2 T cells (Viey *et al*., 2008). The Vδ1 γδT cells, which correspond to the resident subset, preferentially express CXCR1, this suggest that its ligand IL-8 might preferentially act on this subpopulation (Glatzel *et al*., 2002; Roth *et al*., 1998).

In addition to chemokine receptors, the mobility of resident γδT cells also depends on related adhesion molecules. For example, those infiltrating lung tumors express N-Cellular Adhesion Molecules (N-CAM), which allows them to bind to endothelial cells and sub endothelial matrix (Zocchi and Poggi, 1993). In addition, N-CAM might facilitate the extravasation of circulating γδT cells and recirculation of resident γδ Tumor-Infiltrating Lymphocytes (TILs). Similarly, NK cell Receptor Protein 1A (NKRP1A), that may act as an adhesion molecule, is expressed selectively on Vδ2 T cells (Poggi *et al*., 1999). Thus, NKRP1A seems important to drive circulating γδT cells towards the tumor site. The recirculation of NKRP1A+ γδT cells in the damaged tissue is also modulated by IL-12 (Poggi *et al*., 1999; Ferrarini *et al*., 2002).

After activation by IPP, Vδ2 cells release large quantities of the β-chemokines MIP-1α and MIP-1β. The most robust and rapid response is observed or MIP-1β (Cipriani *et al*., 2000). In vitro, human MIP-1α and MIP-1β recruit different populations of T cells, with MIP-1α attracting mainly CD4+ T cells and MIP-1β inducing the chemotaxis of CD8+ T cells (Schall *et al*., 1993; Taub *et al*., 1993). MIP-1α activates CCR1, CCR5 and perhaps CCR4, whereas MIP-1β interacts more selectively with CCR5. Furthermore, MIP-1α has been shown to activate macrophages, eosinophils and basophils, whereas MIP-1β lacks this activity (Baggiolini *et al*., 1993). Taken together, the chemokines released by Vδ2 T cells significantly contribute to a pro-inflammatory microenvironment during infection or others diseases (Boismenu *et al*., 1996). Chemokines produced by activated Vδ2 T cells are also involved in T-cell recruitment and subsequently in their activation, as well as in the recruitment of other immune cells. Therefore, γδT cells have been considered as an interface between innate and adaptive immune responses (Cipriani *et al*., 2000).

What Effector Profiles do γδT Cells Polarize to?

Vδ2 T cells display various functional activities. once activated and cultured in the presence of IL-2, naïve Vδ2 T cells (CD27+CD45RA+) can differentiate into central memory T cells (CD27+CD45RA−), effector memory T cells (CD27−CD45RA−) and terminally differentiated

effector memory cells re-expressing CD45RA (CD27−CD45RA+) cells. Moreover, depending on specific stimulations, Vδ2 T cells may polarize into Th1, Th2, Th17, follicular T helper (Tfh), or regulatory T-cell (Treg) profiles (Dunne *et al*., 2010; Casetti *et al*., 2009) (Table 2). Upon pAg stimulation, in presence of IL-21 and IL-2, Vδ2 T cells polarize to Th1 with increased expression of CD56 and various cytolytic molecules and then acquire a higher tumor-induced degranulation capacity (Thedrez *et al*., 2009). Vδ2 T cells can also polarize into γδ Th17 cells. In neonates, IL-6, IL-1β and TGF-β are required to generate γδ Th17 cells, while in adults, they require the presence of IL-23, IL-1β and TGF-β (Wu *et al*., 2014). It was demonstrated in a colorectal cancer model that activated DCs polarize Vδ2 cells into γδ Th17 profile, with a release of high amount of IL-17, IL-8, TNF-α and Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) (Wu *et al*., 2014). γδ Th17 cells, especially in the cord blood, can also produce IL-22 (Ness-Schwickerath and Morita, 2011). Interestingly, Vδ2 T cells can secret IFN-γ when they polarize into Th1/Th17 cells. In fact, in neonates, γδ Th1/Th17 cells differentiation requires IL-6, IL-1β, IL-23 and TGF-β cytokine's environment, while in adults, memory γδ Th1/Th17 cells need the same cytokines except IL-6 (Ness-Schwickerath *et al*., 2010). Wesch *et al*. (2001) reported that; once stimulated with IPP, Vδ2 T cells can polarize to Th1 profile in the context of Th1-priming conditions (IL-12) and to Th2-cell profile due to Th2-priming conditions (IL4, anti IL-12 Ab). In contrast, it has been shown, that Vδ2 cells can polarize to FOXP3+γδ Treg cells (Hu *et al*., 2013) succeeding stimulation by TGF-β and IL-15 and exert a regulatory activity (Schall *et al*., 1993). Finally, IL-21 can stimulate the differentiation of Vδ2 T cells to a follicular T helper (Tfh)-like phenotype. These cells express different molecules and receptors such as BCL-6 transcription factor, ICOS (Inducible T-cell costimulator), CD40-L, CXCR5, CD244, IL-21R, CXCL10 and CXCL13 to reach germinal centre lymph nodes. This polarization facilitates the maturation of B cells (Caccamo *et al*., 2011). It is important to know if this functional plasticity is a feature of all γδT cells population or it is restricted to Vδ2 subset, since this plasticity leads to either an anti-inflammatory profile or to a pro-inflammatory one (Yan and Huang, 2014) (Table 3).

What about γδT Cells Ambivalence in Cancer?

γδT cells may exert direct antitumor activity through multiple mechanisms like perforin-granzyme pathway (Niu *et al*., 2015), TRAIL (tumornecrosis-factor related apoptosis inducing ligand) and FasL (Hu *et al*., 2013), ADCC (Antibody dependent cellular cytotoxicity) (Todaro *et al*., 2009) and by secreting IFN-γ and TNF-α cytokines. These factors enhance antitumor immunity and inhibit cancer angiogenesis (Niu *et al*., 2015; Todaro *et al*., 2009; Fisher *et al*., 2014; Li *et al*., 2008). They can also interact with B, dendritic, T (αβ) and Natural killer cells and have an indirect antitumor effect in consequence. For instance, in non-immunized mice, Vγ4 and Vγ6 subsets can modulate B cells, hence the antibody synthesis (Caccamo *et al*., 2012). Following the same line of thought, after their co-culture with IPP (Caccamo *et al*., 2012) or HMB-PP (Bansal *et al*., 2012) and IL-21, the Vδ2 T cells polarize to follicular helper T (Tfh) cells (Caccamo *et al*., 2012) that can secrete IL-4, IL-10 and CXCL13 and favours B cells antibodies production. γδT cells are also able to perform as APCs for αβ T-cell priming. In fact, after activation, γδT cells overexpress HLA-DR, a leukocyte activation receptor (CD69), as well as co-stimulatory and adhesion molecules (CD40, CD54, CD80 and CD86) (Kunzmann *et al*., 2000; Moser *et al*., 2005). Another indirect effect is the ability of γδT cells to activate DC maturation.

Table 3: γδT cells functional plasticity (Lafont *et al*., 2014)

γδT cells subsets	TCR activation	Cytokines	Polarization *transcription factors*	Effector molecules
Adult blood Vγ9Vδ2	+	IL-12 or IL-18	Th1-like *T-bet*, *eomesodermin*	IFN-γ, TNF-α
T cells	+	IL-4	Th2-like *GATA-3*	IL-4
	+	IL-15$^+$ TGF-β	Treg-like *Foxp3*	IL-10, TGF-β
	+	IL-6 IL-23$^+$ IL-1β$^+$ TGF-β$^+$ Ahr^a agonists	Th17-like *RORγt*	IL-17
	+	IL-23$^+$ IL-1β$^+$ TGF-β	Th17-like, *RORγt* Th1/17, like, *RORγt*, *T-bet* Th22, *FOX04*	IL-17 IFN-γ, IL-17 IL-22
	+	IL-2	APC functions ND	MHC I and II
Adult blood and tonsillar Vγ9Vδ2 T cells	+	IL-21	Tfh-like *Bcl6*	IL-4, IL-10, CXCL13
Th1 Vγ9Vδ2 T cells	−	IFN type I	Th1-like *ND*	IFN-γ
Cord blood Vγ9Vδ2 T cells	+	IL-6$^+$ IL-1β $^+$ TGF-β	Th17-like, *RORγt* Th22-like, *FOX04*	IL-17 IL-22
	+	IL-6$^+$ IL-1β$^+$ TGF-β $^+$ IL-23	Th1/17 like *RORγt*, *T-bet*	IFN-γ, IL-17
Human Vγ1+ and Vγ2+ thymocytes	−	IL-2 or IL-15	Th1 like *T-bet*, *eomesodermin*	IFN-γ, TNF-α
Murine γδT cells	−	IL-23$^+$ IL-1β	Th17 *RORγt*	IL-17, IL-21, IL-22

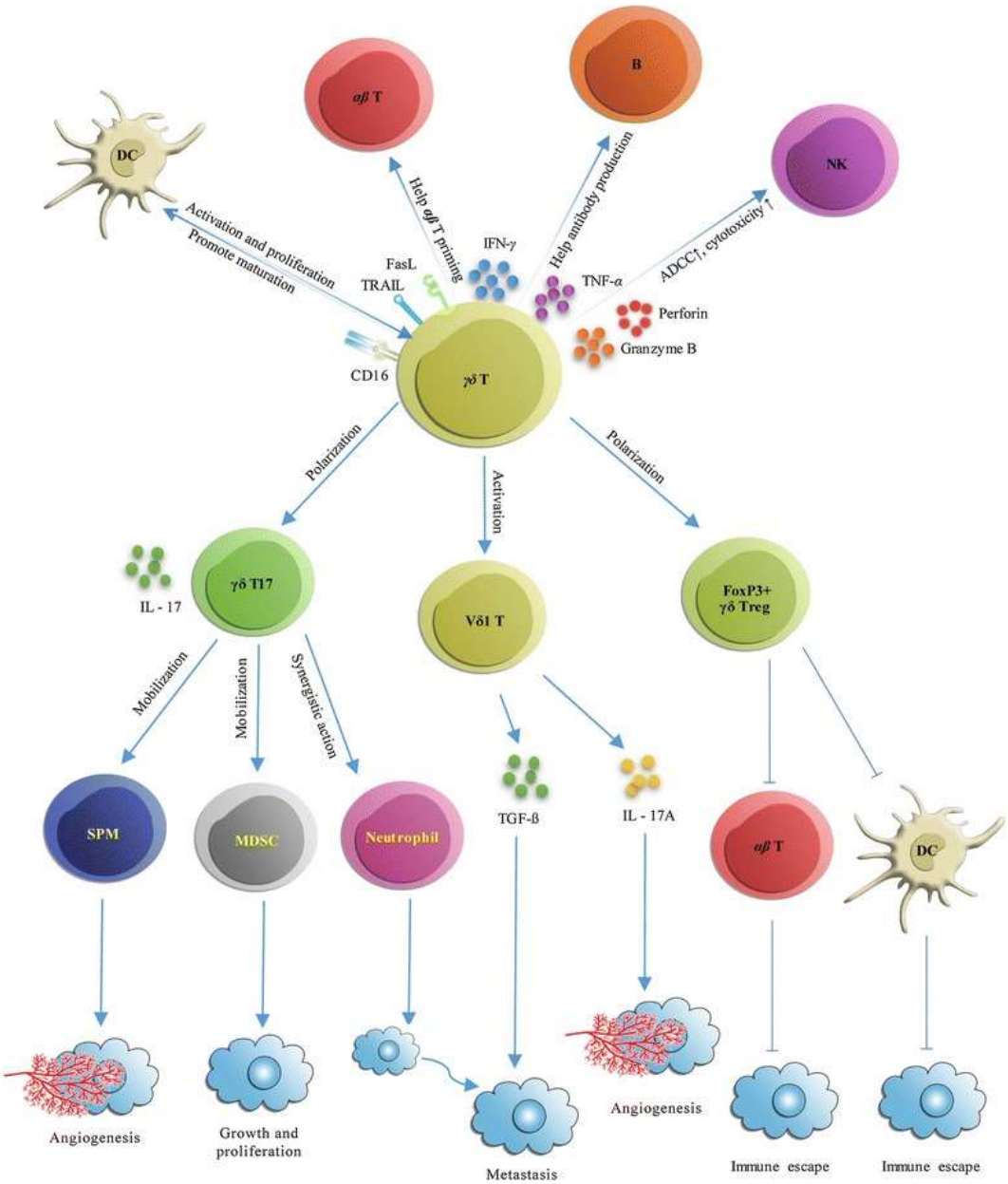

Fig. 5: Antitumoral and protumoral functions of γδT cells (Zhao *et al*., 2018); Antitumor effect of γδT cells: Direct antitumor effects are mediated by perforin-granzyme pathway, inflammatory cytokines, cytotoxicity on Fas+ and TRAIL-R+ malignant cells and ADCC. Polarized γδ Tfh cells mediate an indirect antitumoral action of γδT cells. This stimulates the antibody production, the γδT cell antigen presentation to αβ T cell, the triggering of DC maturation and the activation of NK antitumor activity. Protumoral effect of γδT cells: γδT cells might polarize into FOXP3+ γδ Treg cells and γδ T17 cells. They can directly impair αβ T cells and DC antitumor functions. They also increase MDSC, SPM and neutrophil immunosuppressive functions. Conjointly, these effects enhance tumor angiogenesis, growth, proliferation, metastasis and immune escape

This maturation is mostly mediated by TNF-α and IFN-γ, which can be provided by γδT cells (Conti *et al*., 2005). Finally, the interaction of CD137 and CD137L expressed successively on NK cells and γδT cells may induce solid NK cell-mediated antitumoral cytotoxicity (Kabelitz, 2016) (Fig. 5). However, γδT cells can also directly enhance cancer progression. Actually, through the secretion of IL-17, γδ Th17 cells play an immunosuppressive role; promoting the development of cancer. γδ Th17 cells promote angiogenesis through Vascular Endothelial Growth Factor (VEGF) production, which also helps cancer progression. Such phenomenon has been established in the progression of gallbladder and ovarian cancers (Patil *et al*., 2016). As demonstrated

in vitro, after the polarization of Vδ2 T cells into FOXP3+ via TGF-β and IL-15, they adopted an analogous function as αβ Treg cells (Casetti *et al*., 2009). Moreover, it was shown that Vδ1 Treg cells secrete the chemokine IP-10 and TGF-β in breast cancer (Ye *et al*., 2013a). When produced, TGF-β may provoke a transition from epithelial into mesenchymal fate, which encourages cancer immune escape. This results cancer invasiveness (Yang and Weinberg, 2008). The same subset of Vδ1 cells, specifically the peripheral ones, seems to have more effective regulatory action than αβ Treg cells (CD4+ CD25+) (Van Acker *et al*., 2015). γδT cells may also have an indirect pro-tumor effect through additional mechanisms.

One of them is due to an impairment of the function of antitumor immune cells. For example, infiltrating Vδ1 γδT cells in human breast tumor may indirectly impair the activation of naïve αβT-cells and their differentiation into effector T cells by inhibiting DC maturation and their APC function (Peng *et al*., 2007). Vδ1 γδT cells in pancreatic ductal adeno-carcinoma were demonstrated to express important levels of PD-L1. This promote T cell suppression and then oncogenesis (Daley *et al*., 2016). It was also shown that tumor-derived γδ-Treg cells could inhibit T-lymphocytes cellular cycle and induce senescence in DC (Ye *et al*., 2013b). Another aspect of the pro-tumor activity of γδT cells is IL-17 secretion. This cytokine represents a main chemo-attractant factor that recruits Myeloid-Derived Suppressor Cells (MDSCs) (Welte and Zhang, 2015). Thus, the innate γδ T17 cells might use MDSCs to transform cancer-elicited inflammation into immunosuppression in colorectal cancer (Wu *et al*., 2014). In a mouse ovarian cancer model, (Coffelt *et al*., 2015), demonstrated that small peritoneal macrophages (SPMs) are immobilized under the action of IL-17 secreted by Vγ6 CD27−γδ T-cell phenotype which up-regulated pro-tumor and pro-angiogenic molecular mediators. This in turn enhanced the growth of ovarian cancer. In addition, the production of IL-1β and IL-17 by γδ T17 cells increases the expansion and polarization of neutrophils, which inhibits CD8+ cytotoxic T lymphocytes and promote metastasis (Sabbione *et al*., 2014) (Fig. 5).

How Tumor-Infiltrating γδT Cells Influence Cancer Outcome?

Tumor-infiltrating leukocytes comprise myeloid cells (granulocytes, macrophages and myeloid-derived suppressor cells) and different lymphocyte subsets (T, B and NK cells). All of them might have an impact on tumor progression (Gooden *et al*., 2011). γδT cells can infiltrate solid tumors and exhibit a selective antitumoral lytic activity (Haas *et al*., 1993). It was reported that γδT cells belong to Tumor-Infiltrating Lymphocytes (TILs) in numerous cancers. Nevertheless, because of several controversies their clinical significance remains unclear. In a series of patients suffering from necrotizing choroidal melanoma, the Immunohistochemical (IHC) analysis showed that TILs are present in 76% of samples and that 52% of samples are infiltrated by Vδ1 γδT cells (Bialasiewicz *et al*., 1999). In another series of primary melanoma cases, γδT cells have been shown to be the main group of CD3+ T lymphocytes, equally distributed between Vδ1 and Vδ2. Additionally, Vδ2 subset correlated with an early stage melanoma but Vδ1 T cells did not correlate with melanoma prognosis (Cordova *et al*., 2012). In the context of breast cancer, an IHC study showed that γδT cells were detected in 93% of cancer cases versus 3% in normal breast specimens (Ma *et al*., 2012). The authors, then, showed that the frequency of γδT cell correlates positively with cancer in advanced stage, HER2 expression status and high lymph node metastasis, but correlates negatively with relapse-free and overall survival (Ye *et al*., 2013a). This allowed the authors to suggest that breast tumor infiltrating γδT cells are the most significant prognostic factor in assessing the gravity of breast cancer. Along the same line, in breast cancer, Vδ1 TILs can inhibit the activation of CD4 and CD8 T cells and impair DC's maturation and functioning and thus suppress immune responses (Peng *et al*., 2007). These T and dendritic cells switch to regulatory cells, thus intensifying immunosuppression (Ye *et al*., 2013a). It is worth mentioning that not the proliferation of resident regulatory Vδ1 who is responsible of their accumulation in the context of breast cancer but it is due to their attraction by IP-10 issued from breast cancer cells (Peng *et al*., 2007). Another study conducted on colon cancer samples, indicated that 80 and 20% of γδ Th17 cells corresponded to Vδ1 and Vδ2 subsets respectively. Based on these observations, the authors considered the infiltration of human colon cancer by γδ Th17 cell as a prognostic factor since it correlates with cancer stages and other pathological features (tumor size and infiltration, lymphatic and vascular invasion, lymph node metastasis and serum (Carcinoembryonic Antigens (CEA) levels). On the other hand, among the tumor-infiltrating Vδ1 subset, γδ Th17 cells represent about 25% and secrete TNF-α, IL-8 and GM-CSF, which are responsible for the recruitment, the survival, the activation and the proliferation of MDSC, known to mediate immunosuppression and promote tumor growth (Wu *et al*., 2014).

On the contrary, other authors did not find any correlation between the presence of infiltrating γδT cells and prognosis factors (Inman *et al*., 2008). The tumor type, as well as its localization, the specific γδT cells profiles infiltrating the tumor and the tumor microenvironment perhaps condition the biologic effects

of γδT cells. Linked to some of these aspects, the *ex vivo* expanded colon, ovary, lung, breast, renal and pancreatic cancer derived γδT cells possess an efficient antitumor cytolytic effect and Vδ1 subset is generally considered more cytotoxic than Vδ2 one (Groh *et al*., 1999). Moreover, it was reported that infiltrating γδT cells selectively produce IL-17 but not IFN-γ on a transplantable mouse tumor model (Wakita *et al*., 2010). The lack of IL-17 generally avoids tumor growth, which correlates with reduced tumor angiogenesis and VEGF and Ang-2 expression in tumor cells. This shows that tumor-infiltrating γδ17 T cells enhance angiogenesis and therefore the tumor growth. The same effect was reported in a mouse model of hepatocellular carcinoma but has been attributed to the MDSC recruitment. Once at the tumor site, IL-17 induces the production of IL-1β and IL-23 by MDSC, which amplify the differentiation of γδ Th17 cells. Finally, this mechanism sustains immunosuppression and promotes tumor growth (Ma *et al*., 2014).

What are the Challenges for γδT Cell-Based Immunotherapy?

Clinical trials of γδT cell-Based Immunotherapy (γδT-BI) have been reported in multiple cancers with efficacy and good tolerance (Zhao *et al*., 2018; Zou *et al*., 2017). This immunotherapy depends either on the *in vivo* activation or the *ex vivo* expansion of γδT cells. The first approach consists on the stimulation of γδT cells by systemic administration of pAg or N-bis (Bennouna *et al*., 2008). The second one is based on their expansion using synthetic pAg or N-bis (mostly used as drug, ex: Zoledronate, pamidronate and alkylamine) before the administration of the cultured γδT cells (Zhao *et al*., 2018; Dieli *et al*., 2007). Interestingly, the capacity of γδT cells to be easily and specifically stimulated either by pAg (HMBPP and IPP) or by factors that induce IPP accumulation represent an important characteristic for their use in research (Dieli *et al*., 2007). Thus, in several clinical trials, γδT cells displayed an efficacy in diverse tumors, including renal cell carcinomas (Kobayashi *et al*., 2011), lung carcinomas (Johnson *et al*., 2003), melanomas (Body, 2006), breast cancer (Jemal *et al*., 2005) and many others (Zou *et al*., 2017). In a series of metastatic renal cell carcinoma that underwent γδT cells therapy, 60% of patients displayed a Stable Disease (SD) (Bennouna *et al*., 2008). A Complete Remission (CR) was observed in a patient with advanced renal cell carcinoma who underwent six monthly cycles of autologous γδ T cell therapy and was disease free for more than 3 years without supplementary treatment. In a second time, the authors proceeded to phases I/II clinical trial, using 2-methyl-3-butenyl-1-pyrophosphate associated to zoledronate and IL-2. The findings of the study revealed an increased "Tumor Doubling Time", with 1, 5 and 5 cases of complete remission, stable disease and progressive disease respectively (Kobayashi *et al*., 2010). Numerous *in vitro* and *in vivo* experiments have demonstrated that γδT cells could be suitable for possible exploitation in the transplantation of Hematopoietic Stem Cells (HSCT). Indeed, there is a positive correlation between the allogeneic graft γδT cells and the disease-free survival (Lamb Jr *et al*., 1996; 1999; Godder *et al*., 2007). They are also unlikely to initiate Graft-Versus-Host Disease (GVHD), with an ability to attenuate the GVHD activity of αβ T cells (Drobyski *et al*., 1999; Ellison *et al*., 1995). Moreover, allogeneic transplantation studies give evidences that γδT cells may facilitate alloengraftment (Drobyski and Majewski, 1997; Kawanishi *et al*., 1997; Henslee *et al*., 1987).

However, γδ T-BI still encounters many obstacles linked to many factors:

- The difficulty to infiltrate tumors by Vδ2 T cells, which may explain the poor results in cancers (Olive *et al*., 1997). In fact, this infiltration is negatively impacted by the significantly low expression of adhesion molecules such as ICAM-1 and VCAM-1 on human tumors as well as the overexpression of the endothelin B Receptor (ETBR), which inhibits ICAM-1 expression on human endothelium (Buckanovich *et al*., 2008; Hamzah *et al*., 2008)
- The existence of inhibitory factors produced by cancer cells or their microenvironment, mainly TGF-β, Prostaglandin E2 (PGE2), tumor derived adenosine and NKG2D-L:
    - TGF-β alters Vδ2 T cell antitumor activity by inducing a Treg profile (Ribot *et al*., 2014; Li *et al*., 2006)
    - Prostaglandin E2 (PGE2) is also an important regulator of Vδ2 T cells since they highly express PGE2 receptors EP2 and EP4 and many human cancers express high levels of PGE2 due to up-regulation of COX 2 (a key enzyme of prostaglandin biosynthesis). This may explain the limited efficacy of Vδ2 T cell-based immunotherapy in patients with high levels of PGE2 or COX2 (Martinet *et al*., 2009; Kunzmann *et al*., 2009)
    - Increased production of tumor-derived adenosine, highly secreted in various types of tumors, may alter Vδ2 T cells cytotoxicity (Blay *et al*., 1997)
    - The potential limitation of Vδ2 T cells, because of the proteolysis and the shedding of NKG2D-L (MICA/B and ULBP1-4) from the surface of tumor cells (Rincon-Orozco *et al*., 2005; Groh *et al*., 2002; Doubrovina *et al*., 2003)

The γδ T-BI may also face other challenges related to the activity of immunosuppressive cells. For instance, γδT cells antitumor immune responses are downregulated by Treg cells that act through IL-10, TGF-β and IL-35 (Strauss *et al*., 2007). Additionally, Tregs can also express high levels of perforin and granzyme B exerting a cytotoxic activity against γδT cells (Cao *et al*., 2007). Moreover, Mesenchymal Stem Cells (MSCs) can also display an important immunosuppressive effect on activated Vδ2 T cells (Krampera *et al*., 2003; Prigione *et al*., 2009). This inhibition is mediated by their COX2-dependent production of PGE2. Additionally, MSCs have the ability to migrate towards different types of tumor in vivo including glioma (Sonabend *et al*., 2008), colon carcinoma (Hung *et al*., 2005), ovarian carcinoma (Komarova *et al*., 2006), breast carcinoma (Karnoub *et al*., 2007) and melanoma (Studeny *et al*., 2002). This is possible since several MSC chemo-attracting factors are released from tumor cells and surrounding stromal cells, such as MCP-1 (Dwyer *et al*., 2007), SDF-1 (Menon *et al*., 2007), PDGF, EGF and VEGF (Beckermann *et al*., 2008). On the other side, it was noticed that neutrophils are able to suppress γδT cells impairing their antitumor activity. This suppression would be due to the release of vesicular components of the neutrophils (serine proteases, elastase, cathepsin G) which catalyse the shedding of IL-2 and IL-6 receptors on γδT cells (Bank *et al*., 1999; Bank and Ansorge, 2001). Neutrophils also inhibit γδT cells by producing Reactive Oxygen Species (ROS) and releasing arginase, provoking down regulation of TCRζ on γδT cells with a halt of their cell cycle in the G0-G1 phase. Consequently, the up regulation of PD-L1 expression on γδT cells is associated with interferon-dependent PD1-mediated γδ T-cell apoptosis (Sabbione *et al*., 2014; Leliefeld *et al*., 2015).

Conclusion

The current synthesis aims to provide global answers on central questions on γδT cells, specifically about their structure, their functions and their clinical applications. γδT cells are characterized by diverse subsets among which Vδ2 is the most prevalent and well studied with more clinical applications. The way these cells recognize various antigens-either directly or through antigen presenting cells-widens their contribution to both innate and adaptive immune responses. Functionally, these cells show an interesting plasticity to polarize to diverse immune profiles and then display an anti-inflammatory or a pro-inflammatory fate. Thanks to the diversity of their receptors, these cells play a potent role in both infections and malignancies. Additionally, their proven anti-tumoral potential has allowed γδT cells to enter the immunotherapy universe in some cancers, using multiple strategies. Namely, the use of antibodies to activate Fc receptor-dependent ADCC, the development of γδT cell-based cancer vaccines and the promotion of their IFNγ secretion and not IL-17, with encouraging results. However, some obstacles remain challenging to overcome, mainly due to poor infiltration of target tissues, soluble inhibitory molecules and immunosuppressive cells in the tumor microenvironment. In addition, current and future researches are focusing on the implication of γδT cell in infections particularly the identification of bacterial ligands they can recognize and their behaviour in viral infections especially with HIV and cytomegalovirus. Finally, after decades of investigations, γδT cells still hide many of their characteristics and much remains to be learned.

Author's Contributions

Moulay Yassine Belghali and Brahim Admou: Design, writing and revision of the manuscript.

Saadia Ba-M'hamed: Writing and revision of the manuscript.

Mouna Khouchani: Revision of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Adams, E. J., Strop, P., Shin, S., Chien, YH., & Garcia, K. C. (2008). An autonomous CDR3δ is sufficient for recognition of the nonclassical MHC class I molecules T10 and T22 by γδ T cells. Nature immunology, 9(7), 777-784.

Adams, E. J., Gu, S., & Luoma, A. M. (2015). Human gamma delta T cells: evolution and ligand recognition. Cellular immunology, 296(1), 31-40.

Allison, T. J., & Garboczi, D. N. (2002). Structure of γδ T cell receptors and their recognition of non-peptide antigens. Molecular immunology, 38(14), 1051-1061.

Almeida, A. R., Correia, D. V., Fernandes-Platzgummer, A., da Silva, C. L., da Silva, M. G., Anjos, D. R., & Silva-Santos, B. (2016). Delta one T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept. Clinical Cancer Research, 22(23), 5795-5804.

Ampuero, J. D., Del Campo, J. A., Rojas, L., García-Lozano, R. J., Buti, M., Solá, R., ... & Salmerón, J. (2015). Fine-mapping butyrophilin family genes revealed several polymorphisms influencing viral genotype selection in hepatitis C infection. Genes & Immunity, 16(5), 297-300.

Baccala, R., Witherden, D., Gonzalez-Quintial, R., Dummer, W., Surh, C. D., Havran, W. L., & Theofilopoulos, A. N. (2005). γδ T cell homeostasis is controlled by IL-7 and IL-15 together with subset-specific factors. The Journal of Immunology, 174(8), 4606-4612.

Baggiolini, M., Dewald, B., & Moser, B. (1993). lnterleukin-8 and related chemotactic cytokines—CXC and CC chemokines. In Advances in immunology (Vol. 55, pp. 97-179). Academic Press.

Bank, U., Reinhold, D., Schneemilch, C., Kunz, D., Synowitz, H. J., & Ansorge, S. (1999). Selective proteolytic cleavage of IL-2 receptor and IL-6 receptor ligand binding chains by neutrophil-derived serine proteases at foci of inflammation. Journal of interferon & cytokine research, 19(11), 1277-1287.

Bank, U., & Ansorge, S. (2001). More than destructive: neutrophil-derived serine proteases in cytokine bioactivity control. Journal of leukocyte biology, 69(2), 197-206.

Bansal, R. R., Mackay, C. R., Moser, B., & Eberl, M. (2012). IL-21 enhances the potential of human γδ T cells to provide B-cell help. European journal of immunology, 42(1), 110-119.

Beckermann, B. M., Kallifatidis, G., Groth, A., Frommhold, D., Apel, A., Mattern, J., ... & Saffrich, R. (2008). VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. British journal of cancer, 99(4), 622-631.

Bennouna, J., Bompas, E., Neidhardt, E. M., Rolland, F., Philip, I., Galéa, C., ... & Lafaye-de Micheaux, S. (2008). Phase-I study of Innacell γδ™, an autologous cell-therapy product highly enriched in γ9δ2 T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. Cancer immunology, immunotherapy, 57(11), 1599-1609.

Bialasiewicz, A. A., Ma, J. X., & Richard, G. (1999). α/β− and γ/δ TCR+ lymphocyte infiltration in necrotising choroidal melanomas. British journal of ophthalmology, 83(9), 1069-1073.

Bitter, W., Houben, E. N., Bottai, D., Brodin, P., Brown, E. J., Cox, J. S., ... & Van Pittius, N. C. G. (2009). Systematic genetic nomenclature for type VII secretion systems. PLoS Pathog, 5(10), e1000507.

Blay, J., White, T. D., & Hoskin, D. W. (1997). The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. Cancer research, 57(13), 2602-2605.

Body, J. J. (2006). Bisphosphonates for malignancy-related bone disease: current status, future developments. Supportive care in cancer, 14(5), 408-418.

Boismenu, R., Feng, L., Xia, Y. Y., Chang, J. C., & Havran, W. L. (1996). Chemokine expression by intraepithelial gamma delta T cells. Implications for the recruitment of inflammatory cells to damaged epithelia. The Journal of Immunology, 157(3), 985-992.

Bonneville, M., O'brien, R. L., & Born, W. K. (2010). γδ T cell effector functions: a blend of innate programming and acquired plasticity. Nature Reviews Immunology, 10(7), 467-478.

Born, W., Miles, C., White, J., O'Brien, R., Freed, J. H., Marrack, P., ... & Kubo, R. T. (1987). Peptide sequences of T-cell receptor δ and γ chains are identical to predicted X and γ proteins. Nature, 330(6148), 572-574.

Boyden, L. M., Lewis, J. M., Barbee, S. D., Bas, A., Girardi, M., Hayday, A. C., ... & Lifton, R. P. (2008). Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. Nature genetics, 40(5), 656-662.

Brandes, M., Willimann, K., Lang, A. B., Nam, K. H., Jin, C., Brenner, M. B., ... & Moser, B. (2003). Flexible migration program regulates γδ T-cell involvement in humoral immunity. Blood, 102(10), 3693-3701.

Buckanovich, R. J., Facciabene, A., Kim, S., Benencia, F., Sasaroli, D., Balint, K., ... & Coukos, G. (2008). Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. Nature medicine, 14(1), 28-36.

Bukowski, J. F., Morita, C. T., & Brenner, M. B. (1999). Human γδ T cells recognize alkylamines derived from microbes, edible plants and tea: implications for innate immunity. Immunity, 11(1), 57-65.

Caccamo, N., Todaro, M., La Manna, M. P., Sireci, G., Stassi, G., & Dieli, F. (2012). IL-21 regulates the differentiation of a human γδ T cell subset equipped with B cell helper activity. PloS one, 7(7), e41940.

Caccamo, N., La Mendola, C., Orlando, V., Meraviglia, S., Todaro, M., Stassi, G., ... & Dieli, F. (2011). Differentiation, phenotype and function of interleukin-17–producing human Vγ9Vδ2 T cells. Blood, 118(1), 129-138.

Cao, X., Cai, S. F., Fehniger, T. A., Song, J., Collins, L. I., Piwnica-Worms, D. R., & Ley, T. J. (2007). Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity, 27(4), 635-646.

Casetti, R., Agrati, C., Wallace, M., Sacchi, A., Martini, F., Martino, A., ... & Malkovsky, M. (2009). Cutting edge: TGF-β1 and IL-15 induce FOXP3+ γδ regulatory T cells in the presence of antigen stimulation. The Journal of Immunology, 183(6), 3574-3577.

Casetti, R., Perretta, G., Taglioni, A., Mattei, M., Colizzi, V., Dieli, F., ... & Poccia, F. (2005). Drug-induced expansion and differentiation of Vγ9Vδ2 T cells in vivo: the role of exogenous IL-2. The Journal of Immunology, 175(3), 1593-1598.

Champsaur, M., & Lanier, L. L. (2010). Effect of NKG2D ligand expression on host immune responses. Immunological reviews, 235(1)

Chien, Y. H., Meyer, C., & Bonneville, M. (2014). γδ T cells: first line of defense and beyond. Annual review of immunology, 32, 121-155.

Cipriani, B., Borsellino, G., Poccia, F., Placido, R., Tramonti, D., Bach, S., ... & Brosnan, C. F. (2000). Activation of CC β-chemokines in human peripheral blood γδ T cells by isopentenyl pyrophosphate and regulation by cytokines. Blood, The Journal of the American Society of Hematology, 95(1), 39-47.

Coffelt, S. B., Kersten, K., Doornebal, C. W., Weiden, J., Vrijland, K., Hau, C. S., ... & de Visser, K. E. (2015). IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature, 522(7556), 345-348.

Conti, L., Casetti, R., Cardone, M., Varano, B., Martino, A., Belardelli, F., ... & Gessani, S. (2005). Reciprocal activating interaction between dendritic cells and pamidronate-stimulated γδ T cells: role of CD86 and inflammatory cytokines. The Journal of Immunology, 174(1), 252-260.

Cordova, A., Toia, F., La Mendola, C., Orlando, V., Meraviglia, S., Rinaldi, G., ... & Caccamo, N. (2012). Characterization of human γδ T lymphocytes infiltrating primary malignant melanomas. PLoS One, 7(11), e49878.

Correia, D. V., Lopes, A. C., & Silva-Santos, B. (2013). Tumor cell recognition by γδ T lymphocytes: T-cell receptor vs. NK-cell receptors. Oncoimmunology, 2(1), e22892.

Cubillos-Ruiz, J. R., Martinez, D., Scarlett, U. K., Rutkowski, M. R., Nesbeth, Y. C., Camposeco-Jacobs, A. L., & Conejo-Garcia, J. R. (2010). CD277 is a negative co-stimulatory molecule universally expressed by ovarian cancer microenvironmental cells. Oncotarget, 1(5), 329.

Daley, D., Zambirinis, C. P., Seifert, L., Akkad, N., Mohan, N., & Werba, G. (2016). Gamma delta T cells support pancreatic oncogenesis by restraining alpha beta T cell activation. Cell166, 1485–1499. e15.

Das, H., Groh, V., Kuijl, C., Sugita, M., Morita, C. T., Spies, T., & Bukowski, J. F. (2001). MICA engagement by human Vγ2Vδ2 T cells enhances their antigen-dependent effector function. Immunity, 15(1), 83-93.

Davis, M. M., & Bjorkman, P. J. (1988). T-cell antigen receptor genes and T-cell recognition. Nature, 334(6181), 395-402.

De Libero, G., Lau, S. Y., & Mori, L. (2015). Phosphoantigen presentation to TCR γδ cells, a conundrum getting less gray zones. Frontiers in immunology, 5, 679.

De Rosa, S. C., Andrus, J. P., Perfetto, S. P., Mantovani, J. J., Herzenberg, L. A., Herzenberg, L. A., & Roederer, M. (2004). Ontogeny of γδ T cells in humans. The Journal of Immunology, 172(3), 1637-1645.

Dieli, F., Vermijlen, D., Fulfaro, F., Caccamo, N., Meraviglia, S., Cicero, G., ... & Salerno, A. (2007). Targeting human γδ T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. Cancer research, 67(15), 7450-7457.

Dieudé, M., Striegl, H., Tyznik, A. J., Wang, J., Behar, S. M., Piccirillo, C. A., ... & Rauch, J. (2011). Cardiolipin binds to CD1d and stimulates CD1d-restricted γδ T cells in the normal murine repertoire. The Journal of Immunology, 186(8), 4771-4781.

Doubrovina, E. S., Doubrovin, M. M., Vider, E., Sisson, R. B., O'Reilly, R. J., Dupont, B., & Vyas, Y. M. (2003). Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. The Journal of Immunology, 171(12), 6891-6899.

Drobyski, W. R., & Majewski, D. (1997). Donor γδ T lymphocytes promote allogeneic engraftment across the major histocompatibility barrier in mice. Blood, The Journal of the American Society of Hematology, 89(3), 1100-1109.

Drobyski, W. R., Majewski, D., & Hanson, G. (1999). Graft-facilitating doses of ex vivo activated gammadelta T cells do not cause lethal murine graft-vs.-host disease. Biology of Blood and Marrow Transplantation, 5(4), 222-230.

Dunne, M. R., Mangan, B. A., Madrigal-Estebas, L., & Doherty, D. G. (2010). Preferential Th1 cytokine profile of phosphoantigen-stimulated human Vγ9Vδ2 T cells. Mediators of inflammation, 2010.

Dwyer, R. M., Potter-Beirne, S. M., Harrington, K. A., Lowery, A. J., Hennessy, E., Murphy, J. M., ... & Kerin, M. J. (2007). Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. Clinical Cancer Research, 13(17), 5020-5027.

Eagle, R. A., & Trowsdale, J. (2007). Promiscuity and the single receptor: NKG2D. Nature Reviews Immunology, 7(9), 737-744.

Ellison, C. A., MacDonald, G. C., Rector, E. S., & Gartner, J. G. (1995). Gamma delta T cells in the pathobiology of murine acute graft-versus-host disease. Evidence that gamma delta T cells mediate natural killer-like cytotoxicity in the host and that elimination of these cells from donors significantly reduces mortality. The Journal of Immunology, 155(9), 4189-4198.

Esin, S., Shigematsu, M., Nagai, S., Eklund, A., Wigzell, H., & Grunewald, J. (1996). Different percentages of peripheral blood γδ+ T cells in healthy individuals from different areas of the world. Scandinavian journal of immunology, 43(5), 593-596.

Ferrarini, M., Heltai, S., Pupa, S. M., Menard, S., & Zocchi, M. R. (1996). Killing of laminin receptor-positive human lung cancers by tumor-infiltrating lymphocytes bearing γδ+ T-cell receptors. JNCI: Journal of the National Cancer Institute, 88(7), 436-441.

Ferrarini, M., Ferrero, E., Dagna, L., Poggi, A., & Zocchi, M. R. (2002). Human γδ T cells: a nonredundant system in the immune-surveillance against cancer. Trends in immunology, 23(1), 14-18.

Fisher, J. P., Yan, M., Heuijerjans, J., Carter, L., Abolhassani, A., Frosch, J., ... & Klein, N. (2014). Neuroblastoma killing properties of Vδ2 and Vδ2-negative γδT cells following expansion by artificial antigen-presenting cells. Clinical Cancer Research, 20(22), 5720-5732.

Fox, D. T., & Poulter, C. D. (2002). Synthesis of (E)-4-hydroxydimethylallyl diphosphate. An intermediate in the methyl erythritol phosphate branch of the isoprenoid pathway. The Journal of organic chemistry, 67(14), 5009-5010.

Gertner-Dardenne, J., Fauriat, C., Orlanducci, F., Thibult, M. L., Pastor, S., Fitzgibbon, J., ... & Olive, D. (2013). The co-receptor BTLA negatively regulates human Vγ9Vδ2 T-cell proliferation: a potential way of immune escape for lymphoma cells. Blood, The Journal of the American Society of Hematology, 122(6), 922-931.

Glatzel, A., Wesch, D., Schiemann, F., Brandt, E., Janssen, O., & Kabelitz, D. (2002). Patterns of chemokine receptor expression on peripheral blood γδ T lymphocytes: strong expression of CCR5 is a selective feature of Vδ2/Vγ9 γδ T cells. The Journal of Immunology, 168(10), 4920-4929.

Gober, H. J., Kistowska, M., Angman, L., Jenö, P., Mori, L., & De Libero, G. (2003). Human T cell receptor γδ cells recognize endogenous mevalonate metabolites in tumor cells. Journal of Experimental Medicine, 197(2), 163-168.

Godder, K. T., Henslee-Downey, P. J., Mehta, J., Park, B. S., Chiang, K. Y., Abhyankar, S., & Lamb, L. S. (2007). Long term disease-free survival in acute leukemia patients recovering with increased γδ T cells after partially mismatched related donor bone marrow transplantation. Bone marrow transplantation, 39(12), 751-757.

Gooden, M. J., de Bock, G. H., Leffers, N., Daemen, T., & Nijman, H. W. (2011). The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. British journal of cancer, 105(1), 93-103.

Groh, V., Wu, J., Yee, C., & Spies, T. (2002). Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature, 419(6908), 734-738.

Groh, V., Rhinehart, R., Secrist, H., Bauer, S., Grabstein, K. H., & Spies, T. (1999). Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. Proceedings of the National Academy of Sciences, 96(12), 6879-6884.

Gu, S., Nawrocka, W., & Adams, E. J. (2015). Sensing of pyrophosphate metabolites by Vγ9Vδ2 T cells. Frontiers in immunology, 5, 688.

Haas, W., Pereira, P., & Tonegawa, S. (1993). Gamma/delta cells. Annual review of immunology, 11(1), 637-685.

Hamzah, J., Jugold, M., Kiessling, F., Rigby, P., Manzur, M., Marti, H. H., ... & Arnold, B. (2008). Vascular normalization in Rgs5-deficient tumours promotes immune destruction. Nature, 453(7193), 410-414.

Harly, C., Guillaume, Y., Nedellec, S., Peigné, C. M., Mönkkönen, H., Mönkkönen, J., ... & Déchanet-Merville, J. (2012). Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. Blood, The Journal of the American Society of Hematology, 120(11), 2269-2279.

Hayday, A. C. (2000). γδ cells: a right time and a right place for a conserved third way of protection. Annual review of immunology, 18(1), 975-1026.

Henslee, P. J., Thompson, J. S., Romond, E. H., Doukas, M. A., Metcalfe, M., Marshall, M. E., & MacDonald, J. S. (1987, February). T cell depletion of HLA and haploidentical marrow reduces graft-versus-host disease but it may impair a graft-versus-leukemia effect. In Transplantation proceedings (Vol. 19, No. 1 Pt 3, pp. 2701-2706).

Hintz, M., Reichenberg, A., Altincicek, B., Bahr, U., Gschwind, R. M., Kollas, A. K., ... & Jomaa, H. (2001). Identification of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human γδ T cells in Escherichia coli. FEBS letters, 509(2), 317-322.

Hinz, T., Wesch, D., Halary, F., Marx, S., Choudhary, A., Arden, B., ... & Kabelitz, D. (1997). Identification of the complete expressed human TCR V gamma repertoire by flow cytometry. International immunology, 9(8), 1065-1072.

Holtmeier, W., Witthöft, T., Hennemann, A., Winter, H. S., & Kagnoff, M. F. (1997). The TCR-delta repertoire in human intestine undergoes characteristic changes during fetal to adult development. The Journal of Immunology, 158(12), 5632-5641.

Holtmeier, W., Chowers, Y., Lumeng, A., Morzycka-Wroblewska, E., & Kagnoff, M. F. (1995). The delta T cell receptor repertoire in human colon and peripheral blood is oligoclonal irrespective of V region usage. The Journal of clinical investigation, 96(2), 1108-1117.

Hu, Y., Cui, Q., Gu, Y., Sheng, L., Wu, K., Shi, J., ... & Yu, X. (2013). Decitabine facilitates the generation and immunosuppressive function of regulatory γδT cells derived from human peripheral blood mononuclear cells. Leukemia, 27(7), 1580-1585.

Hung, S. C., Deng, W. P., Yang, W. K., Liu, R. S., Lee, C. C., Su, T. C., ... & Wei, H. J. (2005). Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. Clinical Cancer Research, 11(21), 7749-7756.

Hviid, L., Akanmori, B. D., Loizon, S., Kurtzhals, J. A., Ricke, C. H., Lim, A., ... & Behr, C. (2000). High frequency of circulating γδ T cells with dominance of the Vδ1 subset in a healthy population. International immunology, 12(6), 797-805.

Inman, B. A., Frigola, X., Harris, K. J., Kuntz, S. M., Lohse, C. M., Leibovich, B. C., & Kwon, E. D. (2008). Questionable relevance of γδ T lymphocytes in renal cell carcinoma. The Journal of Immunology, 180(5), 3578-3584.

Iwasaki, M., Tanaka, Y., Kobayashi, H., Murata-Hirai, K., Miyabe, H., Sugie, T., ... & Minato, N. (2011). Expression and function of PD-1 in human γδ T cells that recognize phosphoantigens. European journal of immunology, 41(2), 345-355.

Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R. C., Ghafoor, A., Feuer, E. J., & Thun, M. J. (2005). Cancer Statistics. American Cancer Society, 55, 10-30.

Jensen, K. D., Su, X., Shin, S., Li, L., Youssef, S., Yamasaki, S., ... & Baumgarth, N. (2008). Thymic selection determines γδ T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon γ. Immunity, 29(1), 90-100.

Johnson, J. R., Williams, G., & Pazdur, R. (2003). End points and United States Food and Drug Administration approval of oncology drugs. Journal of Clinical Oncology, 21(7), 1404-1411.

Johnson, R. M., Lancki, D. W., Sperling, A. I., Dick, R. F., Spear, P. G., Fitch, F. W., & Bluestone, J. A. (1992). A murine CD4-, CD8-T cell receptor-gamma delta T lymphocyte clone specific for herpes simplex virus glycoprotein I. The Journal of Immunology, 148(4), 983-988.

Kabelitz, D. (2016). Human γδ T cells: From a neglected lymphocyte population to cellular immunotherapy: A personal reflection of 30years of γδ T cell research. Clinical immunology, 100(172), 90-97.

Kabelitz, D., & Wesch, D. (2003). Features and functions of gd T lymphocytes: focus on chemokines and their receptors. Critical Reviews™ in Immunology, 23(5-6).

Kabelitz, D., Lettau, M., & Janssen, O. (2017). Immunosurveillance by human γδ T lymphocytes: the emerging role of butyrophilins. F1000Research, 6.

Karnoub, A. E., Dash, A. B., Vo, A. P., Sullivan, A., Brooks, M. W., Bell, G. W., ... & Weinberg, R. A. (2007). Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature, 449(7162), 557-563.

Kawanishi, Y., Passweg, J., Drobyski, W. R., Rowlings, P., Cook-Craig, A., Casper, J., ... & Juckett, M. (1997). Effect of T cell subset dose on outcome of T cell-depleted bone marrow transplantation. Bone marrow transplantation, 19(11), 1069-1077.

Kazen, A. R., & Adams, E. J. (2011). Evolution of the V, D and J gene segments used in the primate γδ T-cell receptor reveals a dichotomy of conservation and diversity. Proceedings of the National Academy of Sciences, 108(29), E332-E340.

Khairallah, C., Chu, T. H., & Sheridan, B. S. (2018). Tissue adaptations of memory and tissue-resident gamma delta T cells. Frontiers in Immunology, 9, 2636.

Kobayashi, H., Tanaka, Y., Shimmura, H., Minato, N., & Tanabe, K. (2010). Complete remission of lung metastasis following adoptive immunotherapy using activated autologous γδ T-cells in a patient with renal cell carcinoma. Anticancer research, 30(2), 575-579.

Kobayashi, H., Tanaka, Y., Yagi, J., Minato, N., & Tanabe, K. (2011). Phase I/II study of adoptive transfer of γδ T cells in combination with zoledronic acid and IL-2 to patients with advanced renal cell carcinoma. Cancer Immunology, Immunotherapy, 60(8), 1075-1084.

Komarova, S., Kawakami, Y., Stoff-Khalili, M. A., Curiel, D. T., & Pereboeva, L. (2006). Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Molecular cancer therapeutics, 5(3), 755-766.

Kozbor, D., Cassatella, M. A., Lessin, S., Kagan, J., Finver, S., Faust, J., ... & Croce, C. M. (1990). Expression and function of gamma delta-and alpha beta-T cell receptor heterodimers on human somatic T cell hybrids. The Journal of Immunology, 144(10), 3677-3683.

Krampera, M., Glennie, S., Dyson, J., Scott, D., Laylor, R., Simpson, E., & Dazzi, F. (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood, 101(9), 3722-3729.

Kulpa, D. A., Lawani, M., Cooper, A., Peretz, Y., Ahlers, J., & Sékaly, R. P. (2013, October). PD-1 coinhibitory signals: the link between pathogenesis and protection. In Seminars in immunology (Vol. 25, No. 3, pp. 219-227). Academic Press.

Kunzmann, V., Bauer, E., Feurle, J., Tony, F. W. H. P., & Wilhelm, M. (2000). Stimulation of γδ T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood, The Journal of the American Society of Hematology, 96(2), 384-392.

Kunzmann, V., Kimmel, B., Herrmann, T., Einsele, H., & Wilhelm, M. (2009). Inhibition of phosphoantigen-mediated γδ T-cell proliferation by CD4+ CD25+ FoxP3+ regulatory T cells. Immunology, 126(2), 256-267.

Kunzmann, V., Bauer, E., & Wilhelm, M. (1999). γ/δ T-cell stimulation by pamidronate. New England Journal of Medicine, 340(9), 737-738.

Lafont, V., Sanchez, F., Laprevotte, E., Michaud, H. A., Gros, L., Eliaou, J. F., & Bonnefoy, N. (2014). Plasticity of gamma delta T cells: impact on the anti-tumor response. Frontiers in immunology, 5, 622.

Lamb Jr, L. S., Henslee-Downey, P. J., Parrish, R. S., Godder, K., Thompson, J., Lee, C., & Gee, A. P. (1996). Increased frequency of TCR gamma delta+ T cells in disease-free survivors following T cell-depleted, partially mismatched, related donor bone marrow transplantation for leukemia. Journal of hematotherapy, 5(5), 503-509.

Lamb Jr, L. S., Gee, A. P., Hazlett, L. J., Musk, P., Parrish, R. S., O'Hanlon, T. P., ... & Lee, C. (1999). Influence of T cell depletion method on circulating γδ T cell reconstitution and potential role in the graft-versus-leukemia effect. Cytotherapy, 1(1), 7-19.

Lanca, T., Correia, D. V., Moita, C. F., Raquel, H., Neves-Costa, A., Ferreira, C., ... & Silva-Santos, B. (2010). The MHC class Ib protein ULBP1 is a nonredundant determinant of leukemia/lymphoma susceptibility to γδ T-cell cytotoxicity. Blood, The Journal of the American Society of Hematology, 115(12), 2407-2411.

Lebrero-Fernández, C., & Bas-Forsberg, A. (2016). The ontogeny of Butyrophilin-like (Btnl) 1 and Btnl6 in murine small intestine. Scientific reports, 6(1), 1-8.

Leliefeld, P. H., Koenderman, L., & Pillay, J. (2015). How neutrophils shape adaptive immune responses. Frontiers in immunology, 6, 471.

Li, H., Luo, K., & Pauza, C. D. (2008). TNF-α is a positive regulatory factor for human Vγ2Vδ2 T cells. The Journal of Immunology, 181(10), 7131-7137.

Li, J., Herold, M. J., Kimmel, B., Müller, I., Rincon-Orozco, B., Kunzmann, V., & Herrmann, T. (2009). Reduced expression of the mevalonate pathway enzyme farnesyl pyrophosphate synthase unveils recognition of tumor cells by Vγ9Vδ2 T cells. The Journal of Immunology, 182(12), 8118-8124.

Li, M. O., Wan, Y. Y., Sanjabi, S., Robertson, A. K. L., & Flavell, R. A. (2006). Transforming growth factor-β regulation of immune responses. Annu. Rev. Immunol., 24, 99-146.

Long, E. O. (2002). Versatile signaling through NKG2D. nature immunology, 3(12), 1119-1120.

Ma, C., Zhang, Q., Ye, J., Wang, F., Zhang, Y., Wevers, E., ... & Hsueh, E. C. (2012). Tumor-infiltrating γδ T lymphocytes predict clinical outcome in human breast cancer. The Journal of Immunology, 189(10), 5029-5036.

Ma, S., Cheng, Q., Cai, Y., Gong, H., Wu, Y., Yu, X., ... & Liu, H. (2014). IL-17A produced by γδ T cells promotes tumor growth in hepatocellular carcinoma. Cancer research, 74(7), 1969-1982.

Malissen, M., Pereira, P., Gerber, D. J., Malissen, B., & DiSanto, J. P. (1997). The common cytokine receptor γ chain controls survival of γ/δ T cells. The Journal of experimental medicine, 186(8), 1277-1285.

Martinet, L., Fleury-Cappellesso, S., Gadelorge, M., Dietrich, G., Bourin, P., Fournié, J. J., & Poupot, R. (2009). A regulatory cross-talk between Vγ9Vδ2 T lymphocytes and mesenchymal stem cells. European journal of immunology, 39(3), 752-762.

McVay, L. D., & Carding, S. R. (1996). Extrathymic origin of human gamma delta T cells during fetal development. The Journal of Immunology, 157(7), 2873-2882.

Menon, L. G., Picinich, S., Koneru, R., Gao, H., Lin, S. Y., Koneru, M., ... & Banerjee, D. (2007). Differential gene expression associated with migration of mesenchymal stem cells to conditioned medium from tumor cells or bone marrow cells. Stem cells, 25(2), 520-528.

Morita, C. T., Parker, C. M., Brenner, M. B., & Band, H. (1994). TCR usage and functional capabilities of human gamma delta T cells at birth. The Journal of immunology, 153(9), 3979-3988.

Morita, C. T., Beckman, E. M., Bukowski, J. F., Tanaka, Y., Band, H., Bloom, B. R., ... & Brenner, M. B. (1995). Direct presentation of nonpeptide prenyl pyrophosphate antigens to human γδ T cells. Immunity, 3(4), 495-507.

Moser, B., Brandes, M., & Willimann, K. (2005). Professional antigen-presentation function by human gammadelta T Cells. Science, 309(5732), 264-8.

Ness-Schwickerath, K. J., & Morita, C. T. (2011). Regulation and function of IL-17A-and IL-22-producing γδ T cells. Cellular and Molecular Life Sciences, 68(14), 2371-2390.

Ness-Schwickerath, K. J., Jin, C., & Morita, C. T. (2010). Cytokine requirements for the differentiation and expansion of IL-17A–and IL-22–producing human Vγ2Vδ2 T cells. The Journal of Immunology, 184(12), 7268-7280.

Niu, C., Jin, H., Li, M., Xu, J., Xu, D., Hu, J., ... & Cui, J. (2015). 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 immunology, 16(1), 61.

O'brien, R. L., Fu, Y. X., Cranfill, R., Dallas, A., Ellis, C., Reardon, C., ... & Born, W. (1992). Heat shock protein Hsp60-reactive gamma delta cells: a large, diversified T-lymphocyte subset with highly focused specificity. Proceedings of the National Academy of Sciences, 89(10), 4348-4352.

Olive, C., Nicol, D., & Falk, M. C. (1997). Characterisation of γδ T cells in renal cell carcinoma patients by polymerase chain reaction analysis of T cell receptor transcripts. Cancer Immunology, Immunotherapy, 44(1), 27-34.

Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., ... & Weinberg, R. A. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell, 121(3), 335-348.

Palakodeti, A., Sandstrom, A., Sundaresan, L., Harly, C., Nedellec, S., Olive, D., ... & Adams, E. J. (2012). The molecular basis for modulation of human Vγ9Vδ2 T cell responses by CD277/butyrophilin-3 (BTN3A)-specific antibodies. Journal of Biological Chemistry, 287(39), 32780-32790.

Patil, R. S., Shah, S. U., Shrikhande, S. V., Goel, M., Dikshit, R. P., & Chiplunkar, S. V. (2016). IL17 producing γδT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. International Journal of Cancer, 139(4), 869-881.

Pedoeem, A., Azoulay-Alfaguter, I., Strazza, M., Silverman, G. J., & Mor, A. (2014). Programmed death-1 pathway in cancer and autoimmunity. Clinical Immunology, 153(1), 145-152.

Peng, G., Wang, H. Y., Peng, W., Kiniwa, Y., Seo, K. H., & Wang, R. F. (2007). Tumor-infiltrating γδ T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. Immunity, 27(2), 334-348.

Poggi, A., Zocchi, M. R., Costa, P., Ferrero, E., Borsellino, G., Placido, R., ... & Brosnan, C. F. (1999). IL-12-mediated NKRP1A up-regulation and consequent enhancement of endothelial transmigration of Vδ2+ TCRγδ+ T lymphocytes from healthy donors and multiple sclerosis patients. The Journal of Immunology, 162(7), 4349-4354.

Porritt, H. E., Rumfelt, L. L., Tabrizifard, S., Schmitt, T. M., Zúñiga-Pflücker, J. C., & Petrie, H. T. (2004). Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. Immunity, 20(6), 735-745.

Prigione, I., Benvenuto, F., Bocca, P., Battistini, L., Uccelli, A., & Pistoia, V. (2009). Reciprocal interactions between human mesenchymal stem cells and γδ T cells or invariant natural killer T cells. Stem cells, 27(3), 693-702.

Ramirez, K., Witherden, D. A., & Havran, W. L. (2015). All hands on DE (T) C: Epithelial-resident γδ T cells respond to tissue injury. Cellular immunology, 296(1), 57-61.

Rhodes, D. A., Chen, H. C., Price, A. J., Keeble, A. H., Davey, M. S., James, L. C., ... & Trowsdale, J. (2015). Activation of human γδ T cells by cytosolic interactions of BTN3A1 with soluble phosphoantigens and the cytoskeletal adaptor periplakin. The Journal of Immunology, 194(5), 2390-2398.

Rhodes, D. A., Reith, W., & Trowsdale, J. (2016). Regulation of immunity by butyrophilins. Annual review of immunology, 34, 151-172.

Ribeiro, S. T., Ribot, J. C., & Silva-Santos, B. (2015). Five layers of receptor signalling in γδ T cell differentiation and activation. Frontiers in immunology, 6, 15.

Ribot, J. C., Debarros, A., & Silva-Santos, B. (2011). Searching for "signal 2": costimulation requirements of γδ T cells. Cellular and Molecular Life Sciences, 68(14), 2345-2355.

Ribot, J. C., Debarros, A., Pang, D. J., Neves, J. F., Peperzak, V., Roberts, S. J., ... & Silva-Santos, B. (2009). CD27 is a thymic determinant of the balance between interferon-γ-and interleukin 17–producing γδ T cell subsets. Nature immunology, 10(4), 427-436.

Ribot, J. C., Ribeiro, S. T., Correia, D. V., Sousa, A. E., & Silva-Santos, B. (2014). Human γδ thymocytes are functionally immature and differentiate into cytotoxic type 1 effector T cells upon IL-2/IL-15 signaling. The Journal of Immunology, 192(5), 2237-2243.

Rincon-Orozco, B., Kunzmann, V., Wrobel, P., Kabelitz, D., Steinle, A., & Herrmann, T. (2005). Activation of Vγ9Vδ2 T cells by NKG2D. The Journal of Immunology, 175(4), 2144-2151.

Roth, S. J., Diacovo, T. G., Brenner, M. B., Rosat, J. P., Buccola, J., Morita, C. T., & Springer, T. A. (1998). Transendothelial chemotaxis of human αβ and γδ T lymphocytes to chemokines. European journal of immunology, 28(1), 104-113.

Russano, A. M., Agea, E., Corazzi, L., Postle, A. D., De Libero, G., Porcelli, S., ... & Spinozzi, F. (2006). Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted γδ T cells. Journal of allergy and clinical immunology, 117(5), 1178-1184.

Sabbione, F., Gabelloni, M. L., Ernst, G., Gori, M. S., Salamone, G., Oleastro, M., ... & Jancic, C. C. (2014). Neutrophils suppress γδ T-cell function. European journal of immunology, 44(3), 819-830.

Sandstrom, A., Peigné, C. M., Léger, A., Crooks, J. E., Konczak, F., Gesnel, M. C., ... & Adams, E. J. (2014). The intracellular B30. 2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. Immunity, 40(4), 490-500.

Schall, T. J., Bacon, K., Camp, R. D., Kaspari, J. W., & Goeddel, D. V. (1993). Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. The Journal of experimental medicine, 177(6), 1821-1826.

Schatorjé, E. J., Gemen, E. F., Driessen, G. J., Leuvenink, J., van Hout, R. W., & de Vries, E. (2012). Paediatric reference values for the peripheral T cell compartment. Scandinavian journal of immunology, 75(4), 436-444.

Scotet, E., Martinez, L. O., Grant, E., Barbaras, R., Jenö, P., Guiraud, M. & Lopez, F. (2005). Tumor recognition following Vγ9Vδ2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein AI. Immunity, 22(1), 71-80.

Sebestyen, Z., Scheper, W., Vyborova, A., Gu, S., Rychnavska, Z., Schiffler, M. & Olive, D. (2016). RhoB mediates phosphoantigen recognition by Vγ9Vδ2 T cell receptor. Cell reports, 15(9), 1973-1985.

Siegers, G. M., Swamy, M., Fernández-Malavé, E., Minguet, S., Rathmann, S., Guardo, A. C., ... & Schamel, W. W. (2007). Different composition of the human and the mouse γδ T cell receptor explains different phenotypes of CD3γ and CD3δ immunodeficiencies. The Journal of experimental medicine, 204(11), 2537-2544.

Silva-Santos, B., Serre, K., & Norell, H. (2015). γδ T cells in cancer. Nature reviews Immunology, 15(11), 683-691.

Sonabend, A. M., Ulasov, I. V., Tyler, M. A., Rivera, A. A., Mathis, J. M., & Lesniak, M. S. (2008). Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma. Stem cells, 26(3), 831-841.

Strauss, L., Bergmann, C., Szczepanski, M., Gooding, W., Johnson, J. T., & Whiteside, T. L. (2007). A unique subset of CD4+ CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-β1 mediates suppression in the tumor microenvironment. Clinical Cancer Research, 13(15), 4345-4354.

Studeny, M., Marini, F. C., Champlin, R. E., Zompetta, C., Fidler, I. J., & Andreeff, M. (2002). Bone marrow-derived mesenchymal stem cells as vehicles for interferon-β delivery into tumors. Cancer research, 62(13), 3603-3608.

Sumaria, N., Roediger, B., Ng, L. G., Qin, J., Pinto, R., Cavanagh, L. L., ... & Weninger, W. (2011). Cutaneous immunosurveillance by self-renewing dermal γδ T cells. Journal of Experimental Medicine, 208(3), 505-518.

Tanaka, Y., Morita, C. T., Tanaka, Y., Nieves, E., Brenner, M. B., & Bloom, B. R. (1995). Natural and synthetic non-peptide antigens recognized by human γδ T cells. Nature, 375(6527), 155-158.

Tanaka, Y., Sano, S., Nieves, E., De Libero, G., Rosa, D., Modlin, R. L., ... & Morita, C. T. (1994). Nonpeptide ligands for human gamma delta T cells. Proceedings of the National Academy of Sciences, 91(17), 8175-8179.

Taub, D. D., Conlon, K., Lloyd, A. R., Oppenheim, J. J., & Kelvin, D. J. (1993). Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta. Science, 260(5106), 355-358.

Thedrez, A., Harly, C., Morice, A., Salot, S., Bonneville, M., & Scotet, E. (2009). IL-21-mediated potentiation of antitumor cytolytic and proinflammatory responses of human Vγ9Vδ2 T cells for adoptive immunotherapy. The Journal of Immunology, 182(6), 3423-3431.

Thedrez, A., Sabourin, C., Gertner, J., Devilder, M. C., Allain-Maillet, S., Fournié, J. J., ... & Bonneville, M. (2007). Self/non-self discrimination by human γδ T cells: simple solutions for a complex issue?. Immunological reviews, 215(1), 123-135.

Todaro, M., D'Asaro, M., Caccamo, N., Iovino, F., Francipane, M. G., Meraviglia, S., ... & Dieli, F. (2009). Efficient killing of human colon cancer stem cells by γδ T lymphocytes. The Journal of Immunology, 182(11), 7287-7296.

Van Acker, H. H., Anguille, S., Van Tendeloo, V. F., & Lion, E. (2015). Empowering gamma delta T cells with antitumor immunity by dendritic cell-based immunotherapy. Oncoimmunology, 4(8), e1021538.

Vantourout, P., & Hayday, A. (2013). Six-of-the-best: unique contributions of γδ T cells to immunology. Nature Reviews Immunology, 13(2), 88-100.

Viey, E., Lucas, C., Romagne, F., Escudier, B., Chouaib, S., & Caignard, A. (2008). Chemokine receptors expression and migration potential of tumor-infiltrating and peripheral-expanded Vγ9Vδ2 T cells from renal cell carcinoma patients. Journal of Immunotherapy, 31(3), 313-323.

Wakita, D., Sumida, K., Iwakura, Y., Nishikawa, H., Ohkuri, T., Chamoto, K., ... & Nishimura, T. (2010). Tumor-infiltrating IL-17-producing γδ T cells support the progression of tumor by promoting angiogenesis. European journal of immunology, 40(7), 1927-1937.

Welte, T., & Zhang, X. H. F. (2015). Interleukin-17 could promote breast cancer progression at several stages of the disease. Mediators of inflammation, 2015.

Wesch, D., Glatzel, A., & Kabelitz, D. (2001). Differentiation of resting human peripheral blood γδ T cells toward Th1-or Th2-phenotype. Cellular immunology, 212(2), 110-117.

Willcox, C. R., Pitard, V., Netzer, S., Couzi, L., Salim, M., Silberzahn, T., ... & Déchanet-Merville, J. (2012). Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor. Nature immunology, 13(9), 872-879.

Witherden, D. A., & Havran, W. L. (2012). EPCR: a stress trigger for γδ T cells. Nature immunology, 13(9), 812-814.

Wu, P., Wu, D., Ni, C., Ye, J., Chen, W., Hu, G., ... & Chen, Z. (2014). γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. Immunity, 40(5), 785-800.

Yamaguchi, T., Suzuki, Y., Katakura, R., Ebina, T., Yokoyama, J., & Fujimiya, Y. (1998). Interleukin-15 effectively potentiates the in vitro tumor-specific activity and proliferation of peripheral blood γδT cells isolated from glioblastoma patients. Cancer Immunology, Immunotherapy, 47(2), 97-103.

Yan, J., & Huang, J. (2014). Innate γδT17 cells convert cancer-elicited inflammation into immunosuppression through myeloid-derived suppressor cells. Oncoimmunology, 3(8), e953423.

Yang, J., & Weinberg, R. A. (2008). Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Developmental cell, 14(6), 818-829.

Ye, J., Ma, C., Hsueh, E. C., Eickhoff, C. S., Zhang, Y., Varvares, M. A., ... & Peng, G. (2013a). Tumor-derived γδ regulatory T cells suppress innate and adaptive immunity through the induction of immunosenescence. The Journal of Immunology, 190(5), 2403-2414.

Ye, J., Ma, C., Wang, F., Hsueh, E. C., Toth, K., Huang, Y., ... & Hoft, D. F. (2013b). Specific recruitment of γδ regulatory T cells in human breast cancer. Cancer research, 73(20), 6137-6148.

Yin, Z., Zhang, D. H., Welte, T., Bahtiyar, G., Jung, S., Liu, L., ... & Craft, J. (2000). Dominance of IL-12 over IL-4 in γδ T cell differentiation leads to default production of IFN-γ: failure to down-regulate IL-12 receptor β2-chain expression. The Journal of Immunology, 164(6), 3056-3064.

Zhao, H., Nguyen, H., & Kang, J. (2005). Interleukin 15 controls the generation of the restricted T cell receptor repertoire of γδ intestinal intraepithelial lymphocytes. Nature immunology, 6(12), 1263-1271.

Zhao, Y., Niu, C., & Cui, J. (2018). Gamma-delta (γδ) T cells: friend or foe in cancer development?. Journal of translational medicine, 16(1), 3.

Zocchi, M. R., & Poggi, A. (1993). Lymphocyte-endothelial cell adhesion molecules at the primary tumor site in human lung and renal cell carcinomas. JNCI: Journal of the National Cancer Institute, 85(3), 246-247.

Zou, C., Zhao, P., Xiao, Z., Han, X., Fu, F., & Fu, L. (2017). γδ T cells in cancer immunotherapy. Oncotarget, 8(5), 8900.w