γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer

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ABSTRACT

The potential of cancer immunotherapy relies on the mobilization of immune cells capable of producing anti-tumour cytokines and effectively killing tumour cells. These are major attributes of γδ T cells, a lymphoid lineage that is often underestimated despite its major role in tumour immune surveillance, which has been established in a variety of pre-clinical cancer models. This notwithstanding, in particular instances the tumour microenvironment seemingly mobilizes γδ T cells with immunosuppressive or tumour-promoting functions, thus emphasizing the importance of regulating γδ T cell responses to realize their translation into effective cancer immunotherapies. In this Review we outline both seminal work and recent advances in our understanding of how γδ T cells participate in tumour immunity and how their functions are regulated in experimental models of cancer. We also discuss the current strategies aimed at maximizing the therapeutic potential of human γδ T cells, on the eve of their exploration in cancer clinical trials that may position them as key players in cancer immunotherapy.

[H1] INTRODUCTION

T cells are key components of the tumour microenvironment (TME), and their therapeutic manipulation with immune checkpoint inhibitors or upon adoptive cell transfer has produced recent breakthroughs in the treatment of cancer[1,2]. While most T cell research and clinical application centres on αβ T cells, i.e., T cells expressing a lineage-specific αβ T cell receptor (TCR), γδ TCR-expressing T cells are also important players in cancer immunity[3]. γδ T cells share many qualities with their αβ T cell counterparts, such as cytotoxic effector functions and pro-inflammatory cytokine production, but one major difference between γδ T cells and αβ T cells is their relative dependence on major histocompatibility complex (MHC) molecules. The γδ TCR does not bind MHC molecules, and antigen recognition by γδ T cells has remained elusive, as recently discussed elsewhere[4,5]. This distinction from αβ T cells, coupled with their relatively low numbers in mammals, has slowed down progress on understanding the role of γδ T cells in tumorigenesis. However, the last few years has seen major advances in our knowledge of cancer-associated γδ T cell biology (**Figure 1**): uncovering their powerful influence on tumours and other immune cells; highlighting their multifaceted role as both anti- and pro-tumour mediators; and unravelling the individual contributions of γδ T cell subsets to cancer progression.

An intrinsic difficulty in γδ T cell research is the evolutionary divergence of TCR genes between humans and mice, where most pre-clinical work is performed. In particular, the major γδ T cell subsets in humans do not have orthologs in mice[6]. Moreover, the most relevant mouse γδ T cell subsets are defined by the TCR Vγ chain usage (i.e. Vγ1-7), in contrast with Vδ-based subsets in humans (i.e. Vδ1-3)[3]. Despite this clear discrepancy, functionally analogous γδ T cell populations – i.e., with similar effector functions and (patho)physiological roles – can be found in mice and humans, which has contributed decisively to our increased understanding of the place occupied by γδ T cells in immunity. Along these lines, an important recent finding was the conserved role of butyrophilin family members in homeostatic interactions with functionally equivalent subsets of mouse and human intestinal γδ T cells[7]. In this Review we elaborate on the basic biological behaviour and therapeutic potential of γδ T cells in cancer, from their functional properties and regulation in the TME to the design of new γδ T cell-based approaches for cancer immunotherapy.

[H1] ANTI-TUMOUR FUNCTIONS OF γδ T CELLS

[H2] Direct tumour cell targeting by γδ T cells

The seminal study that established an anti-tumour role for γδ T cells in mice came from the Hayday laboratory and demonstrated that these cells control the development and growth of transplantable squamous cell carcinomas, as well as methylcholanthrene (MCA)- or dimethylbenz[a]anthracene (DMBA)-induced cutaneous tumours[8]. The strong anti-tumour function of mouse γδ T cells in the MCA cancer model was corroborated by other groups[9] and extended to models of spontaneous B cell lymphomas[10], prostate cancer[11] and the widely-used B16 melanoma model[9,12,13]. γδ T cell recognition of cancer cells relies on the engagement of their TCR and/or natural killer cell receptors (NKRs)[14]. In mice, skin exposure to carcinogens leads to expression of the stress ligands, RAE-1 and H60, by keratinocytes that bind the NKG2D receptor expressed on skin-resident Vγ5$^+$ T cells (also called dendritic epidermal T cells (DETCs))[8]. Indeed, acute changes in NKG2D ligand expression in the epidermis induce morphological changes[15,16] and interleukin 13 (IL-13) expression[17] in Vγ5$^+$ T cells to counteract carcinogenesis *in vivo*. The mechanism by which γδ T cell-derived IL-13 protects against tumour formation in the DMBA cancer model is not entirely clear. IL-13 activates keratinocytes via the IL-13 receptor (IL-13Rα1) to produce various cytokines and

IL-13 mediates their migration through the epidermis[17], but whether these effects explain the anti-tumour functions has yet to be formally established. Recent studies have shown that inhibition of mTOR signalling using rapamycin increases NKG2D expression on *ex vivo*-expanded mouse Vγ4$^+$ T cells as well as enhances their cytotoxicity to various cancer cell lines[18]. Human γδ T cells also recognize transformed cells through NKG2D[14,19]. Tumour cells in both solid and haematological malignancies frequently express the human orthologues of RAE-1, MHC class I polypeptide related sequence A (MICA) and MICB, as well as members of the UL16 binding protein (ULBP) family (ULBP1-6) that also activate NKG2D-expressing Vδ1$^+$ cells[20] and Vδ2$^+$ cells[21,22]. Other NKRs, such as DNAM-1, NKp30 and NKp44, which can be expressed by γδ T cells and play a role in recognition of cancer cells, are reviewed elsewhere[14,23].

The mechanisms by which γδ T cells kill cancer cells are similar to that of conventional cytotoxic T cells **(Figure 2)**. In fact, engagement of NKG2D activates cytolytic responses in human γδ T cells[19], which are mediated by the granule exocytosis pathway through the secretion of the pore-forming molecule, perforin, and the pro-apoptotic protease, granzyme B. In mouse studies, γδ T cells and CD8$^+$ T cells infiltrating B16 melanoma lesions express perforin and granzyme B to the same degree[12]. However, specific subsets of γδ T cells are more prone to cancer cell killing than other subpopulations. *In vitro*-expanded splenic Vγ4$^+$ cells express higher levels of perforin and induce greater mouse YAC-1 T cell lymphoma and B16 melanoma cell death than Vγ1$^+$ cells[13]. Similarly, human γδ T cells employ the granule exocytosis pathway to kill various cancer cell types *in vitro*, such as renal cell carcinoma[24], squamous cell carcinoma[25], colorectal carcinoma[25,26], transformed kidney fibroblasts[25] and chronic myeloid leukemia (CML) cells[27]. Besides the perforin–granzyme axis, human Vγ9Vδ2 T cells also induce *in vitro* killing of CML cells[27] and lung cancer cells[28] through the expression of tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). In addition, FAS ligand, another member of the TNF family that induces apoptosis in target cells, mediates human γδ T cell killing of FAS receptor-expressing osteosarcoma cell lines *in vitro*[29]. Human γδ T cells also use antibody-dependent cellular cytotoxicity (ADCC), which is a cell death-inducing mechanism by which immune cells that express Fc receptors recognize antibodies bound to a target cell. Indeed, CD16 (also known as FcγRIII) expression by circulating T lymphocytes is mainly attributed to γδ T cells[30]. Upon activation, Vγ9Vδ2 T cells upregulate CD16 and can induce ADCC on target cells following treatment with antibodies, such as the monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2; also known as ERBB2) trastuzumab[31,32], the B lymphocyte antigen CD20-specific monoclonal antibody rituximab[31,33], bispecific antibodies that bind the TCR complex and HER2[34] or even B lymphocyte antigen CD19-specific triplebodies **[G]** [35]. Interestingly, this category of killing seems specific to Vγ9Vδ2 T cells, as their Vδ1$^+$ T cell counterparts utilize antibody-independent mechanisms – which may include increased production of interferon-γ (IFNγ) and Granzyme B – to induce neuroblastoma cell death *in vitro*[36]. However, ADCC may not be the only outcome of CD16 activation, as IgG-opsonized human cytomegalovirus induces IFNγ production by Vδ2$^-$ T cells in a CD16-dependent manner, but the importance of this mechanism remains unknown for anti-tumour responses[30].

[H2] Indirect effects of γδ T cells on anti-tumour immunity

γδ T cells also influence anti-tumour immunity by orchestrating downstream immune responses **(Figure 2)**. In B16 melanoma, they express IFNγ in the tumour bed to amplify IFNγ production in αβ T cells[9] and induce MHC-I expression on tumour cells[37], thereby increasing the potency of cytotoxic T cells and potentiating recognition of cancer cells. Likewise, human blood- and gastric tumour-derived γδ T cells stimulate αβ T cell activation and proliferation – an effect achieved by the antigen-presenting cell properties of Vγ9Vδ2 T cells[38-42]. In fact, this subset not only expresses similar levels of antigen presentation molecules and co-stimulatory molecules as standard antigen-presenting cells[38], they are also

functionally equivalent to mature dendritic cells in their ability to induce peptide-specific T cell activation and expansion[39]. These antigen-presenting cell functions can be further enhanced by tumour-reactive monoclonal antibodies[41]. The impact of γδ T cells on anti-tumour immunity is not limited to the promotion of αβ T cell responses, since activated human γδ T cells can stimulate NK cell cytotoxicity via costimulation of CD137 (also known as 4-1BB)[43]. However, it should be noted that in co-cultures of zoledronate-activated human γδ T cells, IL-2-primed NK cells and monocyte-derived dendritic cells (moDCs), γδ T cells negatively impacted IFNγ production by NK cells by killing moDCs that supply NK cell-activating cytokines[44]. These data suggest that the effects of γδ T cells on anti-tumour immunity are context-dependent and may be modulated by specific anti-cancer therapies.

Another established function of murine γδ T cells in immunology is the provision of help towards immunoglobulin class switching **[G]**, germinal centre **[G]** formation, production of autoantibodies and shaping of pre-immune peripheral B cell populations[45-47]. These data may also extend to human γδ T cells, as Vγ9Vδ2 T cells stimulated *in vitro* with interleukin-21 (IL-21) and (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) – a microbial metabolite – increased the production of the B cell chemoattractant, C-X-C motif chemokine ligand 13 (CXCL13), increasing their potential to influence B cells[48]. A few studies have begun to elucidate the relevance of this γδ T cell function in anti-tumour responses. In a mouse model of epidermal hyperplasia driven by the loss of *Notch1* in keratinocytes that express an artificial antigen, β-galactosidase, the induction of skin hyperplasia results in an increased production of β-galactosidase-specific immunoglobulin G (IgG), which is dependent on γδ T cells[49]. However, the impact of these tumour-specific, γδ T cell-dependent antibodies on cancer progression in this model is unknown. More recently, a protective response by tumour-specific antibodies that are induced by γδ T cells was shown in a model of DMBA-driven cutaneous tumorigenesis[50], where the anti-tumour functions of NKG2D-expressing Vγ5$^+$ T cells were previously established[8,15]. In this report, topical exposure to DMBA leads to Vγ5$^+$ T cell-dependent B cell class switching to IgE. The accumulation of autoreactive IgE protects against carcinogenesis in an FcεRI-dependent manner, indicating that γδ T cells play an important role in tumour protection by helping B cells to undergo class switching[50].

In mice, γδ T cells can play a beneficial role in chemotherapy and targeted therapy response. Namely, γδ T cells were required for the anti-proliferative effects of doxorubicin on subcutaneously injected AT3 mammary cells[51] and MCA205 fiborsarcoma cells[51,52]. The mechanism proposed for this anti-tumour benefit involves IL-17A-producing γδ T cells that control the influx and activity of IFNγ-expressing CD8 T cells[52]. Similarly, in a cKIT-mutated mouse model of gastrointestinal stromal tumours (GIST), γδ T cells mediated anti-tumour immunity and tumour progression following cKIT inhibitor therapy with imatinib. GM-CSF-expressing γδ T cells regulated the infiltration of CD103$^+$ dendritic cells (and subsequently CD8 T cells), under the direction of macrophages producing IL-1β[53]. Interestingly, γδ T cells co-expressed GM-CSF and IL-17A in the GIST model, even though, the role of IL-17A was not tested. These data stand in contrast to the large body of literature on the pro-tumour functions of IL-17A-producing γδ T cells (discussed in the next section), suggesting that chemotherapy and targeted therapy in some scenarios may alter the natural functions of IL-17-producing γδ T cells.

[H1] PRO-TUMOUR FUNCTIONS OF γδ T CELLS

Much of what we know about the pro-tumorigenic roles of γδ T cells stems from their ability to produce IL-17A (**Box 1**). Various studies have shown that IL-17 (used hereafter to denote IL-17A for simplification) expression is increased by γδ T cells in tumours formed following the

injection of cancer cell lines subcutaneously, orthotopically or intravenously in mice[54-61], and that implanting these same cell lines into IL-17 knockout mice results in reduced tumour growth in models of breast cancer[61], fibrosarcoma[54,57], hepatocellular carcinoma[59], lung cancer[55,58], melanoma[55,58] and ovarian cancer[60]. IL-17-producing γδ T cells are also increased in autochthonous genetically engineered models of cancer, such as the *Mist1-Cre*^{ERT2};*Kras*^{G12D} model of early pancreatic cancer[62], colorectal cancer models driven by the loss of the tumour suppressor, adenomatous polyposis coli (*Apc*)[63,64], the keratin 14 (*K14*)-*Cre;*cadherin-1 (*Cdh1*)$^{F/F}$;*Trp53*$^{F/F}$ lobular breast cancer model[65], the *Kras*^{G12D} or *Kras*^{G12D};*Trp53*$^{F/F}$ lung adenocarcinoma models[66,67] and the *K14*-human papillomavirus 16 (*HPV16*) model of skin squamous cell carcinoma[68,69]. γδ T cells that produce IL-17 in tumour-bearing mice usually express Vγ4 or Vγ6 TCRs[59,60,65,67].

IL-17 from γδ T cells drives cancer progression via several downstream effects on cancer cells, endothelial cells and other immune cell populations **(Figure 3)**. For example, signalling directly through IL-17 receptors on pancreatic acinar cells accelerates pancreatic intraepithelial neoplasia (PanIN) in *Mist1-Cre*^{ERT2};*Kras*^{G12D} mice[62]. IL-17 may act directly on endothelial cells to stimulate tumour growth via angiogenesis[54,68] or to upregulate adhesion molecules and endothelial cell permeability that promotes metastases at secondary sites[58]. In mice bearing mouse ID8 ovarian cancer cells, the expansion of IL-17-producing γδ T cells promoted the recruitment of pro-angiogenic macrophages to tumours and initiated the angiogenic switch **[G]** [60]. There is also a strong reciprocal link between IL-17-producing γδ T cells and neutrophils. These two cell types influence each other by γδ T cell-driven, G-CSF-mediated expansion and polarization of neutrophils towards an immunosuppressive phenotype[56,59,65], as well as neutrophil-mediated upregulation of IL-17 expression in γδ T cells[59]. These mechanisms support tumour growth and metastasis by dampening anti-tumour immunity in mouse models of liver[59] and breast cancer[65]. More recently, it has been shown in lung tumour-bearing *Kras*^{G12D};*Trp53*$^{F/F}$ mice that microbiota-triggered IL-17-producing γδ T cells promote cancer progression[67]. Neutralization of IL-17 in these tumour-bearing mice reduces granulocyte colony-stimulating factor (G-CSF) levels as well as neutrophil infiltration into tumours, which is a mechanism analogous to the γδ T cell–IL-17–G-CSF–neutrophil axis that promotes breast cancer lung metastasis[65].

IL-17-producing γδ T cells are rarely found in healthy individuals[70,71], but these cells accumulate in disease settings, such as meningitis[71] and cancer. Thus, these cells infiltrate into human tumours from patients with gallbladder[72], breast[73], colon[74,75], lung[76], ovarian[73] and cervical[68] cancer as well as cutaneous squamous cell carcinoma[77]. A few of these studies have shown a preference for IL-17 among Vδ1$^+$ T cells[72,77]. However, their existence and importance in humans has been met with some scepticism. The contentiousness surrounding this issue partly stems from disparate studies where γδ T cell numbers and IL-17 expression levels are widely different. A prime example of this comes from opposing findings in colon cancer studies: one concluding that tumour-infiltrating γδ T cells are highly abundant and a major source of IL-17 [74], while another concluding that IL-17-producing γδ T cells are negligible[75]. The contrasting results may be explained by differences between patient cohorts, such as diet, microbiome, tumour microenvironment and treatment regimen. Ultimately, though, research in this area should expand to investigate more patient cohorts, using techniques that examine γδ T cells *in situ* in addition to *ex vivo* flow cytometry analysis of γδ T cells.

Beyond IL-17, γδ T cells can advance cancer progression via other means **(Figure 3)**. One way this can be achieved is through production of IL-4 which can be expressed by both human[78] and mouse[79] γδ T cells. In B16 melanoma, IL-4-producing γδ T cells suppress the killing capacity of other anti-tumour γδ T cell subsets[79]. IL-4 also inhibits the anti-tumour activities of both human Vδ1$^+$ and Vδ2$^+$ T cells *in vitro*[80]. Mouse γδ T cells residing in injected

sarcomas derived from transgenic *Kras*^{G12D};*Trp53*$^{F/F}$ mice can also suppress cytotoxic CD8$^+$ T cells by secreting galectin-1[73], a molecule that binds glycosylated receptors on target cells, sensitizing them to apoptosis or desensitizing them to other stimuli[81]. Galectin-1-expressing Vγ9$^+$ $\gamma\delta$ T cells can also be found infiltrating human ovarian tumours[73]. In subcutaneous and intra-pancreatic mouse models of pancreatic cancer using cell lines derived from *Kras*^{G12D};*Trp53*^{R172H};*Pdx-1-Cre* (KPC) mice, tumour-associated $\gamma\delta$ T cells express programmed cell death protein 1 ligand 1 (PDL1) and galectin-9 that prevent cytotoxic T cells from killing cancer cells to promote tumour growth[82]. Like galectin-1$^+$ $\gamma\delta$ T cells in ovarian cancer, this observation is relevant to human disease, as PDL1 and galectin-9 expression in circulating and tumour-infiltrating $\gamma\delta$ T cells is increased in patients with pancreatic cancer when compared with healthy individuals[82], although $\gamma\delta$ T cell infiltration in this cancer type seems highly variable[83]. Apart from their suppressive functions on T cells, $\gamma\delta$ T cells may also promote cancer progression by acting directly on malignant epithelial cells. $\gamma\delta$ T cells from KRAS^{G12D}-driven lung tumours express amphiregulin[67] – an epidermal growth factor receptor (EGFR) ligand – as well as IL-22[67,84], and genetic deletion of IL-22[84] or preventing IL-22 signalling in lung epithelial cells[67] reduces lung cancer growth.

[H1] REGULATION OF $\gamma\delta$ T CELL FUNCTIONS

[H2] Recruitment of $\gamma\delta$ T cells

Mouse IL-17-producing $\gamma\delta$ T cells constitutively express the chemokine receptors, CC-chemokine receptor 2 (CCR2) and CCR6, which play distinct roles in $\gamma\delta$ T cell trafficking. While CCR6 is important for homeostatic circulation of Vγ4$^+$ and Vγ6$^+$ T cells to the dermis, CCR2 drives their recruitment to inflammatory sites, including B16 melanoma lesions[85]. For optimal recruitment of these T cells to inflamed tissues, downregulation of CCR6 is required, which is mediated by the cytokines IL-1β, IL-23 and IL-7, and the transcription factors, interferon regulatory factor 4 (IRF4) and B cell-activating transcription factor (BATF)[85]. Intriguingly, Vγ1$^+$ T cells, which are IFNγ biased (and cytotoxic), also respond to CCR2 and its ligand, CC-chemokine ligand 2 (CCL2)[12], suggesting a pleiotropic role for this chemokine in $\gamma\delta$ T cell responses. In addition, the CCL2–CCR2 axis may also influence $\gamma\delta$ T cells indirectly, as shown in the *K14-Cre*;*Cdh1*$^{F/F}$;*Trp53*$^{F/F}$ mouse model, where mammary epithelial cells in tumours express high levels of CCL2 that upregulates IL-1β expression in tumour-associated macrophages, which in turn stimulates IL-17 expression in $\gamma\delta$ T cells[86]. In humans, whereas Vδ2$^+$ T cells express CCR5[87], tumour-infiltrating Vδ1$^+$ T cells express CXC-chemokine receptor 3 (CXCR3) and are activated by CXC-chemokine ligand 10 (CXCL10)[88]; and blood-derived Vδ1$^+$ (but not Vδ2$^+$) T cells express CCR2 and respond to CCL2 *in vitro*[12]. A deeper understanding of chemokine receptor profiles and their implications in migration and tumour infiltration may be important to enhance the efficacy of $\gamma\delta$ T cell-based therapeutic strategies.

[H2] Regulation of anti-tumour functions

Cytokines have major effects on $\gamma\delta$ T cell functions. IL-2 and IL-15 are the two main cytokines involved in the acquisition of anti-tumour functions, namely cytotoxicity and IFNγ production **(Figure 2)**, by human naïve $\gamma\delta$ T cell thymocytes **[G]** [89] as well as $\gamma\delta$ T lymphocytes isolated from the peripheral blood of healthy donors[90] or patients with cancer[91]. Moreover, IL-15-cultured dendritic cells, isolated from healthy donors or patients with cancer, were recently reported to induce, through IL-15 production, the proliferation and expression of cytotoxic molecules and IFNγ in $\gamma\delta$ T cells, without concomitant upregulation of inhibitory molecules[92]. Other cytokines, like IL-12, IL-18 and IL-21 also potentiate IFNγ production and cytotoxicity of $\gamma\delta$ T cells *in vitro*[93-95], while IL-36γ upregulates IFNγ in $\gamma\delta$ T cells and slows tumour growth in transplantable melanoma and mammary tumour mouse models[96].

γδ T cells can be negatively impacted by tumour-infiltrating immune cells **(Figure 2)**, such as regulatory T cells, via transforming growth factor β (TGFβ) and IL-10, in hepatocellular carcinoma[97]. Circulating neutrophils can also suppress IFNγ production and cytotoxicity of Vδ2$^+$ T cells *in vitro*, in an arginase-1-dependent manner[98] or through reactive oxygen species (ROS) production[99]. Similarly, myeloid cells can induce γδ T cell exhaustion through PDL1 expression[100], and the PD1–PDL1 axis downregulates IFNγ production, cytotoxicity and ADCC[101-103]. These data suggest that anti-PD1 therapy may enhance γδ T cell functions.

Various cues from the TME, including oxygen tension **[G]** and nutrient availability, may also regulate anti-tumour γδ T cell functions. Hypoxia (simulated using 1-2% oxygen) seems to have variable impact on γδ T cell activities *in vitro*, either promoting them[104] or having no effect[100] when compared to normoxia (20% oxygen). In contrast, low-density lipoprotein (LDL)-mediated cholesterol uptake by activated human γδ T cells decreased IFNγ production and expression of NKRs (NKG2D and DNAM-1 (also known as CD226)) *in vitro*, which translated into diminished anti-tumour function upon adoptive transfer to a xenograft model of breast cancer[105].

Finally, in the context of cancer treatment, it is relevant to understand how commonly used drugs may impact γδ T cell activity. Low doses of commonly used chemotherapeutic drugs, such as, 5-fluorouracyl, doxorubicin and cisplatin sensitize differentiated cell lines[106] or colon cancer initiating cells[107] to Vγ9Vδ2 T cell cytotoxicity. Decitabine, a drug that inhibits DNA methylation, seemingly upregulates NKG2D ligands on osteosarcoma cell lines and enhances their targeting by Vγ9Vδ2 T cells[108]. However, when γδ T cells themselves are subjected to decitabine treatment, their proliferation and cytotoxic features are dampened[109]. The adverse effect of decitabine on γδ T cells occurs through demethylation of the *KIR2DL2/3* promoter, resulting in increased Sp-1-mediated expression of KIR2DL2/3, an inhibitory receptor of the killer-cell immunoglobulin-like receptor (KIR) family, and reduced cytotoxic function[109]. Furthermore, histone deacetylase (HDAC) inhibitors also negatively regulate γδ T cell proliferation and cytotoxic features, although this suppression can be partially reversed by PD1 blockade[110].

[H2] Regulation of pro-tumour functions

The inflammatory cytokines, IL-1β and IL-23, which are often expressed by macrophages[65,86] or other myeloid cells[59,67] in the TME, have been widely implicated in promoting IL-17$^+$ γδ T cell responses **(Figure 3)**. Blockade or depletion of these cytokines reduced the number of IL-17$^+$ γδ T cells in mouse models of breast cancer[65,86], fibrosarcoma[54,57] and melanoma[55]. More recently, a study in *Kras*^{G12D};*Trp53*$^{F/F}$ mice bearing lung tumours demonstrated a role for commensal bacteria in stimulating the production of IL-1β and IL-23 by myeloid cells in a myeloid differentiation primary response 88 (MYD88)-dependent manner. These two cytokines subsequently induced the proliferation and activation of lung IL-17-producing Vγ6$^+$ T cells[67], consistent with the MYD88-dependent mechanisms driving hepatocellular carcinoma[59] and fibrosarcoma[57] progression. Other pieces of evidence indicate that Toll-like receptor (TLR) pathways are important for inducing IL-1β and IL-23 in cancer-associated myeloid cells upstream of IL-17-producing γδ T cells, as colonic bacterium initiate this pathway in carcinogen-induced and *Apc*^{MIN} models of colorectal cancer[64,111]. By contrast, TLR5 negatively regulates IL-17 expression in mammary cancer, ovarian cancer and sarcoma mouse models[73].

The induction of IL-17 expression in mouse and human γδ T cells seems to be conserved between species, since the combination of IL-1β, IL-23, IL-6 and TGFβ stimulates IL-17 production by human Vδ2$^+$ T cells[71]. Accordingly, human dendritic cells treated with microbial products increase their expression of IL-23, which is sufficient to generate human IL-17-producing γδ T cells[74]. Based on these data, IL-1β and IL-23 inhibitors may be useful in

abrogating the pro-tumorigenic functions of IL-17-producing γδ T cells in patients with cancer. Support for this has been provided by the CANTOS study, a randomized, double-blinded trial involving 10,061 patients across 39 countries for the purpose of preventing cardiovascular events. Unexpectedly, this trial found that an IL-1β antibody (Canakinumab) reduced lung cancer incidence and associated mortality[112]. Since IL-17-producing γδ T cells are abundant in patients with lung cancer[76], it is tempting to speculate that some of the protective effects of Canakinumab may be due to dampening pro-tumour γδ T cell functions.

IL-7 is another cytokine that promotes the expansion of both mouse and human IL-17-producing γδ T cells[113]. In the cancer context, we have shown that IL-7 expression in ID8 ovarian tumours correlates with expansion of IL-17-producing γδ T cells that express the IL-7 receptor[60]. More recently, a study using transplantable mammary tumour models showed that IL-7 expression drives IL-17-producing γδ T cells to potentiate tumour growth and metastasis, and type 1 interferon signaling negatively regulates IL-7 expression. This effect was specific to IL-7, as IL-1β and IL-23 expression were unchanged in tumour-bearing interferon-α receptor 1 (*Ifnar1*)$^{-/-}$ mice[61]. These data provide another avenue of therapeutic intervention to counteract IL-17^{+} γδ T cells.

Besides cytokines, other molecular cues promoting IL-17^{+} γδ T cell responses include activation of TCR and NKG2D[54,114] signalling, as blocking antibodies directed against these two molecules dampen IL-17 production by γδ T cells, both *in vitro*[54] and *in vivo*[114]. Additionally, nitric oxide synthase 2 (NOS2), whose expression in γδ T cells is induced by IL-1β and IL-6[115], supports the production of IL-17 while restraining the production of IFNγ[116]. However, since this study employed complete *Nos2*$^{-/-}$ mice, it is unclear whether the effect of NOS2 on γδ T cell phenotype is cell-intrinsic or extrinsic. Furthermore, IL-17^{+} γδ T cell responses are indirectly promoted by cholesterol metabolites that act on neutrophils and enhance γδ T cell-dependent mammary tumour metastasis[117].

By contrast, negative regulators of IL-17^{+} γδ T cells are still scarce. In a carcinogen-induced colorectal cancer model, the E3 ubiquitin ligase, ITCH, controls IL-17 expression, in γδ T cells, as well as in T helper 17 and innate lymphoid cells, via targeting its master transcription factor, retinoic-acid-receptor-related orphan receptor-γt (ROR-γt; an immune cell-specific isoform of RORγ), for degradation[118]. In addition, we showed that tumour-associated neutrophils suppress the proliferation of IL-17^{+} γδ T cells in transplantable hepatocellular carcinoma and melanoma models[119], consistent with a previous report using a transplantable lung cancer model[120]. We further demonstrated that IL-17^{+} γδ T cells are especially susceptible to neutrophil-derived ROS, which is associated with their lower level of the key cellular antioxidant, glutathione (compared with other lymphocyte subsets)[119]. These findings suggest that mild induction of oxidative stress in the TME may have beneficial effects in tumours highly infiltrated by IL-17^{+} γδ T cells.

[H1] CLINICAL PERSPECTIVES AND CHALLENGES

While most of the data on the interaction of γδ T cells with tumour cells has been obtained in mouse models, as reviewed above, there is clear evidence that γδ T cells impact the progression of human tumours, either as natural immune surveillors or as therapeutic agents. We discuss below the three main lines of research that substantiate this claim: (i) the prognostic value of γδ T cell infiltration in human tumours; (ii) the therapeutic proof-of-concept using xenograft models of human tumours in immunodeficient mice; and (iii) the promising albeit limited clinical data on their therapeutic modulation. We then summarize the main strategies being pursued to realise the clinical potential of γδ T cells in the near future **(Figure 4)**.

[H2] Prognostic value in human cancer

Recent data suggest that the dichotomy of IFNγ versus IL-17 expression by γδ T cells in the TME may easily extend from mouse models to human cancer samples from patients. For example, IL-17+ γδ T cells are associated with poor outcome in patients with gallbladder[72] and colon[74] cancers. In the latter cancer type, γδ T cells were shown to constitute the major source of IL-17 in tumour biopsy samples, and IL-17+ γδ T cell infiltration correlated positively with tumour size, invasion, metastasis and overall staging[74]. This contrasts with a subsequent report where patients with colon cancer whose tumour samples were rich in γδ T cells had a significantly longer 5-year disease-free survival rate[75]. Along these lines, other studies scoring either total γδ T cells[121] or specifically IFNγ+ γδ T cells[72] reported their association with increased patient survival. In fact, the most exhaustive study by Gentles et al. on tumour biopsy samples (>18,000 samples from 39 cancer types), analysed at the transcriptomic level, ranked γδ T cells as the number 1 (out of 22) immune cell population associated with favourable prognosis[122], even though the bioinformatics analysis of these data has been subsequently contested due to the inability to distinguish a γδ T cell signature from a $CD4^+$ T cell, $CD8^+$ T cell or NK cell signature[123].

It is interesting to note that, unlike mouse γδ T cells, circulating human γδ T cells are highly biased towards IFNγ production (often co-expressed with TNF)[89,124], which suggests that tumour-associated inflammation may be the driver of IL-17+ γδ T cell differentiation[3]. This is consistent with what has been reported in the infection setting; for example, in bacterial meningitis, where a large proportion of IL-17+ γδ T cells are found in the cerebrospinal fluid[71]. As with mouse γδ T cells, IL-1β, IL-23 and TGFβ seem to be the main drivers of human IL-17+ γδ T cell differentiation[70,71].

Besides IL-17 production, the adoption of suppressive functions that interfere with dendritic cell maturation and functions has also been proposed as a pro-tumour role of human γδ T cells[88,125-127]. In particular, an immunohistochemistry examination on breast cancer primary specimens revealed high infiltration by γδ T cells, which correlated positively with advanced tumour stages and lymph node metastasis, and negatively with patient survival[126].

More recently, γδ T cells infiltrating human pancreatic ductal adenocarcinoma (PDAC; which were ~40% of all tumour-infiltrating lymphocytes (TILs) in one study[82] and <5% of TILs in another study[83]) were shown to express the potent immunosuppressive ligand, PDL1; and to suppress $CD4^+$ and $CD8^+$ T cell infiltration and functionality in a mouse model of PDAC[82]. It remains unclear if abundant PDL1 expression by γδ T cells is exclusive to the pancreatic cancer microenvironment or shared amongst other tumour types. Future research should formally link functional properties like IFNγ, IL-17 or PDL1 expression to the analysis of γδ T cells in human cancer biopsy samples. This will be important to validate the findings of Gentles et al., which at face value suggest that the anti-tumour functions of γδ T cells dominate over their pro-tumour properties in the vast majority of human cancers[122].

[H2] Current strategies to bring γδ T cells to the clinic

All the available clinical experience with γδ T cells derives from the modulation of polyclonal Vγ9Vδ2 T cell activities, either upon *in vivo* stimulation with aminobisphosphonates **[G]**, or adoptive cell transfer following *in vitro* activation and expansion with aminobisphosphonates or synthetic phosphoantigens. The rationale is derived from the unique TCR-dependent reactivity of Vγ9Vδ2 T cells to non-peptidic pyrophosphates (known as phosphoantigens), which can be increased therapeutically upon aminobisphosphonate (zoledronate or pamidronate) administration. Given the upregulation of the mevalonate pathway **[G]** (that produces the pyrophosphate intermediates) in cancer cells, activated Vγ9Vδ2 T cells are

expected to efficiently and selectively target tumour cells. Despite the confirmed safety with this strategy and some interesting responses[128-130], the cumulative clinical results have been largely disappointing, given the low objective response rates obtained in both settings[131]. Various reasons have been put forward to explain the therapeutic failures, including a highly variable tumour recognition capacity of the polyclonal Vγ9Vδ2 TCR repertoire, and the functional instability, dysfunction or exhaustion of chronically activated Vγ9Vδ2 T cells. Critically, new strategies have emerged to tackle the previous limitations, thus creating a renewed momentum in the clinical application of γδ T cells – reinvigorating Vγ9Vδ2 T cells but also betting on their Vδ1+ T cell counterparts (**Figure 4**).

The combination with antibodies neutralizing inhibitory cytokines (such as TGF-β or IL-10) or with immune checkpoint inhibitors targeting PD1 or cytotoxic T lymphocyte antigen 4 (CTLA4) are logical approaches to counteract immune suppression (and exhaustion) *in vivo*. In fact, in patients with melanoma treated with ipilimumab (anti-CTLA4), higher frequencies of $V\delta2^+$ (but not $V\delta1^+$) T cells constituted an independent indicator of improved overall survival[132]. Future studies in various cancer types should give more attention to these aspects of anti-PD1/ CTLA4 therapy, since recent work using MCA-induced sarcoma cells in mice suggests that γδ T cell infiltration and phenotype change very little after anti-PD1/ CTLA4 therapy[133]. Another way to counteract potential dysfunction of patient-derived Vγ9Vδ2 T cells (either *ex vivo* or induced by long-term *in vitro* culture) using combination approaches is the co-activation with autologous monocyte-derived dendritic cells (moDCs), or the addition of the tyrosine kinase inhibitor, ibrutinib (approved for chronic lymphocytic leukaemia (CLL) treatment)[134]. Ibrutinib has direct effects on Vγ9Vδ2 T cells, as it binds to IL-2-inducible T cell kinase (ITK) and promotes an anti-tumour IFNγ-producing phenotype[134]. Finally, bispecific antibodies are also being developed as a means to enhance Vγ9Vδ2 T cell activation and targeting at the tumour site. A nanobody **[G]** -based construct targeting both Vγ9Vδ2 T cells and EGFR induced potent Vγ9Vδ2 T cell activation and tumour cell killing *in vitro* and *in vivo* (in a xenograft model of colon cancer)[135]. Moreover, a $[(HER2)_2xCD16]$ triplebody molecule, which re-directed CD16-expressing γδ T cells and NK cells to the tumour-associated cell surface antigen HER2, showed augmented cytotoxicity (and superior to trastuzumab) against HER2-expressing PDAC, and breast and ovarian tumour cells[136].

A different strategy under clinical development to overcome the low persistence or impaired activation status of Vγ9Vδ2 T cells in patients with advanced cancer is the transduction of selected high affinity Vγ9Vδ2 TCRs[137] into αβ T cells that (under particular settings, including immune checkpoint inhibition) are expected to develop durable, memory-based responses. These hybrid T cells, named TEGs (T cells Engineered with defined Gamma delta TCRs) have been shown to endow highly polyclonal αβ T cells with innate-like responsiveness against multiple tumours, based on the broad reactivity of Vγ9Vδ2 TCRs[138]. The TEG cellular product has already been produced under good manufacturing practice (GMP) conditions[139] and is now being tested in a Phase I clinical trial in patients with haematological malignancies[140] (NTR 6541).

Besides the renewed interest in Vγ9Vδ2 T cells and their receptors, there is a more recent exploration of a $V\delta1^+$ T cell avenue in cancer immunotherapy (**Figure 4**). Although there are still no validated agonist Vδ1+ TCR antibodies that could potentially be employed to activate Vδ1+ T cells *in vivo*, their use in adoptive cell therapy has been made possible owing to methodological breakthroughs in their *in vitro* expansion upon isolation from human epithelial tissues[141] or peripheral blood[142]. In particular, we have developed a 3-week clinical-grade protocol involving TCR and cytokine stimulation that allows >1,000-fold large-scale expansion of $V\delta1^+$ T cells, which thereby increase $V\delta1^+$ T cells from <0.5% of all peripheral blood lymphocytes to >70% of the cellular product (the remaining cells being mostly other γδ T cell subsets); these have been termed Delta One T (DOT) cells[142]. Importantly, TCR-

mediated activation in the presence of IL-15 induces *de novo* expression of NKRs, particularly NKp30 and NKp44, that enhance the capacity of DOT cells to target multiple haematological[90,142,143] and solid tumour (B.S-S., unpublished observations) types *in vitro*. DOT cells did not show any reactivity against normal cell types (including multiple leukocyte subsets and activated lymphocytes, as well as healthy fibroblasts) that have been tested. Antibody blockade and genetic interference (CRISPR) experiments suggest that DOT cells combine TCR and NKR-mediated mechanisms in tumour cell recognition[90,142,143].

A recent paper showed that Vδ1$^+$ cells generated from hematopoietic stem and/or progenitor cells *in vitro* can recognize the melanoma-associated antigens, melanoma antigen recognized by T cells 1 (MART1) and gp100 (also known as melanocyte protein PMEL)[144]. Challenging decades of research, the study showed that MART1 and gp100 reactive γδ TCRs bind human leukocyte antigen A2 (HLA-A2), identifying a MHC-restricted γδ TCR for the first time. While evidence for the natural existence of these cells in human tumours was not provided, the data open up new possibilities for γδ T cell-based adoptive cell therapies.

Finally, chimeric antigen receptors (CARs) are an obvious addition to the γδ T cell-based cancer immunotherapy portfolio[145]. By combining antibody-like high affinity antigen recognition with T cell signalling, CARs have been shown to dramatically increase the potency of adoptive T cell products[146,147], leading to their approval for treatment of refractory B-cell malignancies[148]. Activated γδ T cells are amenable to CAR transduction and may have the advantage of broadly-reactive γδ TCRs to tackle the potential immune evasion of the specific CAR antigen, which has been observed in the clinic[149,150]. Whether CAR-transduced γδ T cells will also be beneficial in terms of minimizing the cytokine release syndrome and neurotoxicity adverse events of conventional CAR T cells remains to be investigated. Indeed, it will also be key to compare their relative persistence in vivo and, ultimately, their efficacy in inducing cancer elimination.

[H2] Therapeutic proof-of-concept and challenges

Although mice (including γδ T cell-deficient mice) have been instrumental in revealing the non-redundant roles played by γδ T cells in cancer development and progression, the evolutionary divergence in the TCRγ and TCRδ genes between rodents and primates[6] make syngeneic models poorly suited to provide proof-of-concept for γδ T cell-based cancer immunotherapies. In particular, Vγ9Vδ2 and Vδ1+ T cells, the two main human γδ T cell subsets, do not have orthologs or equivalents in mice; and the strong reactivity of Vγ9Vδ2 T cells to non-peptidic phosphoantigens (either tumour-derived or synthetic) is not conserved in rodents[3].

Pre-clinical *in vivo* proof-of-concept studies have been mostly performed in xenograft models using human tumour cell lines or primary samples in immunodeficient (such as NSG) mice. Thus, Vγ9Vδ2 T cells have been administered (usually together with IL-2) to multiple mouse models after *in vitro* expansion with aminobisphosphonates or pyrophosphates and were shown to impact tumour load and progression. To name some interesting examples, a single dose of Vγ9Vδ2 T cells had striking impact on tumour burden in a spontaneous and highly immunosuppressive (via PD1 and CTLA4) Epstein-Barr virus (EBV)-driven lymphoma model[151]; a nanobody-based construct targeting both Vγ9Vδ2 T cells and EGFR induced potent Vγ9Vδ2 T cell activation and tumour cell killing in a xenograft model of human colon cancer[135]; and the stereotaxic administration **[G]** of Vγ9Vδ2 T cells in an orthotopic model of glioblastoma led to tumour cell elimination and much improved host survival[152]. Of note, therapeutic success in the latter model required the co-administration of zoledronate with the Vγ9Vδ2 T cells, thus highlighting the importance of 'sensitizing' tumours (by increasing intra-tumoural phosphoantigen concentrations) to Vγ9Vδ2 T cells. As for the TEG approach, i.e.

αβ T cells transduced with high-affinity Vγ9Vδ2 TCRs, it has also been successfully tested in a lymphoma xenograft model[137].

Vδ1$^+$ T cells have also shown substantial *in vivo* efficacy in pre-clinical models of human cancer. In fact, in one of very few studies where the *in vivo* potency of Vδ1$^+$ T cells was compared with that of their Vδ2$^+$ counterparts, both expanded with artificial antigen-presenting cells (derived from K562 CML cells) serving as irradiated feeders, it was observed that Vδ1$^+$ T cells had superior therapeutic activity, as evaluated by improved host (NSG mouse) survival to human CAOV3 ovarian cancer cells[153]. We have subsequently tested Vδ1$^+$ T cells expanded and differentiated with the DOT protocol in 4 xenograft models of leukaemia (acute myeloid leukaemia (AML) or CLL)[142,143]. In all the models, DOT-cell treatment diminished tumour burden and prolonged host survival, and moreover prevented systemic tumour dissemination in the MEC-1 CLL xenograft[142].

Besides efficacy, safety (toxicology) is clearly a key component of (pre-)clinical studies. However, this constitutes a major challenge and intrinsic limitation of xenograft models. For example, although DOT cell administration did not produce any histological alterations in tissues or in the biochemical analyses reporting liver and kidney function, the host tissue cells were mouse, and therefore lacked potentially relevant human self-antigens to evaluate toxic side effects. An alternative, albeit a very expensive one, is the use of non-human primates, which have been shown to induce potent Vγ9Vδ2 T cell responses *in vivo*[154,155]. This notwithstanding, non-human primates also present various limitations as toxicology models: (i) in the setting where macaque-derived T cells and administered to macaques, the cellular product being tested may be considerably different (in terms of phenotype and functionality) to the human counterpart to be used in the clinic; (ii) if injecting the human cellular product into macaques, there are issues with the potential need for immune suppression (to prevent graft rejection); (iii) and the ethical issues posed by tumour challenge, which may be required to mimic the relevant cellular interactions and even to sustain γδ T cell activation *in vivo*.

Given the limitations of *in vivo* models, we believe the pre-clinical therapeutic potential of anti-tumorigenic human γδ T cells is best evaluated by detailed *in vitro* assessment of tumour versus healthy cell targeting, using comprehensive collections of primary tumour samples and normal cell types of multiple origins (for example, haematopoietic, epithelial, endothelial), ahead of regulatory discussions and ultimately clinical trials.

[H1] CONCLUSIONS

As a result of almost two decades of translational and clinical research on γδ T cells in cancer, the time is ripe for developing efficacious therapies based on their *in vivo* activation or upon adoptive cell transfer. The limited success of previous clinical tests with Vγ9Vδ2 T cells may now be overcome by innovative strategies aiming to surmount exhaustion and guarantee persistence and improved tumour cell recognition. At the same time, we now have the means to expand their rarer (in the blood) Vδ1$^+$ T cell counterparts, which have high tropism for tissues, including tumours, and can therefore test them in the clinic for the first time. These are exciting times for γδ T cell application in cancer immunotherapy, as decisive clinical trials will take place in the next couple of years.

One important conclusion arising from the initial modulation of Vγ9Vδ2 T cells in patients with cancer is the overall safety of such strategies in the autologous setting[131]. But a much more ambitious and potentially feasible goal is the development of allogeneic, off-the-shelf γδ T cell-based immunotherapies. γδ T cells are especially suited for allogeneic strategies, since they are largely not restricted by MHC, thus avoiding the graft-versus-host effects of MHC-

mismatched αβ T cells. In fact, γδ T cell (and particularly Vδ1$^+$ T cell) reconstitution and persistence in patients with leukaemia that received partially mismatched but related donor bone marrow transplantations was the best predictor of long-term disease-free survival[156]; and this has promoted the successful application of haploidentical stem cell transplantation **[G]** using αβ T-cell and B-cell depleted grafts[157]. One interesting prospect of allogeneic γδ T cell immunotherapies is using them to treat aggressive haematological tumours derived from the transformation of γδ T cells themselves (**Box 2**).

By not being restricted by MHC, most γδ T cells also bypass one of the most common cancer immune evasion mechanisms, the downregulation of surface MHC class I molecules[158]. However, since they do not recognize mutated peptides, γδ T cells might be especially suited for treating tumours with low mutational burdens, where immune checkpoint inhibition is notably unsuccessful[159].

Based on ample evidence from pre-clinical models, the balance between IFNγ versus IL-17 producing γδ T cells in the TME may strongly impact on the success of their therapeutic modulation. Thus, upcoming clinical trials should track such activities while clearly attempting to promote IFNγ over IL-17 producing γδ T cells *in vivo*. This may require specific cytokine signals that epigenetically 'lock' γδ T cells in an IFNγ-producing programme, such as IL-15, which can be provided during the *in vitro* expansion and differentiation of cellular products; or administered *in vivo* to patients with cancer, which would require formal testing in the clinic. Another important factor to consider is the impact of the microbiome, since at least in the mouse lung it has been shown to drive the expansion of tumour-promoting IL-17$^+$ γδ T cells[67,160]. Finally, the prognostic value of tumour-infiltrating γδ T cells should be revisited in multiple cancer types with the resolution of IFNγ versus IL-17 protein expression by γδ T cells.

From a more fundamental standpoint, future research should address non-IL-17-mediated pro-tumourigenic functions of γδ T cells; and focus on further dissecting the key cellular partners and molecular co-receptors that may regulate γδ T cell activities in the TME. Finally, the identification of tumour antigens recognised by γδ T cells, either through TCRs or NKRs, remains a priority[14]: it will help clarifying the non-redundant role of γδ T cells in immune surveillance of tumours; and may be the key for the rational selection of patients to be treated with γδ T cell-based cancer immunotherapies.

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AUTHOR CONTRIBUTIONS

B.S.-S., S.M. and S.B.C. researched the data for the article, contributed equally to writing the article and to review and/or editing of the manuscript before submission.

CONFLICT OF INTEREST

B.S.-S. is co-founder and shareholder of Lymphact, the company that developed DOT cells, which was acquired in 2018 by GammaDelta Therapeutics (London, UK). S.M. and S.B.C. declare no competing interests.

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[b1] BOX 1. Phenotypic markers of effector γδ T cell subsets

γδ T cell differentiation has been mostly dissected in the C57BL/6 mouse, where the two main effector cytokines implicated in γδ T cell responses are interferon-γ (IFNγ) and interleukin-17A (IL-17A). These are mostly expressed by distinct subsets segregated on the basis of markers such as CD27, CD122, CD45RB (a splice variant of CD45), which are expressed on IFNγ$^+$ γδ T cells; and CC-chemokine receptor 6 (CCR6) and the scavenger receptor SCART-2, which are found on IL-17A$^+$ γδ T cells. IL-17 producers also express higher levels of CD44, whereas NK1.1 marks IFNγ^{hi} γδ T cells[161]. Moreover, effector γδ T cell differentiation varies across thymic developmental waves characterized by T cell receptor (TCR) Vγ□chain usage as result of V(D)J recombination **[G]**; for example, fetal-derived Vγ6$^+$ γδ T cells produce IL-17A but not IFNγ, while perinatal Vγ1$^+$ γδ T cells are biased towards IFNγ expression. Importantly, most of the accumulated evidence suggests that whereas γδ T cells making IFNγ participate in anti-tumour responses, IL-17A production underlies tumour-promoting functions in various tumour mouse models[3].

In humans, the developmental and phenotypic segregation between IL-17A versus IFNγ producing γδ T cells is much less straightforward. For example, IL-17A producers have been found to be mostly Vδ1$^+$ and to lack CD27 expression, but the majority of cells with this phenotype are actually IFNγ producers[72,77]. Thus, unlike in the mouse, the definition of effector γδ T cell subsets in humans must always rely on cytokine production itself (as assessed by intracellular staining).

[b2] BOX 2. When γδ T cells become malignant

γδ T cell lymphomas are aggressive and rare haematological malignancies that develop from the transformation of mature γδ T cells, and include hepatosplenic γδ T cell lymphoma (HSGDTL) and primary cutaneous γδ T cell lymphoma (PCGDTL). HSGDTL, which is more common among young males, presents with splenomegaly (abnormally enlarged spleen) and thrombocytopenia (a low blood platelet count), often in the absence of nodal involvement; it progresses rapidly, responding poorly to treatment and associating with high mortality[162]. PCGDTL represents less than 1% of all primary cutaneous lymphomas, but is highly aggressive and deadly[163].

γδ T-cell acute lymphoblastic leukaemia (γδ T-ALL) derives from the transformation of immature γδ thymocytes, and presents with clinical features distinct from αβ T-ALL[164]. Albeit rare, γδ T-ALL accounts for up to 10% of all T-ALL cases, which is substantially higher than the proportion (around 1%) of γδ thymocytes from the total number of thymocytes in the human thymus, thus raising the possibility that γδ thymocytes have increased potential for malignant transformation[164,165].

FIGURE LEGENDS

Figure 1. Timeline of developments in the research of γδ T cell function in cancer and their exploitation for immunotherapy

Discovery of γδ T cells – 1984-7[166-169]

Phosphoantigens identified as agonists for human Vγ9Vδ2 T cells – 1994-1996[170]

Anti-tumour role of γδ T cells recognized in mice – 2001[8]

Development of γδ T CARs – 2004[146]

Antigen-presenting cell functions of human Vγ9Vδ2 T cells discovered – 2005[38]

Academic-run trials of adoptive Vγ9Vδ2 T cell therapy in humans conducted – 2003[128]

Pro-tumoural IL-17-producing γδ T cells found in mice and humans – 2010-2014[54,55,59,60,69,71,74]

Development of TEGs – 2011[138]

BTN3A1 identified as a phosphoantigen sensing molecule – 2012[171]

γδ T cells reported as the most favourable prognostic indicator among 22 different immune cell populations in 39 cancer types – 2015[122]

Proof-of-concept demonstrated for DOT cells – 2016[142]

Clinical development of γδ T cell-based therapies by 8 companies world-wide – 2018

BTN3A1, butyrophilin subfamily 3 member A1; CAR, chimeric antigen receptor; DOT, delta One T; IL-17, interleukin-17; TEGs, T cells engineered with defined gamma delta T cell receptors.

Figure 2. Anti-tumour γδ T cell functions and their regulation

γδ T cells directly recognize tumour cells through the T cell receptor (TCR) and natural killer cell receptors (NKRs). Tumour cell-killing can be mediated by the expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), FAS or the granule exocytosis pathway (leading to secretion of perforin and granzyme). Moreover, γδ T cells can target tumour cells through antibody-dependent cellular cytotoxicity (ADCC) upon treatment with tumour-specific antibodies. Alternatively, γδ T cells induce anti-tumour immune responses through IFNγ production, and antigen-presenting cell functions, which lead to αβ T cell

activation, while 4-1BB ligand (4-1BBL) expression stimulates NK cells. In addition, γδ T cells induce antibody class switching in B cells, contributing to a protective humoral response. The anti-tumour features of γδ T cells are mainly potentiated by interleukin-15 (IL-15) and IL-2, while the expression of programmed cell death protein 1 (PD1), the presence of secreted major histocompatibility complex class I polypeptide related sequence A (sMICA) or treatment with the DNA methylation inhibitor decitabine and histone deacetylase (HDAC) inhibitors dampen their killing capacity. Other immune cell subsets like regulatory T (T_{reg}) cells and neutrophils can also inhibit anti-tumour γδ T cell features through IL-10 and TGFβ or Arginase-1 and reactive oxygen species (ROS) production, respectively. DC, dendritic cell; FASL, FAS ligand; FcγRIII, Fcγ receptor III; HLA-DR, human leukocyte antigen-DR; Ig, immunoglobulin; LDL, low-density lipoprotein; LDL-R, LDL receptor; sTRAIL, secreted TRAIL; TGFβ, transforming growth factor β; TRAIL-R, TRAIL receptor.

Figure 3. Pro-tumour γδ T cell functions and their regulation

The pro-tumour functions of γδ T cells are mainly associated with interleukin-17A (IL-17) production, which has several different roles, such as stimulation of tumour cell proliferation, induction of angiogenesis and mobilization of pro-inflammatory or immunosuppressive myeloid cells. Commensal bacteria, 27-hydroxycholesterol (27-HC) or IL-17 itself can mobilize myeloid cells, which produce IL-17-promoting cytokines like IL-1β and IL-23. Both IL-1β and IL-6 can induce the expression of nitric oxide synthase 2 (NOS2), which promotes IL-17+ γδ T cell responses. IL-7 is another factor involved in the survival and proliferation of IL-17-producing γδ T cells. Other tumour-promoting roles of γδ T cells include inhibition of dendritic cell (DC) maturation, suppression of T cell responses through galectin, programmed cell death protein 1 ligand 1 (PDL1), IL-4 expression, and induction of tumour-cell proliferation by IL-22 and amphiregulin production. Inhibition of IL-17-producing γδ T cells can be achieved through reactive oxygen species (ROS) generated by neutrophils or by the E3 ubiquitin ligase ITCH that targets retinoic-acid-receptor-related orphan receptor-γt (RORγt) for degradation. P, phosphorylation; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor β.

Figure 4. Current strategies for therapeutic manipulation of human γδ T cells

Current strategies for therapeutic use of human γδ T cells involve both Vδ1 and Vδ2 subsets. Vδ1 can be isolated from tissues and expanded *in vitro*, or from peripheral blood and expanded with the Delta One T (DOT) cell-generating protocol (a 3-week clinical grade protocol involving T cell receptor (TCR) and cytokine stimulation), which gives rise to Vδ1+ T cells expressing the natural killer (NK) cell receptors NKp30 and NKp44 and the ability to target both solid and haematological tumours. Vδ2-based strategies also involve peripheral blood extraction and *in vitro* activation with phosphoantigens (PAg). Another strategy relies on the generation of T cells Engineered with defined Gamma delta TCRs (TEGs), which consists of the cloning and transfer of Vγ9Vδ2 T cell receptors into αβ T cells. CAR, chimeric antigen receptor; PBL, peripheral blood lymphocyte.

Glossary

Triplebodies. Immunoligands consisting of three tandem single-chain variable fragments with three distinct specificities.

Immunoglobulin class switching. Mechanism by which B cells change the isotype of immunoglobulin produced, altering its effector function.

Germinal centres. Sites within spleen and lymph nodes where B cells proliferate, differentiate and perform immunoglobulin class switching.

Angiogenic switch. Timepoint during tumour progression when the pro-angiogenic factors outcompete the anti-angiogenic ones, leading to the transition between a dormant avascularized hyperplasia and an outgrowing vascularized tumour.

Thymocytes. Hematopoietic progenitor cells present in the thymus gland.

Oxygen tension. Partial pressure of oxygen molecules dissolved in a liquid (such as blood plasma).

Aminobisphosphonates. A drug type that derives from bisphosphonates and is commonly used in bone-related disorders to avoid excessive bone resorption.

Mevalonate or isoprenoid pathway. An essential metabolic pathway that gives rise to two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl purophosphate (DMAPP) which are converted into isoprenoids. Metabolites of this pathway accumulate in metabolically distressed cells.

Nanobody. An antibody with a single monomeric domain.

Stereotaxic administration. Delivery of a compound in the brain using an external, three-dimentional frame of reference usually based on the Cartesian coordinate system.

Haploidentical stem cell transplantation. Treatment of blood disorders involving the replacement of the patient's hematopoietic cells by healthy partially (50%) HLA-matched hematopoietic progenitors

V(D)J or somatic recombination. The somatic rearrangement of variable (V), diversity (D) and joining (J) regions of the genes that encode antigen receptors, leading to repertoire diversity of both T cell and B cell receptors

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Subject Categories

Biological sciences / Immunology / Lymphocytes / T cells / Gammadelta T cells [URI /631/250/1619/554/2509];
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Table of Contents summary

This Review article discusses the rapidly accumulating preclinical evidence in support of anti-tumour but also some pro-tumour roles for γδ T cells in cancer progression. It also outlines the potential of manipulating their functions for use as an unconventional form of cancer immunotherapy.