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

Bruno Silva-Santos[1], Sofia Mensurado[1] & Seth B. Coffelt[2]

[1]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal
[2]Institute of Cancer Sciences, University of Glasgow and Cancer Research UK Beatson Institute, Glasgow, UK.

Correspondence to B.S.-S. (bssantos@medicina.ulisboa.pt) or S.B.C. (Seth.Coffelt@glasgow.ac.uk)

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 Vδ2$^+$ (but not Vδ1$^+$) 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)$_2$xCD16] 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 Vδ1$^+$ 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 Vδ1$^+$ T cells, which thereby increase Vδ1$^+$ 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.

ACKNOWLEDGEMENTS

We thank Daniel Correia, André Simões, Haakan Norell and Karine Serre (iMM JLA) for insightful discussions on this topic. We acknowledge funding from European Research Council (CoG_646701 to B.S.-S.), Cancer Research UK Glasgow Centre (A25142 to S.B.C.), Breast Cancer Now (2018JulPR1101 to S.B.C.), Wellcome Trust (208990/Z/17/Z to S.B.C.), Tenovus Scotland (Project S17-17 to S.B.C.) and Fundação para a Ciência e a Tecnologia (FCT)/ Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through Fundos do Orçamento de Estado (refs. UID/BIM/50005/2019 and PD/BD/114099/2015).

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.

REFERENCES

1 Rosenberg, S. A. & Restifo, N. P. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* **348**, 62-68 (2015).

2 Wei, S. C., Duffy, C. R. & Allison, J. P. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discov* **8**, 1069-1086 (2018).

3 Silva-Santos, B., Serre, K. & Norell, H. gammadelta T cells in cancer. *Nat Rev Immunol* **15**, 683-691 (2015).

4 Willcox, B. E. & Willcox, C. R. gammadelta TCR ligands: the quest to solve a 500-million-year-old mystery. *Nat Immunol* **20**, 121-128 (2019).

5 Vermijlen, D., Gatti, D., Kouzeli, A., Rus, T. & Eberl, M. gammadelta T cell responses: How many ligands will it take till we know? *Semin Cell Dev Biol* **84**, 75-86 (2018).

6 Adams, E. J., Gu, S. & Luoma, A. M. Human gamma delta T cells: Evolution and ligand recognition. *Cell Immunol* **296**, 31-40 (2015).

7 Melandri, D. *et al.* The gammadeltaTCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. *Nat Immunol* **19**, 1352-1365 (2018).

8 Girardi, M. *et al.* Regulation of cutaneous malignancy by gammadelta T cells. *Science* **294**, 605-609 (2001).
Seminal work using *Tcrd*$^{-/-}$ mice to demonstrate a protective role for mouse γδ T cells in chemically-induced skin cancer.

9 Gao, Y. *et al.* Gamma delta T cells provide an early source of interferon gamma in tumor immunity. *J Exp Med* **198**, 433-442 (2003).

10 Street, S. E. *et al.* Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. *J Exp Med* **199**, 879-884 (2004).

11 Liu, Z. *et al.* Protective immunosurveillance and therapeutic antitumor activity of gammadelta T cells demonstrated in a mouse model of prostate cancer. *J Immunol* **180**, 6044-6053 (2008).

12 Lanca, T. *et al.* Protective role of the inflammatory CCR2/CCL2 chemokine pathway through recruitment of type 1 cytotoxic gammadelta T lymphocytes to tumor beds. *J Immunol* **190**, 6673-6680 (2013).

13 He, W. *et al.* Naturally activated V gamma 4 gamma delta T cells play a protective role in tumor immunity through expression of eomesodermin. *J Immunol* **185**, 126-133 (2010).

14 Simoes, A. E., Di Lorenzo, B. & Silva-Santos, B. Molecular Determinants of Target Cell Recognition by Human gammadelta T Cells. *Front Immunol* **9**, 929 (2018).

15 Strid, J. *et al.* Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. *Nat Immunol* **9**, 146-154 (2008).

16 Strid, J., Sobolev, O., Zafirova, B., Polic, B. & Hayday, A. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. *Science* **334**, 1293-1297 (2011).

17 Dalessandri, T., Crawford, G., Hayes, M., Castro Seoane, R. & Strid, J. IL-13 from intraepithelial lymphocytes regulates tissue homeostasis and protects against carcinogenesis in the skin. *Nat Commun* **7**, 12080 (2016).

Study showing a crucial effect of IL-13-producing γδ T cells in maintaining skin integrity and therefore protecting from carcinogenesis.

18 Cao, G. *et al.* mTOR inhibition potentiates cytotoxicity of Vgamma4 gammadelta T cells via up-regulating NKG2D and TNF-alpha. *J Leukoc Biol* **100**, 1181-1189 (2016).

19 Bauer, S. *et al.* Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285**, 727-729 (1999).

20 Groh, V. *et al.* Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc Natl Acad Sci U S A* **96**, 6879-6884 (1999).

21 Kong, Y. *et al.* The NKG2D ligand ULBP4 binds to TCRgamma9/delta2 and induces cytotoxicity to tumor cells through both TCRgammadelta and NKG2D. *Blood* **114**, 310-317 (2009).

22 Lanca, T. *et al.* The MHC class Ib protein ULBP1 is a nonredundant determinant of leukemia/lymphoma susceptibility to gammadelta T-cell cytotoxicity. *Blood* **115**, 2407-2411 (2010).

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

24 Viey, E. *et al.* Phosphostim-activated gamma delta T cells kill autologous metastatic renal cell carcinoma. *J Immunol* **174**, 1338-1347 (2005).

25 Alexander, A. A. *et al.* Isopentenyl pyrophosphate-activated CD56+ T lymphocytes display potent antitumor activity toward human squamous cell carcinoma. *Clin Cancer Res* **14**, 4232-4240 (2008).

26 Todaro, M. *et al.* Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. *J Immunol* **182**, 7287-7296 (2009).

27 D'Asaro, M. *et al.* V gamma 9V delta 2 T lymphocytes efficiently recognize and kill zoledronate-sensitized, imatinib-sensitive, and imatinib-resistant chronic myelogenous leukemia cells. *J Immunol* **184**, 3260-3268 (2010).

28 Dokouhaki, P. *et al.* NKG2D regulates production of soluble TRAIL by ex vivo expanded human gammadelta T cells. *Eur J Immunol* **43**, 3175-3182 (2013).

29 Li, Z. *et al.* IFN-gamma enhances HOS and U2OS cell lines susceptibility to gammadelta T cell-mediated killing through the Fas/Fas ligand pathway. *Int Immunopharmacol* **11**, 496-503 (2011).

30 Couzi, L. *et al.* Antibody-dependent anti-cytomegalovirus activity of human gammadelta T cells expressing CD16 (FcgammaRIIIa). *Blood* **119**, 1418-1427 (2012).

31 Tokuyama, H. *et al.* V gamma 9 V delta 2 T cell cytotoxicity against tumor cells is enhanced by monoclonal antibody drugs--rituximab and trastuzumab. *Int J Cancer* **122**, 2526-2534 (2008).

32 Capietto, A. H., Martinet, L. & Fournie, J. J. Stimulated gammadelta T cells increase the in vivo efficacy of trastuzumab in HER-2+ breast cancer. *J Immunol* **187**, 1031-1038 (2011).

33 Gertner-Dardenne, J. *et al.* Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies. *Blood* **113**, 4875-4884 (2009).

34 Oberg, H. H. *et al.* Novel bispecific antibodies increase gammadelta T-cell cytotoxicity against pancreatic cancer cells. *Cancer Res* **74**, 1349-1360 (2014).

35 Schiller, C. B. *et al.* CD19-specific triplebody SPM-1 engages NK and gammadelta T cells for rapid and efficient lysis of malignant B-lymphoid cells. *Oncotarget* **7**, 83392-83408 (2016).

36 Fisher, J. P. *et al.* Neuroblastoma killing properties of Vdelta2 and Vdelta2-negative gammadeltaT cells following expansion by artificial antigen-presenting cells. *Clin Cancer Res* **20**, 5720-5732 (2014).

37 Riond, J., Rodriguez, S., Nicolau, M. L., al Saati, T. & Gairin, J. E. In vivo major histocompatibility complex class I (MHCI) expression on MHCIlow tumor cells is regulated by gammadelta T and NK cells during the early steps of tumor growth. *Cancer Immun* **9**, 10 (2009).

38 Brandes, M., Willimann, K. & Moser, B. Professional antigen-presentation function by human gammadelta T Cells. *Science* **309**, 264-268 (2005).

39 Altvater, B. *et al.* Activated human gammadelta T cells induce peptide-specific CD8+ T-cell responses to tumor-associated self-antigens. *Cancer Immunol Immunother* **61**, 385-396 (2012).

40 Mao, C. *et al.* Tumor-activated TCRgammadelta(+) T cells from gastric cancer patients induce the antitumor immune response of TCRalphabeta(+) T cells via their antigen-presenting cell-like effects. *J Immunol Res* **2014**, 593562 (2014).

41 Himoudi, N. *et al.* Human gammadelta T lymphocytes are licensed for professional antigen presentation by interaction with opsonized target cells. *J Immunol* **188**, 1708-1716 (2012).

42 Muto, M., Baghdadi, M., Maekawa, R., Wada, H. & Seino, K. Myeloid molecular characteristics of human gammadelta T cells support their acquisition of tumor antigen-presenting capacity. *Cancer Immunol Immunother* **64**, 941-949 (2015).

43 Maniar, A. *et al.* Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. *Blood* **116**, 1726-1733 (2010).

44 Nussbaumer, O., Gruenbacher, G., Gander, H. & Thurnher, M. DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by gammadelta T lymphocytes. *Blood* **118**, 2743-2751 (2011).

45 Wen, L. *et al.* Germinal center formation, immunoglobulin class switching, and autoantibody production driven by "non alpha/beta" T cells. *J Exp Med* **183**, 2271-2282 (1996).

46 Huang, Y. *et al.* gammadelta T cells affect IL-4 production and B-cell tolerance. *Proc Natl Acad Sci U S A* **112**, E39-48 (2015).

47 Rezende, R. M. *et al.* gammadelta T cells control humoral immune response by inducing T follicular helper cell differentiation. *Nat Commun* **9**, 3151 (2018).

48 Bansal, R. R., Mackay, C. R., Moser, B. & Eberl, M. IL-21 enhances the potential of human gammadelta T cells to provide B-cell help. *Eur J Immunol* **42**, 110-119 (2012).

49 Shimura, E. *et al.* Epidermal gammadelta T cells sense precancerous cellular dysregulation and initiate immune responses. *Int Immunol* **22**, 329-340 (2010).

50 Crawford, G. *et al.* Epithelial damage and tissue gammadelta T cells promote a unique tumor-protective IgE response. *Nat Immunol* **19**, 859-870 (2018).
This work demonstrates that γδ T cells provide help for B cells to class-switch to IgE production, resulting in a protective response against tumour development.

51 Mattarollo, S. R. *et al.* Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res* **71**, 4809-4820 (2011).

52 Ma, Y. *et al.* Contribution of IL-17-producing gamma delta T cells to the efficacy of anticancer chemotherapy. *J Exp Med* **208**, 491-503 (2011).

53 Medina, B. D. *et al.* Oncogenic kinase inhibition limits Batf3-dependent dendritic cell development and antitumor immunity. *J Exp Med* (2019).

54 Wakita, D. *et al.* Tumor-infiltrating IL-17-producing gammadelta T cells support the progression of tumor by promoting angiogenesis. *Eur J Immunol* **40**, 1927-1937 (2010).

55 Carmi, Y. *et al.* Microenvironment-derived IL-1 and IL-17 interact in the control of lung metastasis. *J Immunol* **186**, 3462-3471 (2011).

56 Benevides, L. *et al.* IL17 Promotes Mammary Tumor Progression by Changing the Behavior of Tumor Cells and Eliciting Tumorigenic Neutrophils Recruitment. *Cancer Res* **75**, 3788-3799 (2015).

57 Kimura, Y. *et al.* IL-17A-producing CD30(+) Vdelta1 T cells drive inflammation-induced cancer progression. *Cancer Sci* **107**, 1206-1214 (2016).

58 Kulig, P. *et al.* IL17A-Mediated Endothelial Breach Promotes Metastasis Formation. *Cancer Immunol Res* **4**, 26-32 (2016).

59 Ma, S. *et al.* IL-17A produced by gammadelta T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res* **74**, 1969-1982 (2014).

60 Rei, M. *et al.* Murine CD27(-) Vgamma6(+) gammadelta T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. *Proc Natl Acad Sci U S A* **111**, E3562-3570 (2014).
Description of a pro-tumour axis between mouse IL-17-producing γδ T cells and pro inflammatory/pro-angiogenic macrophages in an ovarian cancer model.

61 Patin, E. C. *et al.* Type I IFN Receptor Signaling Controls IL7-Dependent Accumulation and Activity of Protumoral IL17A-Producing gammadeltaT Cells in Breast Cancer. *Cancer Res* **78**, 195-204 (2018).

62 McAllister, F. *et al.* Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. *Cancer Cell* **25**, 621-637 (2014).
This study shows a pathogenic role for IL-17, provided in the tumour microenvironment by γδ T cells and Th17 cells, in pancreatic tumour development and progression.

63 Housseau, F. *et al.* Redundant Innate and Adaptive Sources of IL17 Production Drive Colon Tumorigenesis. *Cancer Res* **76**, 2115-2124 (2016).

64 Wu, S. *et al.* A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med* **15**, 1016-1022 (2009).

65 Coffelt, S. B. *et al.* IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* **522**, 345-348 (2015).
This study reveals for the first time a pro-metastatic role for mouse IL-17-producing γδ T cells in a breast cancer model.

66 Busch, S. E. *et al.* Lung Cancer Subtypes Generate Unique Immune Responses. *J Immunol* **197**, 4493-4503 (2016).

67 Jin, C. *et al.* Commensal Microbiota Promote Lung Cancer Development via gammadelta T Cells. *Cell* **176**, 998-1013 e1016 (2019).

68 Van Hede, D. *et al.* Human papillomavirus oncoproteins induce a reorganization of epithelial-associated gammadelta T cells promoting tumor formation. *Proc Natl Acad Sci U S A* **114**, E9056-E9065 (2017).

69 Gosmann, C., Mattarollo, S. R., Bridge, J. A., Frazer, I. H. & Blumenthal, A. IL-17 suppresses immune effector functions in human papillomavirus-associated epithelial hyperplasia. *J Immunol* **193**, 2248-2257 (2014).

70 Ness-Schwickerath, K. J., Jin, C. & Morita, C. T. Cytokine requirements for the differentiation and expansion of IL-17A- and IL-22-producing human Vgamma2Vdelta2 T cells. *J Immunol* **184**, 7268-7280 (2010).

71 Caccamo, N. *et al.* Differentiation, phenotype, and function of interleukin-17-producing human Vgamma9Vdelta2 T cells. *Blood* **118**, 129-138 (2011).

72 Patil, R. S. *et al.* IL17 producing gammadeltaT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. *Int J Cancer* **139**, 869-881 (2016).

73 Rutkowski, M. R. *et al.* Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. *Cancer Cell* **27**, 27-40 (2015).

74 Wu, P. *et al.* gammadeltaT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity* **40**, 785-800 (2014).
First report of a pro-tumour role for human IL-17-producing γδ T cells in colorectal

cancer.

75 Meraviglia, S. *et al.* Distinctive features of tumor-infiltrating gammadelta T lymphocytes in human colorectal cancer. *Oncoimmunology* **6**, e1347742 (2017).

76 Kargl, J. *et al.* Neutrophils dominate the immune cell composition in non-small cell lung cancer. *Nat Commun* **8**, 14381 (2017).

77 Lo Presti, E. *et al.* Squamous Cell Tumors Recruit gammadelta T Cells Producing either IL17 or IFNgamma Depending on the Tumor Stage. *Cancer Immunol Res* **5**, 397-407 (2017).

78 Wesch, D., Glatzel, A. & Kabelitz, D. Differentiation of resting human peripheral blood gamma delta T cells toward Th1- or Th2-phenotype. *Cell Immunol* **212**, 110-117 (2001).

79 Hao, J. *et al.* Regulatory role of Vgamma1 gammadelta T cells in tumor immunity through IL-4 production. *J Immunol* **187**, 4979-4986 (2011).

80 Mao, Y. *et al.* A new effect of IL-4 on human gammadelta T cells: promoting regulatory Vdelta1 T cells via IL-10 production and inhibiting function of Vdelta2 T cells. *Cell Mol Immunol* **13**, 217-228 (2016).

81 Sundblad, V., Morosi, L. G., Geffner, J. R. & Rabinovich, G. A. Galectin-1: A Jack-of-All-Trades in the Resolution of Acute and Chronic Inflammation. *J Immunol* **199**, 3721-3730 (2017).

82 Daley, D. *et al.* gammadelta T Cells Support Pancreatic Oncogenesis by Restraining alphabeta T Cell Activation. *Cell* **166**, 1485-1499 e1415 (2016).

83 Gunderson, A. J. *et al.* Bruton Tyrosine Kinase-Dependent Immune Cell Cross-talk Drives Pancreas Cancer. *Cancer Discov* **6**, 270-285 (2016).

84 Khosravi, N. *et al.* IL22 Promotes Kras-Mutant Lung Cancer by Induction of a Protumor Immune Response and Protection of Stemness Properties. *Cancer Immunol Res* **6**, 788-797 (2018).

85 McKenzie, D. R. *et al.* IL-17-producing gammadelta T cells switch migratory patterns between resting and activated states. *Nat Commun* **8**, 15632 (2017).

86 Kersten, K. *et al.* Mammary tumor-derived CCL2 enhances pro-metastatic systemic inflammation through upregulation of IL1beta in tumor-associated macrophages. *Oncoimmunology* **6**, e1334744 (2017).

87 Glatzel, A. *et al.* Patterns of chemokine receptor expression on peripheral blood gamma delta T lymphocytes: strong expression of CCR5 is a selective feature of V delta 2/V gamma 9 gamma delta T cells. *J Immunol* **168**, 4920-4929 (2002).

88 Ye, J. *et al.* Specific recruitment of gammadelta regulatory T cells in human breast cancer. *Cancer Res* **73**, 6137-6148 (2013).

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

90 Correia, D. V. *et al.* Differentiation of human peripheral blood Vdelta1+ T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. *Blood* **118**, 992-1001 (2011).

91 Yamaguchi, T. *et al.* Interleukin-15 effectively potentiates the in vitro tumor-specific activity and proliferation of peripheral blood gammadeltaT cells isolated from glioblastoma patients. *Cancer Immunol Immunother* **47**, 97-103 (1998).

92 Van Acker, H. H. *et al.* Interleukin-15-Cultured Dendritic Cells Enhance Anti-Tumor Gamma Delta T Cell Functions through IL-15 Secretion. *Front Immunol* **9**, 658 (2018).

93 Haas, J. D. *et al.* CCR6 and NK1.1 distinguish between IL-17A and IFN-gamma-producing gammadelta effector T cells. *Eur J Immunol* **39**, 3488-3497 (2009).

94 Li, W. *et al.* Effect of IL-18 on expansion of gammadelta T cells stimulated by zoledronate and IL-2. *J Immunother* **33**, 287-296 (2010).

95 Thedrez, A. *et al.* IL-21-mediated potentiation of antitumor cytolytic and proinflammatory responses of human V gamma 9V delta 2 T cells for adoptive immunotherapy. *J Immunol* **182**, 3423-3431 (2009).

96 Wang, X. *et al.* IL-36gamma Transforms the Tumor Microenvironment and Promotes Type 1 Lymphocyte-Mediated Antitumor Immune Responses. *Cancer Cell* **28**, 296-306 (2015).

97 Yi, Y. *et al.* The functional impairment of HCC-infiltrating gammadelta T cells, partially mediated by regulatory T cells in a TGFbeta- and IL-10-dependent manner. *Journal of hepatology* **58**, 977-983 (2013).

98 Sacchi, A. *et al.* Myeloid-Derived Suppressor Cells Specifically Suppress IFN-gamma Production and Antitumor Cytotoxic Activity of Vdelta2 T Cells. *Front Immunol* **9**, 1271 (2018).

99 Sabbione, F. *et al.* Neutrophils suppress gammadelta T-cell function. *Eur J Immunol* **44**, 819-830 (2014).

100 Li, L. *et al.* Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral gammadelta T cell equilibrium via tumor-derived exosomes. *Oncogene* (2018).

101 Rossi, C. *et al.* Boosting gammadelta T cell-mediated antibody-dependent cellular cytotoxicity by PD-1 blockade in follicular lymphoma. *Oncoimmunology* **8**, 1554175 (2019).

102 Iwasaki, M. *et al.* Expression and function of PD-1 in human gammadelta T cells that recognize phosphoantigens. *Eur J Immunol* **41**, 345-355 (2011).

103 Hoeres, T., Holzmann, E., Smetak, M., Birkmann, J. & Wilhelm, M. PD-1 signaling modulates interferon-gamma production by Gamma Delta (gammadelta) T-Cells in response to leukemia. *Oncoimmunology* **8**, 1550618 (2019).

104 Siegers, G. M., Dutta, I., Lai, R. & Postovit, L. M. Functional Plasticity of Gamma Delta T Cells and Breast Tumor Targets in Hypoxia. *Front Immunol* **9**, 1367 (2018).

105 Rodrigues, N. V. *et al.* Low-Density Lipoprotein Uptake Inhibits the Activation and Antitumor Functions of Human Vgamma9Vdelta2 T Cells. *Cancer Immunol Res* **6**, 448-457 (2018).

106 Mattarollo, S. R., Kenna, T., Nieda, M. & Nicol, A. J. Chemotherapy and zoledronate sensitize solid tumour cells to Vgamma9Vdelta2 T cell cytotoxicity. *Cancer Immunol Immunother* **56**, 1285-1297 (2007).

107 Todaro, M., Meraviglia, S., Caccamo, N., Stassi, G. & Dieli, F. Combining conventional chemotherapy and gammadelta T cell-based immunotherapy to target cancer-initiating cells. *Oncoimmunology* **2**, e25821 (2013).

108 Wang, Z. *et al.* Decitabine Enhances Vgamma9Vdelta2 T Cell-Mediated Cytotoxic Effects on Osteosarcoma Cells via the NKG2DL-NKG2D Axis. *Front Immunol* **9**, 1239 (2018).

109 Niu, C. *et al.* Decitabine Inhibits Gamma Delta T Cell Cytotoxicity by Promoting KIR2DL2/3 Expression. *Front Immunol* **9**, 617 (2018).

110 Bhat, S. A., Vedpathak, D. M. & Chiplunkar, S. V. Checkpoint Blockade Rescues the Repressive Effect of Histone Deacetylases Inhibitors on gammadelta T Cell Function. *Front Immunol* **9**, 1615 (2018).

111 Dejea, C. M. *et al.* Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* **359**, 592-597 (2018).

112 Ridker, P. M. *et al.* Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet* **390**, 1833-1842 (2017).

113 Michel, M. L. *et al.* Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing gammadelta cells. *Proc Natl Acad Sci U S A* **109**, 17549-17554 (2012).

114 Tang, Q. *et al.* Hmgb1-IL-23-IL-17-IL-6-Stat3 axis promotes tumor growth in murine models of melanoma. *Mediators Inflamm* **2013**, 713859 (2013).

115 Douguet, L. *et al.* Inflammation drives nitric oxide synthase 2 expression by gammadelta T cells and affects the balance between melanoma and vitiligo associated melanoma. *Oncoimmunology* **7**, e1484979 (2018).

116 Douguet, L. *et al.* Nitric oxide synthase 2 is involved in the pro-tumorigenic potential of gammadelta17 T cells in melanoma. *Oncoimmunology* **5**, e1208878 (2016).

117 Baek, A. E. *et al.* The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat Commun* **8**, 864 (2017).

118 Kathania, M. *et al.* Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-gammat ubiquitination. *Nat Immunol* **17**, 997-1004 (2016).

119 Mensurado, S. *et al.* Tumor-associated neutrophils suppress pro-tumoral IL-17+ gammadelta T cells through induction of oxidative stress. *PLoS Biol* **16**, e2004990 (2018).

120 Liu, Y. *et al.* CD11b+Ly6G+ cells inhibit tumor growth by suppressing IL-17 production at early stages of tumorigenesis. *Oncoimmunology* **5**, e1061175 (2016).

121 Bialasiewicz, A. A., Ma, J. X. & Richard, G. Alpha/beta- and gamma/delta TCR(+) lymphocyte infiltration in necrotising choroidal melanomas. *Br J Ophthalmol* **83**, 1069-1073 (1999).

122 Gentles, A. J. *et al.* The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med* **21**, 938-945 (2015).

123 Tosolini, M. *et al.* Assessment of tumor-infiltrating TCRVgamma9Vdelta2 gammadelta lymphocyte abundance by deconvolution of human cancers microarrays. *Oncoimmunology* **6**, e1284723 (2017).

124 Gibbons, D. L. *et al.* Neonates harbour highly active gammadelta T cells with selective impairments in preterm infants. *Eur J Immunol* **39**, 1794-1806 (2009).

125 Peng, G. *et al.* Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity* **27**, 334-348 (2007).

126 Ma, C. *et al.* Tumor-infiltrating gammadelta T lymphocytes predict clinical outcome in human breast cancer. *J Immunol* **189**, 5029-5036 (2012).

127 Ye, J. *et al.* Tumor-derived gammadelta regulatory T cells suppress innate and adaptive immunity through the induction of immunosenescence. *J Immunol* **190**, 2403-2414 (2013).

128 Wilhelm, M. *et al.* Gammadelta T cells for immune therapy of patients with lymphoid malignancies. *Blood* **102**, 200-206 (2003).

Pioneer clinical trial showing safety and efficacy upon in vivo Vγ9Vδ2 T cell activation in lymphoma patients

129 Kobayashi, H. *et al.* Safety profile and anti-tumor effects of adoptive immunotherapy using gamma-delta T cells against advanced renal cell carcinoma: a pilot study. *Cancer Immunol Immunother* **56**, 469-476 (2007).

130 Nicol, A. J. *et al.* Clinical evaluation of autologous gamma delta T cell-based immunotherapy for metastatic solid tumours. *Br J Cancer* **105**, 778-786 (2011).

131 Fournie, J. J. *et al.* What lessons can be learned from gammadelta T cell-based cancer immunotherapy trials? *Cell Mol Immunol* **10**, 35-41 (2013).

This review provides an insightful discussion on the limited success of cancer clinical trials based on Vγ9Vδ22 T cells.

132 Wistuba-Hamprecht, K. *et al.* Proportions of blood-borne Vdelta1+ and Vdelta2+ T-cells are associated with overall survival of melanoma patients treated with ipilimumab. *Eur J Cancer* **64**, 116-126 (2016).

133 Gubin, M. M. *et al.* High-Dimensional Analysis Delineates Myeloid and Lymphoid Compartment Remodeling during Successful Immune-Checkpoint Cancer Therapy. *Cell* **175**, 1443 (2018).

134 de Weerdt, I. *et al.* Improving CLL Vgamma9Vdelta2-T-cell fitness for cellular therapy by ex vivo activation and ibrutinib. *Blood* **132**, 2260-2272 (2018).

135 de Bruin, R. C. G. *et al.* A bispecific nanobody approach to leverage the potent and widely applicable tumor cytolytic capacity of Vgamma9Vdelta2-T cells. *Oncoimmunology* **7**, e1375641 (2017).

136 Oberg, H. H. *et al.* Tribody [(HER2)2xCD16] Is More Effective Than Trastuzumab in Enhancing gammadelta T Cell and Natural Killer Cell Cytotoxicity Against HER2-Expressing Cancer Cells. *Front Immunol* **9**, 814 (2018).

137 Grunder, C. *et al.* gamma9 and delta2CDR3 domains regulate functional avidity of T cells harboring gamma9delta2TCRs. *Blood* **120**, 5153-5162 (2012).

138 Marcu-Malina, V. *et al.* Redirecting alphabeta T cells against cancer cells by transfer of a broadly tumor-reactive gammadeltaT-cell receptor. *Blood* **118**, 50-59 (2011).
This work establishes a proof-of-principle for transferring Vγ9Vδ2 TCRs into αβ T cells to improve their anti-tumour efficacy and safety.

139 Straetemans, T. *et al.* GMP-Grade Manufacturing of T Cells Engineered to Express a Defined gammadeltaTCR. *Front Immunol* **9**, 1062 (2018).

140 Netherlands Trial Register. Trialregister.nl, https://www.trialregister.nl/trial/6357. (2017).

141 Di Marco Barros, R. *et al.* Epithelia Use Butyrophilin-like Molecules to Shape Organ-Specific gammadelta T Cell Compartments. *Cell* **167**, 203-218 e217 (2016).

142 Almeida, A. R. *et al.* Delta One T Cells for Immunotherapy of Chronic Lymphocytic Leukemia: Clinical-Grade Expansion/Differentiation and Preclinical Proof of Concept. *Clin Cancer Res* **22**, 5795-5804 (2016).
Study providing preclinical proof-of-principle for application of Vδ1$^+$ (DOT) cells in adoptive immunotherapy of chronic lymphocytic leukemia

143 Di Lorenzo, B. *et al.* Broad Cytotoxic Targeting of Acute Myeloid Leukemia by Polyclonal Delta One T Cells. *Cancer Immunol Res* **7**, 552-558 (2019).

144 Benveniste, P. M. *et al.* Generation and molecular recognition of melanoma-associated antigen-specific human gammadelta T cells. *Sci Immunol* **3** (2018).

145 Mirzaei, H. R., Mirzaei, H., Lee, S. Y., Hadjati, J. & Till, B. G. Prospects for chimeric antigen receptor (CAR) gammadelta T cells: A potential game changer for adoptive T cell cancer immunotherapy. *Cancer Lett* **380**, 413-423 (2016).

146 Rischer, M. *et al.* Human gammadelta T cells as mediators of chimaeric-receptor redirected anti-tumour immunity. *Br J Haematol* **126**, 583-592 (2004).

147 Deniger, D. C. *et al.* Bispecific T-cells expressing polyclonal repertoire of endogenous gammadelta T-cell receptors and introduced CD19-specific chimeric antigen receptor. *Molecular therapy : the journal of the American Society of Gene Therapy* **21**, 638-647 (2013).

148 Salter, A. I., Pont, M. J. & Riddell, S. R. Chimeric antigen receptor-modified T cells: CD19 and the road beyond. *Blood* **131**, 2621-2629 (2018).

149 Orlando, E. J. *et al.* Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat Med* **24**, 1504-1506 (2018).

150 Ruella, M. *et al.* Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat Med* **24**, 1499-1503 (2018).

151 Zumwalde, N. A. *et al.* Adoptively transferred Vgamma9Vdelta2 T cells show potent antitumor effects in a preclinical B cell lymphomagenesis model. *JCI Insight* **2** (2017).

152 Jarry, U. *et al.* Stereotaxic administrations of allogeneic human Vgamma9Vdelta2 T cells efficiently control the development of human glioblastoma brain tumors. *Oncoimmunology* **5**, e1168554 (2016).

153 Deniger, D. C. *et al.* Activating and propagating polyclonal gamma delta T cells with broad specificity for malignancies. *Clin Cancer Res* **20**, 5708-5719 (2014).

154 Qaqish, A. *et al.* Adoptive Transfer of Phosphoantigen-Specific gammadelta T Cell Subset Attenuates Mycobacterium tuberculosis Infection in Nonhuman Primates. *J Immunol* **198**, 4753-4763 (2017).

155 Sicard, H. *et al.* In vivo immunomanipulation of V gamma 9V delta 2 T cells with a synthetic phosphoantigen in a preclinical nonhuman primate model. *J Immunol* **175**, 5471-5480 (2005).

156 Godder, K. T. *et al.* Long term disease-free survival in acute leukemia patients recovering with increased gammadelta T cells after partially mismatched related donor bone marrow transplantation. *Bone Marrow Transplant* **39**, 751-757 (2007).
Important paper showing that upon stem cell transplantation efficient Vδ1⁺ T cell reconstitution associates with an improved clinical outcome in leukemia patients.

157 Airoldi, I. *et al.* gammadelta T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-alphabeta+/CD19+ lymphocytes. *Blood* **125**, 2349-2358 (2015).

158 Mittal, D., Gubin, M. M., Schreiber, R. D. & Smyth, M. J. New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. *Current opinion in immunology* **27**, 16-25 (2014).

159 Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69-74 (2015).

160 Cheng, M. *et al.* Microbiota modulate tumoral immune surveillance in lung through a gammadeltaT17 immune cell-dependent mechanism. *Cancer Res* **74**, 4030-4041 (2014).

161 Munoz-Ruiz, M., Sumaria, N., Pennington, D. J. & Silva-Santos, B. Thymic Determinants of gammadelta T Cell Differentiation. *Trends Immunol* **38**, 336-344 (2017).

162 Tripodo, C. *et al.* Gamma-delta T-cell lymphomas. *Nature reviews. Clinical oncology* **6**, 707-717 (2009).

163 Foppoli, M. & Ferreri, A. J. Gamma-delta t-cell lymphomas. *Eur J Haematol* **94**, 206-218 (2015).

164 Matos, D. M., Rizzatti, E. G., Fernandes, M., Buccheri, V. & Falcao, R. P. Gammadelta and alphabeta T-cell acute lymphoblastic leukemia: comparison of their clinical and immunophenotypic features. *Haematologica* **90**, 264-266 (2005).

165 Ribeiro, S. T. *et al.* Casein kinase 2 controls the survival of normal thymic and leukemic gammadelta T cells via promotion of AKT signaling. *Leukemia* **31**, 1603-1610 (2017).

166 Saito, H. *et al.* Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature* **309**, 757-762 (1984).

167 Brenner, M. B. *et al.* Identification of a putative second T-cell receptor. *Nature* **322**, 145-149 (1986).

168 Bank, I. *et al.* A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature* **322**, 179-181 (1986).

169 Borst, J. *et al.* A T-cell receptor gamma/CD3 complex found on cloned functional lymphocytes. *Nature* **325**, 683-688 (1987).

170 Fournie, J. J. & Bonneville, M. Stimulation of gamma delta T cells by phosphoantigens. *Res Immunol* **147**, 338-347 (1996).

171 Harly, C. *et al.* Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. *Blood* **120**, 2269-2279 (2012).
Seminal paper on BTN3A1 and its role in inducing cellular stress-sensing by human Vγ9Vδ2T cells.

[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

Online Only

Subject Categories

Biological sciences / Immunology / Lymphocytes / T cells / Gammadelta T cells [URI /631/250/1619/554/2509];
Biological sciences / Cancer / Cancer microenvironment [URI /631/67/327];
Biological sciences / Cancer / Tumour immunology [URI /631/67/580];
Biological sciences / Cancer / Cancer therapy / Cancer immunotherapy [URI /631/67/1059/2325]

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.