γδ T cells in cancer

Bruno Silva-Santos, Karine Serre and Håkan Norell

Abstract | With the promise of T cell-based therapy for cancer finally becoming reality, this Review focuses on the less-studied γδ T cell lineage and its diverse responses to tumours. γδ T cells have well-established protective roles in cancer, largely on the basis of their potent cytotoxicity and interferon-γ production. Besides this, recent studies have revealed a series of tumour-promoting functions that are linked to interleukin-17-producing γδ T cells. Here, we integrate the current knowledge from both human and mouse studies to highlight the potential of γδ T cell modulation to improve cancer immunotherapy.

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; it is also known as V(D)J recombination.

NKG2D
A C-type lectin-like receptor that binds to multiple stress or transformation-inducible ligands of the non-classical MHC (class Ib) family H60, murine UL16-binding protein-like transcript 1 (MULT1) and members of the retinoic acid early inducible 1 (RAE1) proteins in mice; and MHC class I-related chain A or B (MICA and MICB) and UL16-binding proteins (ULBP1–ULBP6) in humans.

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Avenida Prof. Egas Moniz, 1649–028 Lisboa, Portugal.
Correspondence to B.S.-S. and K.S.
e-mails: bssantos@medicina.ulisboa.pt; karineserre@medicina.ulisboa.pt
doi:10.1038/nri3904
Published online 9 October 2015

γδ T cells are one of only three immune cell types that express antigen receptors that undergo somatic recombination, and they contribute to immune responses to infection, to cellular transformation and to tissue damage responses (reviewed in REF. 1). The non-redundant roles of γδ T cells in host protective responses are likely to derive from a combination of unique antigen specificities, high clonal frequencies and a pre-activated differentiation status that allows for very rapid responses[2]. In fact, mouse γδ T cells develop in the thymus into fully functional subsets that secrete high levels of pro-inflammatory cytokines, such as interferon-γ (IFNγ) or interleukin-17 (IL-17), upon activation in the periphery[3–5]. Notably, in the context of cancer, recent reports[6–9] have described unanticipated tumour-promoting roles for mouse and human IL-17-producing γδ T cells. Nonetheless, these recent developments must not eclipse the extensive evidence for antitumour γδ T cell responses in both species. Here, we aim to provide a balanced insight into the pleiotropic functions of tumour-reactive γδ T cells.

Mouse and human γδ T cells share many developmental and functional properties[1,2]. γδ T cells are highly effective at killing tumour cells and providing IFNγ-mediated protective responses against cancer, but in some cases they can promote tumour growth by IL-17 production[10]. Moreover, the main determinants of tumour cell recognition — namely the γδ T cell receptor (TCR) and natural killer (NK) cell receptors (NKRs), such as NKG2D (natural killer group 2, member D) — are shared by both species[11]. However, owing to evolutionary divergence of the TCRγ and TCRδ loci between rodents and primates[12], a direct comparison between mouse and human γδ T cell subsets is not straightforward (see TABLE 1). Consequently, we first describe γδ T cells in mouse tumour models and then discuss the current understanding and future prospects of their human counterparts in experimental and clinical settings.

γδ T cell responses to mouse tumours

γδ T cells constitute a heterogeneous population of cells that have increasingly recognized developmental and functional complexities, often associated with their tissue homing or localization[13]. Mouse γδ T cells with preferential effector functions (such as IFNγ or IL-17 production) can be categorized according to usage of TCR γ-chain variable region (Vγ)[14] or other cell surface markers, such as CD27, NK1.1 (also known as KLRB1C), CD122 (also known as IL-2Rβ) and CC-chemokine receptor 6 (CCR6)[3,4,15]. As these aspects fall beyond the scope of this article, we provide TABLE 1, which summarizes the main γδ T cell subtypes and their associated features in mice and humans, and refer the reader to previous reviews[2,14]. Nonetheless, we highlight, whenever possible, the division of labour and subset-specific roles of γδ T cell subpopulations; in that regard, FIG. 1 summarizes the diversified responses of mouse γδ T cell subsets to tumours (described below).

Antitumour functions of γδ T cells. Following the seminal paper by Hayday and colleagues on cutaneous malignancy[16], multiple studies in various mouse cancer models firmly established the protective role of γδ T cells during tumour development. In most cases, this was inferred by comparing tumour progression in γδ T cell-deficient (owing to genetic inactivation of the TCRδ locus) versus γδ T cell-sufficient (wild-type) mice. γδ T cells prevented both the chemically induced development of papillomas and their progression into cutaneous squamous cell carcinomas, whereas αβ T cells seemed to promote tumour progression[17]. Moreover, γδ T cells were also protective against spontaneous B cell lymphomas[18], prostate cancer[19] and in the widely used B16-F0 melanoma transplantable model[20,21]. Importantly, a therapeutic effect was demonstrated following adoptive transfer of γδ T cells into mice bearing established adenocarcinomas of the prostate (TRAMP mice)[19] or B16-F0 melanomas[22].

Table 1 | **Features of mouse and human γδ T cell subsets***

Subset	Most common VγVδ pairs	V(D)J diversity	Tissue distribution	Key cytokines produced
Mouse				
Vγ1	Vγ1Vδ6.3	High	Liver and lymphoid tissue	• IFNγ and TNF • Can produce IL-4 and IL-17
Vγ2	Not defined		Very rare	Not defined
Vγ4	Not defined	High	Lymphoid tissue, lungs, liver and inflamed dermis	• IL-17 • IFNγ
Vγ5	Vγ5Vδ1	Invariant	Epidermis	• IFNγ • Can produce IL-22 and TNF
Vγ6	Vγ6Vδ1	Invariant	Uterus, lungs, tongue, liver, placenta and kidney	• IL-17 and IL-22 • Can produce IFNγ
Vγ7	Vγ7Vδ4 Vγ7Vδ5 Vγ7Vδ6	Intermediate	Intestinal mucosa	• IFNγ
Human				
Vδ1	Not defined	High	Epithelia, dermis, spleen and liver	• IFNγ and TNF • Can produce IL-17
Vδ2	Vγ9Vδ2	Intermediate	Blood	• IFNγ and TNF • Can produce IL-17
Vδ3	Not defined	High	Liver and gut epithelium	Not defined

*The nomenclature used for T cell receptor genes is based on the Heilig and Tonegawa's system[92] for mouse γδ T cells and the Lefranc and Rabbits' system[93] for human γδ T cells. IFNγ, interferon-γ; IL, interleukin; TNF, tumour necrosis factor.

Tumour cell recognition and associated γδ T cell activation have been attributed to engagement of the TCR and/or NKRs, mostly NKG2D. For example, the antitumour cytotoxicity of skin-resident Vγ5$^+$ T cells depends on both TCR[17] and NKG2D[16,23] expression. NKG2D is a crucial innate receptor in tumour surveillance, as demonstrated by the high cancer susceptibility of NKG2D-deficient mice[24]. NKG2D is known to sense molecular stress signatures (such as RAE1 and H60) that are largely absent from healthy cells but often upregulated by transformed cells[16,23]. By contrast, tumour-associated antigens that are directly recognized by γδ TCRs are yet to be described.

Efficient target cell recognition by γδ T cells activates cytotoxic mechanisms that involve the release of perforin and granzymes, as well as the expression of ligands, such as CD95 ligand (also known as TNFSF6) and TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10), that engage death receptors (CD95 (also known as TNFRSF6) and TRAIL receptors, respectively)[25]. In addition, γδ T cells promptly secrete IFNγ, which inhibits angiogenesis and enhances MHC class I expression by tumour cells, thus promoting CD8$^+$ T cell responses[20–22,26]. It is interesting to note that, in the adoptive γδ T cell transfer model against B16-F0 melanoma, only Vγ4$^+$ (but not Vγ1$^+$) T cells were protective, which was dependent on their high eomesodermin expression and IFNγ production[22].

Although IFNγ is the major cytokine produced by mouse CD27$^+$ γδ T cells, their CD27$^-$ counterparts preferentially secrete IL-17 (REFS 4,27). IL-17 was also implicated in protective γδ T cell responses in other cancer models. IL-17-producing γδ T cells seemingly cooperated with *Mycobacterium bovis* bacillus Calmette–Guerin (BCG) vaccination to mediate bladder cancer regression[28]. This effect was abolished in both TCRδ-deficient and IL-17-deficient mice, and γδ T cells were the main intratumour source of IL-17, which recruited antitumour neutrophils to the bladder. In another study, IL-17-producing γδ T cells synergized with chemotherapeutic agents (such as doxorubicin) in several transplantation models of epithelial tumours[29]. Mechanistically, tumour-infiltrating IL-1β-activated IL-17$^+$ γδ T cells, which were approximately 60% Vγ4$^+$ and 35% Vγ6$^+$, enhanced the priming and recruitment of IFNγ-producing CD8$^+$ T cells that mediated the antitumour effect. Similarly, the mechanism of action of doxorubicin in transplantable mammary carcinomas and fibrosarcomas that were induced *de novo* by methylcholanthrene (MCA) relied on both IFNγ-producing CD8$^+$ T cells and IL-17$^+$ γδ T cells[30]. Specifically, the antitumor activity of doxorubicin was abolished in both TCRδ-deficient and IL-17-deficient mice, as well as in animals treated with IL-17 receptor-specific antibodies, again supporting a role for IL-17-producing γδ T cells in mediating immune-dependent antitumour actions of standard chemotherapeutic regimens. Whereas these reports suggest a protective adjuvant effect for IL-17-producing γδ T cells in therapeutic settings, several studies in different cancer models have demonstrated an overtly pathogenic role for the CD27$^-$IL-17$^+$ γδ T cell subset during tumour development.

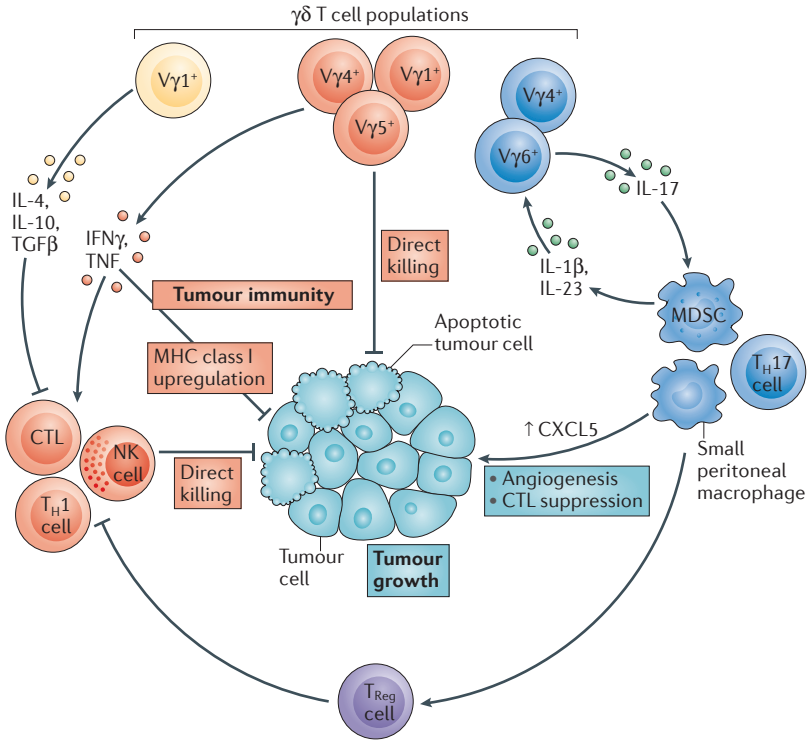

Figure 1 | **Antitumour versus protumour roles of mouse γδ T cells.** Antitumour functions of γδ T cells have been associated with cytotoxicity and the production of interferon-γ (IFNγ), which can act directly on tumour cells to induce the upregulation of MHC class I expression, and thus enhance $CD8^+$ T cell responses. By contrast, interleukin-17 (IL-17) production by certain γδ T cell subsets recruits immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) or small peritoneal macrophages, which can promote angiogenesis, tumour cell growth and inducible regulatory T (T_{Reg}) cell differentiation. Furthermore, γδ T cells producing IL-4, IL-10 and/or transforming growth factor-β (TGFβ) seem to suppress antitumour immune responses by inhibiting dendritic cell maturation and/or the effector functions of γδ T cells, and $CD4^+$ and $CD8^+$ αβ T cells. The T cell receptor (TCR) γ-chain variable regions (Vγ) that are preferentially associated with each functional output are also shown. CTL, cytotoxic T lymphocyte; CXCL5, CXC-chemokine ligand 5; NK cell, natural killer cell; T_H, T helper; TNF, tumour necrosis factor.

Protumour activities of mouse γδ T cells. Recently, IL-17-producing γδ T cells have been consistently associated with tumour growth and metastasis in mice (FIG. 1). Given the lack of γδ T cell-specific (that is, conditional) ablation tools, these conclusions have generally been drawn from the following observations: first, γδ T cells are a major source of IL-17 in the tumour microenvironment and second, reduced tumour growth was reported in both γδ T cell-deficient (TCRδ-deficient) and IL-17-deficient mice. This was observed in spontaneous (that is, oncogene-driven) models of breast cancer metastasis[6] and pancreatic intraepithelial neoplasia[31] and in several transplantable tumour models (with diverse tumour cell lines and various routes of injection: subcutaneous hepa 1–6 murine hepatocellular carcinoma[7] and B16-F10 melanoma[32]; intravenous Lewis lung carcinoma (3LL) and B16-F10 melanoma[33]; intraperitoneal ID8 ovarian cancer[8] and MCA-induced CMS-G4 tumour cell lines[34]). Of note, IL-17-producing cells were either $Vγ4^+$ cells, in hepatocellular and breast cancers[6,7], or $Vγ6^+$ cells, most notably in the ovarian cancer model[8]. The preferential accumulation of IL-17- (compared to IFNγ-) producing γδ T cells in these tumour models has been attributed to the local expression of T helper 17-driving cytokines, in various combinations: IL-1β[6,33] or IL-6 (REF. 31); IL-1β and IL-23 (REF. 7); IL-6, IL-23 and transforming growth factor-β (TGFβ)[34]; or IL-1β, IL-6 and IL-7 (REF. 8).

The protumour mechanisms downstream of IL-17 production by γδ T cells seem to involve different cellular intermediates: endothelial cells, lymphocytes and myeloid cells. Using a MCA-induced fibrosarcoma model, it was first shown that tumour growth was reduced in the genetic absence of IL-17, which correlated with limited angiogenesis within the tumour tissue[34]. The production of IL-17 was essentially restricted to $CD27^-$ γδ T cells, with a minor $CD4^+$ T cell contribution, and the authors validated their main results in separate skin and colon carcinoma models. Conversely, IL-17 treatment increased the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and angiopoietin 2 by tumour cells, which is consistent with our recent data in a peritoneal ovarian cancer model[8].

Subsequent studies have identified an IL-17-mediated crosstalk between γδ T cells and myeloid cells that promotes tumour cell growth and dissemination. In our ovarian cancer model, IL-17 production by γδ T cells enabled the recruitment of small peritoneal macrophages that supported ID8 cancer cell proliferation[8]. By contrast, it was shown that hepatocellular carcinoma cells responded to IL-17 by expressing CXC-chemokine ligand 5 (CXCL5) that recruited $CD11b^+GR1^+$ myeloid-derived suppressor cells (MDSCs)[7], which were also implicated in lung carcinoma growth and metastasis[33]. The strong protumour activities of MDSCs, including the inhibition of both IFNγ production and $CD8^+$ T cell cytotoxicity[7,33], have been reviewed elsewhere[35].

Most recently, the myeloid cells that partner with IL-17-producing γδ T cells in a spontaneous breast cancer model were identified as $CD11b^+LY6G^+$ neutrophils, which functionally correspond to granulocytic MDSCs[6]. In tumour-bearing mice, γδ T cells and IL-17 were required for systemic expansion and polarization of neutrophils, which suppressed protective $CD8^+$ T cell responses and enhanced pulmonary and lymph node metastases (although there was no impact on primary tumour development). Collectively, these mouse studies established a protumour γδ T cell–IL-17–myeloid cell axis that may be a novel target for cancer immunotherapy as also recently proposed in humans (see below).

γδ T cells in human cancer

Human γδ TCR-expressing cells constitute 1–5% of total T cells in the peripheral blood but make up a major lymphoid subset (20–50%) in tissues such as the intestine and the dermis[1]. Similarly to their mouse counterparts, human γδ T cells can be categorized on the basis of their TCR V gene usage, but the TCRγ and TCRδ loci have diverged substantially between rodents and primates[12], preventing comparison of direct orthologues. Human T cells expressing heterodimers of Vδ2 and Vγ9 chains account for most (50–95%) of the γδ T cells in peripheral

Myeloid-derived suppressor cells
(MDSCs). A group of immature $CD11b^+GR1^+$ cells (which include precursors of macrophages, granulocytes, dendritic cells and myeloid cells) that are produced in response to various tumour-derived cytokines. These cells have been shown to induce tolerance in tumour-associated $CD8^+$ T cells.

blood, whereas Vδ1-expressing T cells (paired with different Vγ chains) are most abundant among lymphocytes that populate healthy epithelia or infiltrate solid tumours (reviewed in REFS 36,37).

Antitumour functions of type 1 cytotoxic γδ T cells. Contrary to mouse γδ thymocytes, human γδ thymocytes are functionally immature, but nonetheless are highly poised to become type 1 cytotoxic (but not type 17) effector cells. In fact, exogenous IL-2 or IL-15 signals alone, in the absence of TCR stimulation, can upregulate type 1 transcription factors and endow γδ thymocytes with IFNγ-producing and tumour-killing functions[38]. Therefore, when expanded *in vitro* in the presence of IL-2, γδ T cells isolated from patients with melanoma, glioblastoma, neuroblastoma or renal, breast, lung, ovarian, colon, pancreatic or blood cancers efficiently killed tumour cell lines and/or primary cancer samples[36]. Although all the underlying studies cannot be individually cited here, they have clearly established the potent antitumour functions of human γδ T cells. Of note, Vδ1$^+$ T cell lines generally outperformed their Vδ2$^+$ counterparts[39] (reviewed in REF. 36), which makes it somewhat paradoxical that all the clinical applications of γδ T cells have thus far concentrated on Vγ9Vδ2$^+$ T cells (see below).

Protumour roles of human γδ T cells. Recently, a protumour role for IL-17-producing γδ T cells was reported for the first time in human cancer[9]. Specifically, Vδ1$^+$ T cells were the major source of IL-17 involved in chronic inflammation in colorectal cancer. Moreover, IL-17-producing γδ T cells secreted IL-8, tumour necrosis factor (TNF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), which recruited immunosuppressive MDSCs into the malignant microenvironment, further driving protumour inflammation. Importantly, the extent of IL-17-producing γδ T cell infiltration positively correlated with the clinical stage of the disease, highlighting the potential cancer-promoting role of IL-17-producing γδ T cells in human colorectal cancer[9]. IL-17-producing γδ T cells have also been found to be overrepresented in both the tumour tissue and peripheral blood of patients with gallbladder cancer (S.V. Chiplunkar, personal communication, 2015). Of note, human γδ T cells, unlike their mouse counterparts, are not programmed to produce IL-17 under normal physiological conditions, but instead require a highly inflammatory milieu. For example, IL-17-producing Vγ9Vδ2$^+$ T cells accumulate in large numbers in children suffering from bacterial meningitis[40] and have also been observed in patients with non-melanoma skin cancer (E. Lo Presti, F. Dieli and S. Meraviglia, personal communication, 2015).

Strikingly, tumour-infiltrating γδ T cells were shown to be the most significant predictor of relapse and poor survival in patients with breast cancer[41]. Although the underlying mechanism was not assessed in that study, the same group had previously suggested that breast cancer-infiltrating Vδ1$^+$ T cells could have immunosuppressive roles similar to those ascribed to regulatory T cells, such as suppression of dendritic cell maturation, T cell proliferation and IL-2 secretion[42]. However, as discussed elsewhere[43], various experimental issues, including potential biases conferred by *in vitro* expansion protocols for the establishment of Vδ1$^+$ T cell lines, limit the scope of these findings and urge further investigations.

Tumour cell recognition

By Vγ9Vδ2$^+$ T cells. Two decades ago, human Vγ9Vδ2$^+$ T cells were found to selectively recognize small non-peptidic prenyl-pyrophosphate metabolites of isoprenoid biosynthesis, termed phosphoantigens, in a TCR-dependent but MHC-independent manner[44,45]. Although the most potent phosphoantigens (such as *(E)*-4-hydroxy-3-methyl-butenyl pyrophosphate (HMB-PP)) are generated by the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway used by bacteria and parasites, stressed or transformed vertebrate cells accumulate metabolite intermediates of the mevalonate pathway (such as isopentenyl pyrophosphate (IPP)) that can also activate primate Vγ9Vδ2$^+$ T cells[44,46]. How the accumulation of IPP at high levels in cancer cells may allow it (and other phosphoantigens) to be specifically recognized as a tumour-associated antigen remains highly controversial.

Previous studies indicating that an ecto-F1 ATPase structure in a complex with apolipoprotein A1 constituted the phosphoantigen-sensing ligand of Vγ9Vδ2$^+$ TCRs have been recently challenged by studies on butyrophilin 3A1 (BTN3A1; also known as CD277)[47–49]. BTN3A1 is a B7 superfamily member that seems to be pivotal in providing the motifs that are recognized by Vγ9Vδ2$^+$ TCRs[48,49]. The lack of a BTN3A1 orthologue in rodents for example may indeed explain why only primates have phosphoantigen-reactive T cells[1]. The molecular mechanisms by which phosphoantigens interact with BTN3A1 are currently a matter of intense debate. Direct phosphoantigen presentation in an extracellular domain of BTN3A1 or phosphoantigen binding to an intracellular domain (B30.2) of BTN3A1 are key alternative proposals[49,50]. In the latter case, phosphoantigen-binding would affect TCR engagement indirectly by altering membrane mobility and/or the structure of extracellular BTN3A1 domains. This topic has been thoroughly discussed elsewhere[51–53].

Besides the TCRs, NKRs, particularly NKG2D, also make important contributions to the tumour reactivity of Vγ9Vδ2$^+$ T cells[54]. Overexpression of the NKG2D ligands ULBP1 (UL16-binding protein 1)[55] and ULBP4 (REF. 56) by haematological and epithelial tumours, respectively, drive efficient cytotoxic responses by Vγ9Vδ2$^+$ T cells. FIGURE 2 provides an overview of TCR and NKR interactions with their ligands that mediate tumour cell recognition by human γδ T cells.

By Vδ1$^+$ T cells. Very few TCR ligands have been described so far for Vδ1$^+$ T cells. Of these, only MHC class I-related chain A (MICA) is a transformation-inducible protein that can be clearly linked to tumour cell recognition. Intestinal Vδ1$^+$ T cells were found to target MICA-expressing tumour cells[57], seemingly by direct binding of MICA to a Vδ1 TCR[58], although

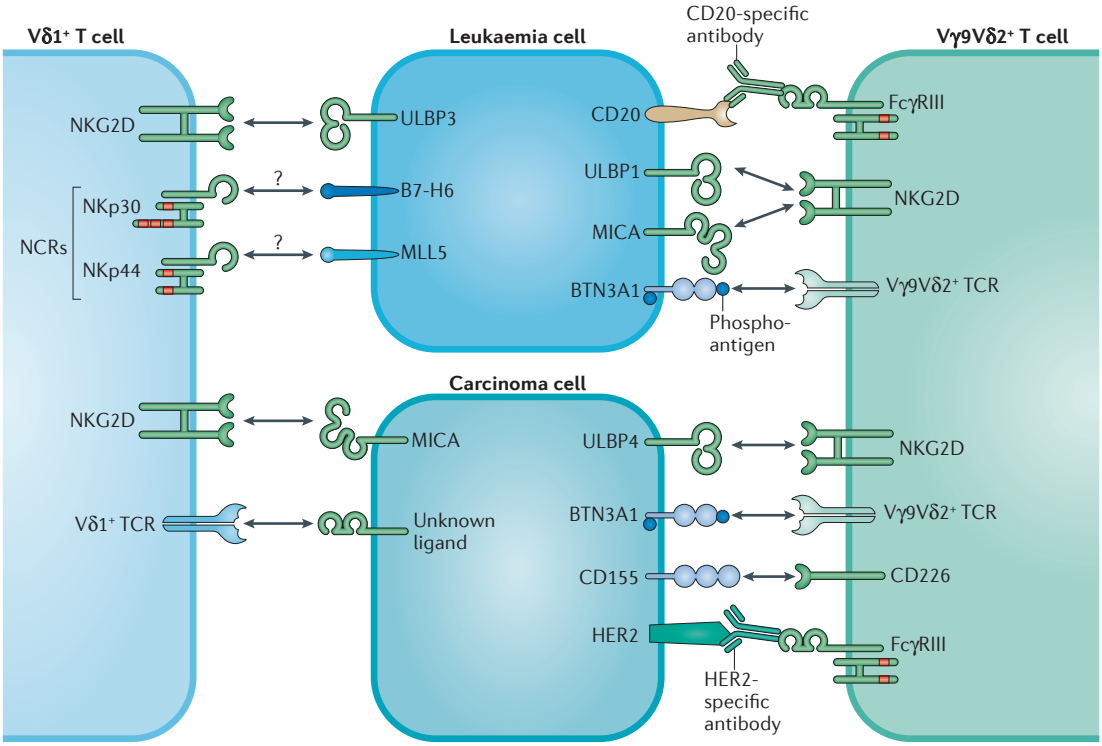

Figure 2 | **Receptor–ligand interactions mediating tumour cell recognition by human γδ T cells.** Interactions mediated between γδ T cell receptors (γδ TCRs) and natural killer cell receptors (NKRs; such as NKG2D) and their ligands on the surface of leukaemia and carcinoma cells are shown. Although the cancer cell ligands recognized by natural cytotoxicity receptors (NCRs) in this context have not been formally characterized, other systems suggest that B7 homologue 6 (B7-H6) could be the putative ligand for NKp30 and mixed lineage leukaemia 5 protein (MLL5), a ligand for NKp44. Tumour specificity for signalling through the Fc receptor for IgG (FcγRIII; also known as CD16) can be achieved by tumour antigen-specific monoclonal antibodies, such as CD20-specific antibody (rituximab) and human epidermal growth factor receptor 2 (HER2)-specific antibody (trastuzumab), used in combination with Vγ9Vδ2$^+$ T cell therapy. BTN3A1, butyrophilin 3A1; MICA, MHC class I-related chain A; ULBP, UL16-binding protein.

NKG2D is also involved in the recognition. Notably, the interaction of MICA with NKG2D was approximately 1,000-fold stronger than with Vδ1 TCR[58]. Another subfamily of MHC-related molecules (MHC class Ib) that are reported to bind Vδ1 TCRs are the glycolipid-presenting CD1 molecules, CD1c and CD1d[59,60], although their relevance to tumour cell targeting remains unclear. It was recently shown that germline-encoded residues in the extracellular domains of the Vδ1 chain mediate binding to CD1d, presenting either the self-lipid sulfatide[60] or exogenous α-galactosylceramide[59], which is a well-known NKT cell agonist. While we await the identification of additional Vδ1 TCR ligands, it is interesting to note that a MHC-like protein, the endothelial protein C receptor (EPCR), which is upregulated in carcinomas and in cytomegalovirus-infected cells, was recently identified as a cognate ligand for a Vγ4Vδ5$^+$ T cell clone isolated from a patient infected with cytomegalovirus[61].

Another NKG2D ligand, ULBP3 (also known as NKG2DL3), was recognized by Vδ1$^+$ T cells on B cell chronic lymphocytic leukaemia (B-CLL) blasts[62]. Moreover, we have found that CLL targeting by Vδ1$^+$ T cells can be notably enhanced upon expression of natural cytotoxicity receptors (NCRs), especially NKp30, following *in vitro* stimulation with strong TCR agonists and cytokines[39]. Interestingly, NKp30, previously regarded as an NK cell-specific receptor, is only expressed *de novo* on Vδ1$^+$ (not Vγ9Vδ2$^+$) T cells[39]. This, together with their increased resistance to both activation-induced cell death (AICD) and T cell exhaustion upon continuous stimulation[37], may render Vδ1$^+$ T cells most suitable for adoptive cell therapy in cancer patients.

T cell exhaustion
The impaired ability of effector T cells to carry out their functions, such as cytotoxicity and cytokine secretion, owing to chronic stimulation by antigens. It is typified by increased surface expression of programmed cell death 1.

Therapeutic opportunities

The concept of boosting type 1 cytotoxic γδ T cells in patients with cancer is particularly attractive given their independence of MHC class I presentation (which tackles a common immune evasion mechanism[63]) and of mutated epitopes (thus making them ideal effectors against tumours with low mutation loads[64,65]). All clinical trials to date have focused on the Vγ9Vδ2$^+$ T cell subset, given their relative abundance in peripheral blood and the availability of chemical methods to selectively activate and expand these cells both *in vitro* and *in vivo*. Aminobisphosphonates, such as pamidronate or zoledronate, are FDA-approved drugs that are regularly used for the treatment of osteoporosis and bone metastasis in patients with cancer. Notably, these drugs interfere with phosphoantigen-processing enzymes and thereby increase the intracellular levels of IPP

in tumour cells[66]. Thus, the clinical manipulation of γδ T cells has relied on the administration of such drugs or the synthetic phosphoantigen bromohydrin pyrophosphate (BrHPP), together with IL-2, either *in vivo* or *ex vivo* (to activate and expand autologous Vγ9Vδ2$^+$ T cells for re-infusion)[67–70]. In addition to being effectors, Vγ9Vδ2$^+$ T cells can also act as potent antigen-presenting cells, thus directly priming adaptive immunity. Notably, such antigen-presenting γδ T cells can even mediate robust cross-presentation of extracellular antigens, in the context of MHC class I, to CD8$^+$ T cells[71]. Although this opens up interesting possibilities in cancer immunotherapy[72], *in vivo* proof-of-concept of adaptive immunity against cancer mediated by antigen-presenting γδ T cells is still missing. Overall, despite their promise in preclinical settings, the clinical performance of Vγ9Vδ2$^+$ T cells (reviewed in REF. 70) may have been limited by their susceptibility to T cell exhaustion and AICD.

Vδ1$^+$ T cells are less susceptible to AICD and exhaustion[37], which may underpin their preferential *in vivo* persistence that seemingly prevents leukaemia relapse in some patients receiving haematopoietic stem cell transplantation (HSCT)[69]. In fact, the period following haploidentical HSCT in patients with leukaemia has emerged as a very promising therapeutic window for γδ T cells[73,74]. However, no clinical trial has yet focused on harnessing the antitumour potential of Vδ1$^+$ T cells, mostly due to the lack of a clinical-grade protocol to selectively activate and expand these cells to sufficient numbers either *in vivo* or *ex vivo*[36,37].

As detailed above and in FIG. 2, NKG2D and some other NKRs play a central role in governing the antitumour reactivity of γδ T cells[11], and this establishes an important parallel with NK cells. These two lymphoid lineages also share similar cytokine dependence, functional properties (potent cytotoxicity and IFNγ production) and dynamics of response[75]. The lack of a strong influence of MHC class I on effector reactivity and the ability of TCR-mediated activation, however, firmly distinguish γδ T cells from NK cells, and these cell types may, in fact, act as complementary effectors against cancer and infections[73].

Conclusions, future challenges and perspectives

Recent reports have added a substantial degree of complexity to our understanding of the crosstalk between γδ T cells and tumours. Notably, a dichotomy between protective IFNγ-based versus pathogenic IL-17-driven γδ T cell responses has emerged from various mouse tumour models[10]. However, it is important to note that whereas IL-17 is readily produced by mouse γδ T cells, it is rarely observed in human γδ T cells, except in highly inflammatory conditions[40,76]. To what extent this includes multiple cancers is still to be established by further investigations.

Assuming its physiological relevance in human cancer, we need to elucidate how the balance between IFNγ- versus IL-17-producing γδ T cells is set within the tumour microenvironment. Is it differential recruitment (from draining lymph nodes, for example) or expansion *in situ*[8]? Or can there be a functional reprogramming of γδ T cells in the inflammatory tumour milieu[36]?

γδ T cells are well equipped to detect and react to inflammation, which is intimately associated with cancer development, progression and metastasis when chronic[77]. It is therefore important to understand how inflammatory stimuli may shape γδ T cell responses to tumours. In this context, the microbiota is recognized as a principal co-evolved regulator of the mammalian immune system[77,78]. The vast intestinal luminal surface represents the principal host–microorganism interface[79] and is surveyed by specialized γδ intraepithelial lymphocytes (IELs)[80]. γδ IELs mediate early protective immunity against gut-resident bacteria that penetrate the intestinal mucosal epithelium[81], and in turn are themselves regulated by commensal microorganisms. The microbiota has also been shown to drive the generation of tumour-responsive IL-17-producing γδ T cells in the mouse lungs[82]. Tumour-induced disruption of the epithelial barrier may mimic mucosal injury[83], thus inducing the release of commensal bacterial products, leading to *in situ* activation of dendritic cells, production of IL-23 and differentiation of IL-17-producing γδ T cells; this is a plausible scenario in human colon cancer[9].

Although the differentiation of IL-17-producing γδ T cells within the tumour microenvironment is a possibility[36] that requires further experimental confirmation, our data in mice suggest that functional plasticity is restricted to the acquisition of IFNγ expression by IL-17-producing CD27$^-$ γδ T cells[84], and not the reverse. However, if type 17 functional re-programming of γδ T cells is observed in human tumours, this will have profound consequences for adoptive cell therapies. Of note, Vδ1$^+$ T cells polarized *ex vivo* to secrete IFNγ before being transplanted to target established human tumours in xenograft models failed to show any signs of conversion towards IL-17 production, even after two months *in vivo* (B.S.-S., unpublished observations). In any case, potent ways to prevent such conversion already exist and include monoclonal antibodies that block type 17-inducing cytokines.

Furthermore, it will be important to tackle additional tumour-derived mechanisms that may limit protective γδ T cell responses. For example, what is the impact of immunosuppressive populations, such as regulatory T cells and MDSCs, on γδ T cell functions? And how strongly does the PD1–PDL1 inhibitory axis suppress efficient γδ T cell activation and/or responses *in situ*? Targeting these and other factors, especially through combination therapies, may help to improve the clinical efficacy of γδ T cells (BOX 1).

Migration and homing properties are also important aspects of γδ T cell physiology to consider for cancer immunotherapy. The migration of γδ T cells is guided by chemokine receptors[85,86]; for example, CCR9 promotes cell homing to the small intestine, whereas CCR6 is required for epidermal trafficking or accumulation in the injured liver[87,88]. A study of the migration patterns of adoptively transferred Vγ9Vδ2$^+$ T cells in humans showed that these cells trafficked predominantly to the lungs, liver and spleen, as well as (in some patients) to metastatic tumour sites outside of these organs[89]. This reflected the profile of chemokine receptors displayed by *ex vivo*-expanded Vγ9Vδ2$^+$ T cells, which preferentially

Box 1 | **Emerging avenues for γδ T cell-based cancer immunotherapy**

Vγ9Vδ2$^+$ T cells

Use of a combination of established strategies for activation and/or expansion *ex vivo* and/or *in vivo* of γδ T cells by phosphoantigens and interleukin-2 (IL-2) with the following novel approaches:

- Tumour cell-specific monoclonal antibodies that induce FcγRIII-dependent antibody-dependent cellular cytotoxicity (ADCC).
- Chemotherapies to sensitize tumours to the effector γδ T cells, which can be engineered to be chemoresistant.
- Toll-like receptor agonists, for example polyinosinic:polycytidylic acid or CpG-containing oligodeoxynucleotides.

Use of agonist butyrophilin 3A1 (BTN3A1)-specific monoclonal antibodies and IL-2 to:

- Activate and/or expand effector γδ T cells *ex vivo* and/or *in vivo*.
- Sensitize all BTN3A1$^+$ tumour target cells, including those expressing only phosphoantigen-non-responsive BTN3A1 isoforms, for recognition by γδ T cells.

Vγ9Vδ2$^+$ T cell receptors

Use of cloned high-affinity Vγ9Vδ2$^+$ T cell receptors (TCRs) to engineer fusion proteins that link the extracellular region of the γδ TCRs to the Fc domains of IgG1, producing reagents that induce ADCC.

Transfer of γδ TCR specificity to αβ T cells using cloned TCRs or chimeric antigen receptors.

Vγ9Vδ2$^-$ and Vδ1$^+$ T cells

Use of CD137L$^+$ artificial antigen-presenting cells and/or γδ TCR- or CD3-specific crosslinking monoclonal antibodies together with IL-2, IL-4, IL-15 and IL-21 to generate clinically relevant expanded polyclonal γδ T cell lineages with broad antitumour activity.

Ex vivo differentiation of Vδ1$^+$ T cells to enhance expression of functional natural cytotoxicity receptors, in particular NKp30, by synergistic TCR and γc cytokine signalling (namely through IL-2 receptor or IL-15 receptor).

Future prospects

Incorporation of complementary strategies with any of the emerging approaches listed above:

- Immune checkpoint blockade, for example, using PD1- or PDL1-specific blocking antibodies.
- Redirection of effector γδ T cells by chimeric antigen receptors that target cell surface tumour-associated antigens.
- Enhancing tumour tropism, such as by engineered chemokine receptor expression.

In vivo targeting of endogenous protumour γδ T cells and their functions, such as IL-17-producing γδ T cells.

expressed peripheral tissue-homing CCR5 and CXC-chemokine receptor 3 (CXCR3) rather than lymphoid-homing CCR7 and CXCR5. Therefore, chemokine receptors are key parameters to consider (and optimize) in future adoptive γδ T cell immunotherapies.

Within the tumour, the current data on the prognostic value of (total) γδ T cell infiltrates show marked variability: from positive (melanoma) to neutral (renal cancer) to negative (breast cancer and colorectal cancer) correlations with patient outcomes (reviewed in REF. 36). However, a very recent analysis of a collection of 39 cancer types revealed intratumoral γδ T cells as the most significant favourable prognostic immune population[90]. It will be important to revisit and clarify this issue in multiple cancer types, with the resolution of IFNγ- versus IL-17-expressing γδ T cells. Furthermore, tumour-infiltrating γδ T cells dynamically interact with myeloid and lymphoid cells, including engaging in regulatory crosstalk between γδ T cell subpopulations[14,32]. These interactions can influence the functions of intratumour γδ T cell subsets and thus the net outcome of their response to tumours.

Finally, it is still unclear how the wealth of recent knowledge on BTN3A1 and its role on Vγ9Vδ2$^+$ T cell activation may translate to the clinic, but the first steps are now being taken in humanized mouse models. An alternative to manipulating polyclonal Vγ9Vδ2$^+$ T cells, which have variable antitumour reactivity owing to the diverse complementarity-determining region 3 domains of their individual TCRs, is to transfer selected high-affinity Vγ9Vδ2$^+$ TCRs to αβ T cells[91]; this successful approach aims to target a broad range of tumour types.

Alternatively, we and others are making great efforts to devise clinical-grade protocols to markedly expand Vδ2$^-$ T cell populations under type 1-polarizing conditions and thus allow the first adoptive cell therapy with activated Vδ1$^+$ T cells (BOX 1). From a fundamental standpoint, we need to understand the tumour-associated antigens that are recognized by γδ T cells (most notably by human Vδ1$^+$ T cells) via their TCRs or NKRs. This understanding will be crucial to grasp the potential of combining TCR and NKR targeting, and to rationally select patients for future γδ T cell-based cancer immunotherapies.

1. Vantourout, P. & Hayday, A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat. Rev. Immunol.* **13**, 88–100 (2013).
2. Bonneville, M., O'Brien, R. L. & Born, W. K. γδ T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* **10**, 467–478 (2010).
3. Jensen, K. D. *et al.* Thymic selection determines γδ T cell effector functions: a blend of innate programming and acquired plasticity T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon γ. *Immunity* **29**, 90–100 (2008).
4. Ribot, J. C. *et al.* CD27 is a thymic determinant of the balance between interferon-γ- and interleukin-17-producing γδ T cell subsets. *Nat. Immunol.* **10**, 427–436 (2009).
5. Turchinovich, G. & Hayday, A. C. Skint-1 identifies a common molecular mechanism for the development of interferon-γ-secreting versus interleukin-17-secreting γδ T cells. *Immunity* **35**, 59–68 (2011).
6. Coffelt, S. B. *et al.* IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* **522**, 345–348 (2015).
This study describes a protumour role for mouse IL-17-producing γδ T cells in a breast cancer metastasis model.

7. Ma, S. *et al.* IL-17A produced by γδ T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res.* **74**, 1969–1982 (2014).
8. Rei, M. *et al.* Murine CD27$^{(-)}$ Vγ6$^{(+)}$ γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. *Proc. Natl Acad. Sci. USA* **111**, e3562–e3570 (2014).
Description of a protumour role for mouse IL-17-producing γδ T cells in an ovarian cancer model.
9. Wu, P. *et al.* γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity* **40**, 785–800 (2014).
First report of a protumour role for human IL-17-producing γδ T cells in colorectal cancer.
10. Rei, M., Pennington, D. J. & Silva-Santos, B. The emerging protumor role of γδ T lymphocytes: implications for cancer immunotherapy. *Cancer Res.* **75**, 798–802 (2015).
11. Correia, D. V., Lopes, A. & Silva-Santos, B. Tumor cell recognition by γδ T lymphocytes: T-cell receptor versus NK-cell receptors. *Oncoimmunology* **2**, e22892 (2013).
12. Kazen, A. R. & Adams, E. J. Evolution of the V, D, and J gene segments used in the primate γδ T-cell receptor reveals a dichotomy of conservation and diversity. *Proc. Natl Acad. Sci. USA* **108**, e332–e340 (2011).
13. Carding, S. R. & Egan, P. J. γδ T cells: functional plasticity and heterogeneity. *Nat. Rev. Immunol.* **2**, 336–345 (2002).
14. O'Brien, R. L. & Born, W. K. γδ T cell subsets: a link between TCR and function? *Semin. Immunol.* **22**, 193–198 (2010).
15. Haas, J. D. *et al.* CCR6 and NK1.1 distinguish between IL-17A and IFN-γ-producing γδ effector T cells. *Eur. J. Immunol.* **39**, 3488–3497 (2009).
16. Girardi, M. *et al.* Regulation of cutaneous malignancy by γδ T cells. *Science* **294**, 605–609 (2001).
Seminal work demonstrating the antitumour role for mouse γδ T cells in chemically induced skin cancer. This paper established the concept of cancer immunosurveillance by γδ T cells.
17. Girardi, M. *et al.* The distinct contributions of murine T cell receptor (TCR)γδ$^+$ and TCRαβ$^+$ T cells to different stages of chemically induced skin cancer. *J. Exp. Med.* **198**, 747–755 (2003).
18. Street, S. E. *et al.* Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and γδ T cells. *J. Exp. Med.* **199**, 879–884 (2004).
19. Liu, Z. *et al.* Protective immunosurveillance and therapeutic antitumor activity of γδ T cells demonstrated in a mouse model of prostate cancer. *J. Immunol.* **180**, 6044–6053 (2008).
20. Gao, Y. *et al.* γδ T cells provide an early source of interferon γ in tumor immunity. *J. Exp. Med.* **198**, 433–442 (2003).
This study demonstrates that γδ T cells constitute an early and important source of IFNγ following tumour challenge.
21. Lanca, T. *et al.* Protective role of the inflammatory CCR2/CCL2 chemokine pathway through recruitment of type 1 cytotoxic γδ T lymphocytes to tumor beds. *J. Immunol.* **190**, 6673–6680 (2013).
22. He, W. *et al.* Naturally activated Vγ4 γδ T cells play a protective role in tumor immunity through expression of eomesodermin. *J. Immunol.* **185**, 126–133 (2010).
23. 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).
Elucidation of the dramatic impact of NKG2D ligands on the antitumour functions of γδ T cells.
24. Guerra, N. *et al.* NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity* **28**, 571–580 (2008).
25. Pennington, D. J. *et al.* The integration of conventional and unconventional T cells that characterizes cell-mediated responses. *Adv. Immunol.* **87**, 27–59 (2005).
26. Riond, J., Rodriguez, S., Nicolau, M. L., al Saati, T. & Gairin, J. E. *In vivo* major histocompatibility complex class I (MHCI) expression on MHCI^{low} tumor cells is regulated by γδ T and NK cells during the early steps of tumor growth. *Cancer Immun.* **9**, 10 (2009).
27. Haas, J. D. *et al.* Development of interleukin-17-producing γδ T cells is restricted to a functional embryonic wave. *Immunity* **37**, 48–59 (2012).
28. Takeuchi, A. *et al.* IL-17 production by γδ T cells is important for the antitumor effect of *Mycobacterium bovis* bacillus Calmette-Guerin treatment against bladder cancer. *Eur. J. Immunol.* **41**, 246–251 (2011).
29. Ma, Y. *et al.* Contribution of IL-17-producing γδ T cells to the efficacy of anticancer chemotherapy. *J. Exp. Med.* **208**, 491–503 (2011).
This study unravelled a protective role for IL-17-producing γδ T cells following doxorubicin treatment by promoting the priming and recruitment of antitumour CD8$^+$ T cells.
30. Mattarollo, S. R. *et al.* Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res.* **71**, 4809–4820 (2011).
31. 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).
32. Hao, J. *et al.* Regulatory role of Vγ1 γδ T cells in tumor immunity through IL-4 production. *J. Immunol.* **187**, 4979–4986 (2011).
33. Carmi, Y. *et al.* Microenvironment-derived IL-1 and IL-17 interact in the control of lung metastasis. *J. Immunol.* **186**, 3462–3471 (2011).
34. Wakita, D. *et al.* Tumor-infiltrating IL-17-producing γδ T cells support the progression of tumor by promoting angiogenesis. *Eur. J. Immunol.* **40**, 1927–1937 (2010).
35. Gabrilovich, D. I., Ostrand-Rosenberg, S. & Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253–268 (2012).
36. Lo Presti, E., Dieli, F. & Meraviglia, S. Tumor-infiltrating γδ T lymphocytes: pathogenic role, clinical significance, and differential programing in the tumor microenvironment. *Front. Immunol.* **5**, 607 (2014).
37. Siegers, G. M. & Lamb, L. S. Jr. Cytotoxic and regulatory properties of circulating Vδ1$^+$ γδ T cells: a new player on the cell therapy field? *Mol. Ther.* **22**, 1416–1422 (2014).
38. Ribot, J. C., Ribeiro, S. T., Correia, D. V., Sousa, A. E. & Silva-Santos, B. Human γδ 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).
39. Correia, D. V. *et al.* Differentiation of human peripheral blood Vδ1$^+$ T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. *Blood* **118**, 992–1001 (2011).
40. Caccamo, N. *et al.* Differentiation, phenotype, and function of interleukin-17-producing human Vγ9Vδ2 T cells. *Blood* **118**, 129–138 (2011).
41. Ma, C. *et al.* Tumor-infiltrating γδ T lymphocytes predict clinical outcome in human breast cancer. *J. Immunol.* **189**, 5029–5036 (2012).
42. Ye, J. *et al.* Specific recruitment of γδ regulatory T cells in human breast cancer. *Cancer Res.* **73**, 6137–6148 (2013).
43. Wesch, D., Peters, C. & Siegers, G. M. Human γδ T regulatory cells in cancer: fact or fiction? *Front. Immunol.* **5**, 598 (2014).
44. Tanaka, Y., Morita, C. T., Nieves, E., Brenner, M. B. & Bloom, B. R. Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature* **375**, 155–158 (1995).
45. Morita, C. T. *et al.* Direct presentation of nonpeptide prenyl pyrophosphate antigens to human γδ T cells. *Immunity* **3**, 495–507 (1995).
46. Gober, H. J. *et al.* Human T cell receptor γδ cells recognize endogenous mevalonate metabolites in tumor cells. *J. Exp. Med.* **197**, 163–168 (2003).
47. Scotet, E. *et al.* Tumor recognition following Vγ9Vδ2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. *Immunity* **22**, 71–80 (2005).
48. Harly, C. *et al.* Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood* **120**, 2269–2279 (2012).
Seminal paper on BTN3A1 and its role in human Vγ9Vδ2$^+$ T cell activation.
49. Vavassori, S. *et al.* Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells. *Nat. Immunol.* **14**, 908–916 (2013).
50. Sandstrom, A. *et al.* The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity* **40**, 490–500 (2014).
51. Gu, S., Nawrocka, W. & Adams, E. J. Sensing of pyrophosphate metabolites by Vγ9Vδ2 T cells. *Front. Immunol.* **5**, 688 (2014).
52. De Libero, G., Lau, S. Y. & Mori, L. Phosphoantigen presentation to TCR γδ cells, a conundrum getting less gray zones. *Front. Immunol.* **5**, 679 (2014).
53. Harly, C., Peigne, C. M. & Scotet, E. Molecules and mechanisms implicated in the peculiar antigenic activation process of human Vγ9Vδ2 T cells. *Front. Immunol.* **5**, 657 (2014).
54. Girlanda, S. *et al.* MICA expressed by multiple myeloma and monoclonal gammopathy of undetermined significance plasma cells costimulates pamidronate-activated γδ lymphocytes. *Cancer Res.* **65**, 7502–7508 (2005).
55. Lanca, T. *et al.* The MHC class Ib protein ULBP1 is a nonredundant determinant of leukemia/lymphoma susceptibility to γδ T-cell cytotoxicity. *Blood* **115**, 2407–2411 (2010).
56. Kong, Y. *et al.* The NKG2D ligand ULBP4 binds to TCRγ9/δ2 and induces cytotoxicity to tumor cells through both TCRγδ and NKG2D. *Blood* **114**, 310–317 (2009).
57. Groh, V. *et al.* Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. *Proc. Natl Acad. Sci. USA* **96**, 6879–6884 (1999).
58. Xu, B. *et al.* Crystal structure of a γδ T-cell receptor specific for the human MHC class I homolog MICA. *Proc. Natl Acad. Sci. USA* **108**, 2414–2419 (2011).
59. Uldrich, A. P. *et al.* CD1d-lipid antigen recognition by the γδ TCR. *Nat. Immunol.* **14**, 1137–1145 (2013).
60. Luoma, A. M. *et al.* Crystal structure of Vδ1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human γδ T cells. *Immunity* **39**, 1032–1042 (2013).
61. Willcox, C. R. *et al.* Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor. *Nat. Immunol.* **13**, 872–879 (2012).
62. Poggi, A. *et al.* Vδ1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by *trans*-retinoic acid. *Cancer Res.* **64**, 9172–9179 (2004).
63. 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. *Curr. Opin. Immunol.* **27**, 16–25 (2014).
64. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
65. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
66. Kunzmann, V., Bauer, E. & Wilhelm, M. γδ T-cell stimulation by pamidronate. *N. Engl. J. Med.* **340**, 737–738 (1999).
67. Gomes, A. Q., Martins, D. S. & Silva-Santos, B. Targeting γδT lymphocytes for cancer immunotherapy: from novel mechanistic insight to clinical application. *Cancer Res.* **70**, 10024–10027 (2010).
68. Hannani, D. *et al.* Harnessing γδ T cells in anticancer immunotherapy. *Trends Immunol.* **33**, 199–206 (2012).
69. Godder, K. T. *et al.* Long term disease-free survival in acute leukemia patients recovering with increased γδ T cells after partially mismatched related donor bone marrow transplantation. *Bone Marrow Transplant* **39**, 751–757 (2007).
70. Fournie, J. J. *et al.* What lessons can be learned from γδ T cell-based cancer immunotherapy trials? *Cell. Mol. Immunol.* **10**, 35–41 (2013).
71. Brandes, M. *et al.* Cross-presenting human γδ T cells induce robust CD8$^+$ αβ T cell responses. *Proc. Natl Acad. Sci. USA* **106**, 2307–2312 (2009).
72. Moser, B. & Eberl, M. γδ T-APCs: a novel tool for immunotherapy? *Cell. Mol. Life Sci.* **68**, 2443–2452 (2011).
73. Norell, H., Moretta, A., Silva-Santos, B. & Moretta, L. At the bench: preclinical rationale for exploiting NK cells and γδ T lymphocytes for the treatment of high-risk leukemias. *J. Leukoc. Biol.* **94**, 1123–1139 (2013).
74. Airoldi, I. *et al.* γδ T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-αβ$^+$/CD19$^+$ lymphocytes. *Blood* **125**, 2349–2358 (2015).
75. Childs, R. W. & Carlsten, M. Therapeutic approaches to enhance natural killer cell cytotoxicity against cancer: the force awakens. *Nat. Rev. Drug Discov.* **14**, 487–498 (2015).
76. Silva-Santos, B. γδ cells making IL-17. *Blood* **118**, 3–5 (2011).
77. Elinav, E. *et al.* Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat. Rev. Cancer* **13**, 759–771 (2013).
78. Hooper, L. V. & Macpherson, A. J. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat. Rev. Immunol.* **10**, 159–169 (2010).

79. Duerkop, B. A., Vaishnava, S. & Hooper, L. V. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity* **31**, 368–376 (2009).
80. Cheroutre, H., Lambolez, F. & Mucida, D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat. Rev. Immunol.* **11**, 445–456 (2011).
81. Ismail, A. S. *et al.* γδ intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc. Natl Acad. Sci. USA* **108**, 8743–8748 (2011).
82. Cheng, M. *et al.* Microbiota modulate tumoral immune surveillance in lung through a γδT17 immune cell-dependent mechanism. *Cancer Res.* **74**, 4030–4041 (2014).
83. Ismail, A. S., Behrendt, C. L. & Hooper, L. V. Reciprocal interactions between commensal bacteria and γδ intraepithelial lymphocytes during mucosal injury. *J. Immunol.* **182**, 3047–3054 (2009).
84. Schmolka, N. *et al.* Epigenetic and transcriptional signatures of stable versus plastic differentiation of proinflammatory γδ T cell subsets. *Nat. Immunol.* **14**, 1093–1100 (2013).
85. Brandes, M. *et al.* Flexible migration program regulates γδ T-cell involvement in humoral immunity. *Blood* **102**, 3693–3701 (2003).
86. Kabelitz, D. & Wesch, D. Features and functions of γδ T lymphocytes: focus on chemokines and their receptors. *Crit. Rev. Immunol.* **23**, 339–370 (2003).
87. Hammerich, L. *et al.* Chemokine receptor CCR6-dependent accumulation of γδ T cells in injured liver restricts hepatic inflammation and fibrosis. *Hepatology* **59**, 630–642 (2014).
88. Mabuchi, T., Takekoshi, T. & Hwang, S. T. Epidermal CCR6$^+$ γδ T cells are major producers of IL-22 and IL-17 in a murine model of psoriasiform dermatitis. *J. Immunol.* **187**, 5026–5031 (2011).
89. Nicol, A. J. *et al.* Clinical evaluation of autologous γδ T cell-based immunotherapy for metastatic solid tumours. *Br. J. Cancer* **105**, 778–786 (2011).
90. Gentles, A. J. *et al.* The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat. Med.* **21**, 938–945 (2015).
Groundbreaking demonstration of tumour-infiltrating γδ T cells as the most significant favourable prognostic immune population across a large collection of cancer types.
91. Grunder, C. *et al.* γ9 and δ2CDR3 domains regulate functional avidity of T cells harboring γ9δ2TCRs. *Blood* **120**, 5153–5162 (2012).
92. Heilig, J. S. & Tonegawa, S. Diversity of murine γ genes and expression in fetal and adult T lymphocytes. *Nature* **322**, 836–840 (1986).
93. LeFranc, M. P., Forster, A., Baer, R., Stinson, M. A. & Rabbitts, T. H. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. *Cell* **45**, 237–246 (1986).

Acknowledgements
The authors thank A. Hayday for critical reading of the manuscript and D. Pennington, M. Rei, T. Lança, D. Correia, J. Ribot, S. Chiplunkar and F. Dieli for insightful discussions on this topic. Their work was funded by the European Research Council (StG_260352 to B.S.-S.), and the Investigator FCT (to K.S.) and Welcome II (to H.N.) programmes of Fundação para a Ciência e Tecnologia.

Competing interests statement
The authors declare competing interests: see Web version for details.