Received: 8 July 2020 | Revised: 24 July 2020 | Accepted: 26 July 2020

DOI: 10.1111/imr.12916

INVITED REVIEW

Immunological Reviews WILEY

Gut γδ T cells as guardians, disruptors, and instigators of cancer

Toshiyasu Suzuki[1,2] | Liam Hayman[1] | Anna Kilbey[1,2] | Joanne Edwards[1] | Seth B. Coffelt[1,2]

[1]Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

[2]Cancer Research UK Beatson Institute, Glasgow, UK

Correspondence
Seth B. Coffelt, PhD, Cancer Research UK Beatson Institute, Garscube Estate, Switchback Road, G61 1BD Glasgow, UK.
Email: seth.coffelt@glasgow.ac.uk

Joanne Edwards, PhD, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Garscube Estate, Switchback Road, G61 1QH Glasgow, UK
Email: joanne.edwards@glasgow.ac.uk

Funding information
European Commission Marie Curie Fellowship, Grant/Award Number: GDCOLCA 800112; Naito Foundation; Wellcome Trust, Grant/Award Number: 208990/Z/17/Z; Cancer Research UK, Grant/Award Number: A25142; Medical Research Council, Grant/Award Number: MR/R502327/1

Abstract

Colorectal cancer is the third most common cancer worldwide with nearly 2 million cases per year. Immune cells and inflammation are a critical component of colorectal cancer progression, and they are used as reliable prognostic indicators of patient outcome. With the growing appreciation for immunology in colorectal cancer, interest is growing on the role γδ T cells have to play, as they represent one of the most prominent immune cell populations in gut tissue. This group of cells consists of both resident populations—γδ intraepithelial lymphocytes (γδ IELs)—and transient populations that each has unique functions. The homeostatic role of these γδ T cell subsets is to maintain barrier integrity and prevent microorganisms from breaching the mucosal layer, which is accomplished through crosstalk with enterocytes and other immune cells. Recent years have seen a surge in discoveries regarding the regulation of γδ IELs in the intestine and the colon with particular new insights into the butyrophilin family. In this review, we discuss the development, specialities, and functions of γδ T cell subsets during cancer progression. We discuss how these cells may be used to predict patient outcome, as well as how to exploit their behavior for cancer immunotherapy.

KEYWORDS
colorectal cancer, gut, IL-17, intestine, intraepithelial lymphocyte, γδ T cells

1 | INTRODUCTION

One of the most prevalent immune cell populations in gut tissue are γδ T cells. γδ T cells represent a collection of diverse subsets with independent phenotypes and functions—some subsets reside in tissue-resident and other subsets circulate in blood. In humans, these subsets are defined by their expression of the δ chain (Vδ1, Vδ2, and Vδ3), whereas γδ T cell subsets in mice are characterized by the expression of the γ chain (Vγ1, Vγ4, Vγ5, Vγ6, and Vγ7). One interesting aspect of γδ T cells is that the γδTCR often dictates where the cells localize anatomically. Human Vδ1 and Vδ3 cells are frequently found in organs, including the gut, skin, and liver,[1-3] while Vδ2 cells circulate in the peripheral blood. Similarly, mouse Vγ5, Vγ6, and Vγ7 cells are tissue-resident, whereas Vγ1 and Vγ4 cells traffic from tissue to lymph nodes. Human Vγ4Vδ1 cells and mouse Vγ7 cells account for the majority of gut-resident γδ T cells,[1,4] but other subsets can infiltrate diseased, damaged, and dysplastic gut

Liam Hayman and Toshiyasu Suzuki should be considered joint first authors.

This article is part of a series of reviews covering γδ T cells appearing in Volume 298 of *Immunological Reviews*.

tissue. This review will focus on the development, phenotype, and function of both resident and infiltrating γδ T cells in the gut during cancer progression. We highlight the individual contribution of γδ T cell subsets in colorectal cancer (CRC), as well as strategies to boost their anti-tumor properties or methods to mitigate their pro-tumor functions. The role of γδ T cells in other cancer types has recently been reviewed elsewhere.[5]

2 | DEVELOPMENT OF CIRCULATING AND GUT-RESIDENT γδ T CELLS

γδ T cell generation in humans and mice is highly complex and tightly regulated. Recent years have seen a surge of new findings in this area. Here, we describe the aspects of γδ T cell development related to the subsets found in gut tissue. More detailed information can be found in recent review articles.[6-8]

In mice, the rearrangement of the Vγ4/5/6/7 locus is evident from embryonic day 12 (E12),[9] preceding the start of αβ T cell development at E17.[10,11] E14 is just after completion of thymus development in the embryo, whereas hematopoiesis in the liver occurs from E12 and then in the bone marrow from E16.5.[12] Beginning at E12-E14, γδ T cells develop in ontogenic waves by functional subtype in the fetal thymus, starting with Vγ5 cells. Generation of Vγ5 cells is completed by E18.[10,13] After Vγ5 cells, Vγ6 cells start to expand from E16 in mice followed by Vγ4 and Vγ1 cells.[10,11,14,15] Recently, a monoclonal antibody specific for Vγ6 chain was developed, which has allowed the acquisition of more specific information about Vγ6 cell development.[15] Vγ6 cells arise in the fetal thymus, like other subpopulations. Their numbers (together with Vγ5 cells) start diminishing after birth, while Vγ1 and Vγ4 cells continue to expand.[15] Vγ5 cells, which express an invariant TCR chain, then migrate to the skin, proliferate within the skin, and exist there for their entire life as dendritic epidermal T cells (DETCs).[16] Vγ6 cells also express a semi-invariant TCR,[17,18] and these cells localize primarily to reproductive organs, tongue, and lung.[19-21] In adult mice, Vγ5 and Vγ6 cells (and some IL-17–producing Vγ4 cells in the colon) no longer require the thymus for development. They exist mostly as self-generating, tissue-resident cells.[15,22-26] The development of Vγ1 and Vγ4 cells in the thymus continues throughout adult life, where these cells emerge in a naive state, similar to αβ T cells. Vγ1 and Vγ4 cells travel throughout the body, and they are enriched in secondary lymphoid organs.[27] Gene expression datasets, including single-cell sequencing, have provided more insight into the hierarchical development of γδ T cells in the thymus.[28,29]

The generation of γδ T cells in the fetal or adult thymus of mice results in two main functional populations—not including skin Vγ5 cells or gut Vγ7 cells. These two groups of γδ T cells are defined by the cytokines they secrete, IFNγ and IL-17, rather than the TCR they express. IFNγ–producing γδ T cells are associated with Vγ1 and Vγ4 TCRs, while IL-17–producing γδ T cells are associated with Vγ4 and Vγ6 TCRs. The programming of each effector population undergoes a distinct developmental process. Upon stimulation of γδTCR in thymic development, the common αβ/γδ precursors are committed into γδ T cell progenitors, upregulating CD24 as an immature marker. After this, the fate of both effector types is also regulated by TCR stimulation, lymphotoxin, and IL-7 signaling.[30-33] Strong signaling on the γδTCR together with CD27 co-stimulation and lymphotoxin signaling directs progenitor cells into IFNγ–producing γδ T cells, leading to downregulation of CD24 and CD25.[30,31] On the other hand, the development of IL-17–producing γδ T cells is dependent on weak TCR signaling, the lack of CD27 signaling, strong IL-7α signaling, and positive cues from the Notch pathway.[30,31,34-36] During this process, IL-17–producing γδ T cells lose CD27 expression and upregulate other receptors, such as CCR6 and CD44.[37] Certain transcription factors are required for the generation of IL-17–producing γδ T cells, such as RORγt, MAF, HES1, and STAT5.[30,38-40] However, there are different developmental requirements of transcription factors between IL-17–producing Vγ4 and Vγ6 cells. For example, SOX4 and SOX13 are essential for the differentiation of Vγ4 cells, whereas they are dispensable for Vγ6 cells.[41] Conversely, PLZF is important for Vγ6 cells, but dispensable for Vγ4 cells.[42] These data imply that IL-17–producing effector subsets are driven by both shared and discretely independent mechanisms. Whether these mechanisms are related to the timing of development—embryonic development (Vγ6) versus postnatal thymic development (Vγ4)—is unknown. In adult mice, three developmental pathways for γδ T cells have been identified that can be defined using CD24, CD25, CD73, CD117, CD200, and CD371.[43]

Human γδ T cells also develop in an embryonic wave.[44,45] These cells emerge from the fetal or adult thymus as naive cells with polyclonal TCRs or imprinted effector cells, some of which express an invariant TCR.[46-51] Interestingly, Vγ9Vδ2 cells generated during fetal development are replaced by Vγ9Vδ2 cells with a different TCR in adults,[52,53] indicating a TCR switch of unknown importance. After birth, Vδ1 cells dominate γδ T cell development, overtaking Vδ2 cell numbers in the thymus, gut, and skin.[45,54-57] With age, however, Vγ9Vδ2 cells expand to become the most abundant subset in the blood and spleen.

The origin of mouse liver-resident and gut-resident γδ T cells—whose functions will obviously be important in primary CRC and liver metastasis—is somewhat unclear. Thus far, at least two subsets of liver-resident γδ T cells have been identified: one CD1d-expressing subset that produces IL-17 governed by lipid antigens from gut commensal bacteria,[58] and another CD8αα$^+$ subset regulated by the MHC class I–related molecule, H2-Q10.[59] Similar populations of resident cells are also found in human livers, characterized by the expression of Vδ1 as well as established resident markers, such as CD69, CXCR3, and CXCR6.[3] However, the original source of γδ T liver cells in humans or mice is currently unknown. The origin of gut-resident γδ T cells is controversial. Rearrangement of the Vγ7 locus occurs as early as E11 in the liver and gut before T cell progenitors migrate into developing thymic lobes.[9,60] Vγ7 cells can be exported from the fetal thymus during the perinatal period, and the adult thymus can generate Vγ7 cells.[61-64] Thymic development of Vγ7 cells is dependent on IL-15–activated STAT5.[65] However, nude mice, which lack a thymus, still contain Vγ7 cells,[1,66-68] and parabiosis experiments using adult mice have demonstrated that Vγ7 cells are rarely replaced

by circulating cells.[69,70] The appearance of human gut Vγ4 cells may also occur independently of the thymus, but the ontogeny of these human cells is still not well addressed.[71] Thus, the contribution of gut-resident γδ T cell development from the thymus seems minimal.

3 | LOCATION AND FUNCTION OF γδ T CELL SUBSETS IN THE GUT

Covering the surface of the intestinal lumen is a tight layer of epithelia organized as crypts and villi in the small intestine (SI) and crypts in the colon (Figure 1). This single layer of cells separates the gut tissue from digested food, dietary antigens, and microbes. Intestinal stem cells at the base of the crypts, marked by the leucine-rich repeat-containing G protein–coupled receptor 5 (Lgr5) protein, give rise to all types of intestinal epithelial cells (IECs), including enterocytes (or absorptive epithelial cells), goblet cells, enteroendocrine cells, Paneth cells, and Tuft cells.[72] Lgr5$^+$ stem cells divide about every 24 hours to self-maintain. These cells also exit the cell cycle, move upward toward the villus tip, and differentiate to replenish the various cell types that form the luminal layer of the gut. Once cells reach the top of the villus (except for Paneth cells) in a process that takes about 3-5 days, cells are most exposed to gut luminal contents and the microbiome. Tip cells undergo apoptosis and slough off into the lumen. The rapid turnover of new epithelial cells is necessary to prevent a break in the barrier.

Enterocytes are the most abundant cell type in the epithelium and form tight junctions with their neighboring cells to seal the barrier. Enterocytes and goblet cells synthesize transmembrane mucins to protect apical surfaces and expedite food waste. Cells in the colon form two thick layers of mucus compared with the SI, and the mucus has a high viscosity with a gradient from proximal to distal. The mucus layer in the SI is much thinner but is augmented by anti-microbial peptides (AMPs) synthesized by Paneth cells and enterocytes. Paneth cells are found only in the SI; the AMPs they produce include defensins, cathelicidin, lysozyme, and regenerating islet-derived protein 3 gamma (REG3G or REGIIIγ). In the colon, Paneth-like cells, also known as deep crypt secretory cells, support Lgr5$^+$ stem cells, but it is unknown whether these cells can secret AMPs.[73,74]

The constituents of the microbiome are very different between the SI and colon. There is a gradient of bacterial load from proximal

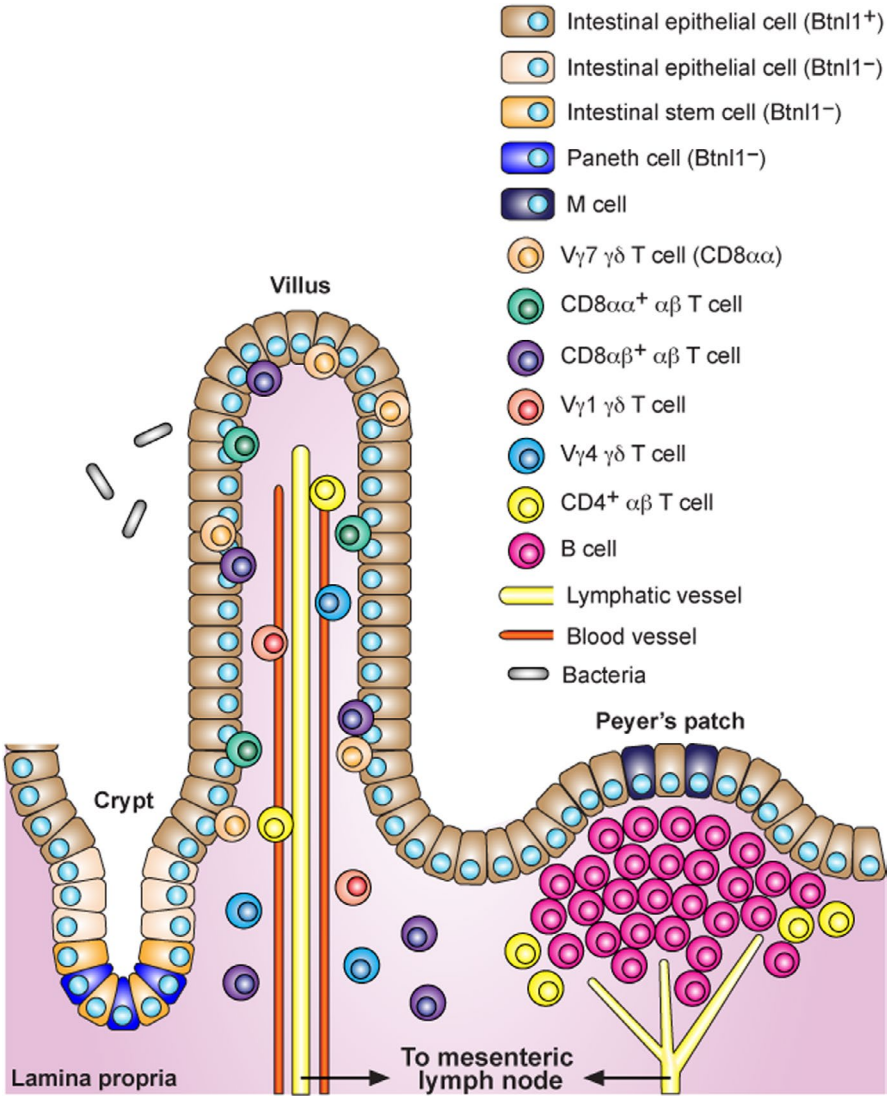

FIGURE 1 T cell location in the intestine. Stem cells in the crypt receive support and survival cues from Paneth cells. These stem cells give rise to epithelial cells that line the gut and protect internal organs from microorganisms in the gut lumen. During differentiation, epithelial cells express BTNL1 in mice (and BTNL3 in humans), which localizes Vγ7 cells (or Vγ4 cells in humans) adjacent to epithelial cells and maintains their survival. αβ T cells that express CD8αα homodimers or CD8αβ heterodimers also reside next to epithelial cells. In the lamina properia, a variety of other γδ and αβ T cells traffic in and out of the tissue through blood vessels or lymphatic vessels. M cells, specialized epithelial cells, transport antigen to Peyer's patch, which contains mostly B cells and CD4 T cells

to distal gastrointestinal tract: ordered by duodenum, jejunum, ileum, cecum, proximal colon, and distal colon.[75] Bacterial load is much higher in the colon than the SI, partly because of the inhospitable environment of the SI, which is more acidic and more abundant in AMPs than the colon.[76] Therefore, it is easily presumed that immunity in the SI and colon is suitably distinct. Coincidentally, human tumors develop mainly in the colon and rarely in the SI. Accumulating evidence suggests that certain species of bacteria are associated with and drive CRC development.[77]

γδ T cells in the SI and colon serve as one of the first lines of defense in the immune surveillance program of gut tissue (Figure 1). γδ T cells in this organ consist of both resident and infiltrating populations. The resident subset, called intraepithelial lymphocytes (IELs), roams up and down the villus along the basement membrane behind enterocytes in the lateral intercellular space that separates epithelial cells from the lamina propria.[78] IELs can express either a γδTCR or αβTCR, and these TCRs are oligoclonal in humans and mice.[79-82] γδ and αβ IELs that express the CD8αα co-receptor but not CD8β or CD4 are the so-called natural or type B IELs. SI enterocytes express the ligand for CD8αα, thymus leukemia antigen (TLA), which is a non-classical MHC class I molecule.[83,84] There is another group of induced or type A IELs that arise from αβTCR-expressing T cells, and these cells can express CD4, CD8αα, and/or CD8αβ (not discussed further) [reviewed in Ref. 85]. γδ IELs account for 20%-30% of IELs in humans and about 50% of IELs in mice, where they express the Vγ4 and Vγ7 chains, respectively.[1,66,68,86]

A few key molecules control the localization and active migration patterns of IELs. CD103, also known as αE integrin, is one of these molecules expressed by IELs. CD103 dimerizes with β7 integrin, and this complex binds to E-cadherin on epithelial cells.[87,88] In both CD103- and β7-deficient mice, IEL number is reduced with a greater reduction seen in β7-deficient mice.[89,90] The milder effect of CD103 loss compared with β7 loss on IELs might be explained by the fact that β7 can form another heterodimer with α_4 integrin, which is also involved in homing to the gut.[91] Interestingly, αβ IELs are more affected by CD103 loss than γδ IELs. CD103 expression on IELs is stimulated by TGFβ and runt-related transcription factor 3 (RUNX3),[92,93] as well as by the CCL25-CCR9 axis.[94,95] CCL25 is a chemokine highly expressed by SI epithelial cells,[96] and its receptor CCR9 is expressed on IELs.[97] Despite ubiquitous expression of CCR9 by all IELs, CCL25- and CCR9-deficient mice exhibit a specific reduction in γδ IELs to half their number in wildtype mice.[98,99] CCR9 expression and IEL homing to SI seem to be regulated in part by the vitamin D receptor (VDR), as VDR knockout mice exhibit reduced CD8αα-expressing IEL that coincides with decreased IL-10 levels.[100] IL-15 is another key molecule that regulates both γδ and αβ IEL maintenance and localization.[101-106] This cytokine and its receptor, IL-15Rα, are expressed by enterocytes and lamina propria DCs, where they form a complex that is transpresented to IELs. The use of sophisticated mouse models and advanced live cell imaging techniques has shown that IL-15 controls Vγ7 cell localization and migratory behavior within the lateral intercellular space, as blocking IL-15 signaling causes their patrolling nature to idle.[104] In addition, the G protein–coupled receptor, GPR18, regulates γδ IEL abundance in the gut and this molecule is important for positioning of IELs next to epithelial cells as opposed to the lamina propria.[107,108] By contrast, GPR55 negatively regulates γδ IELs, as confirmed by intravital imaging in GPR55-deficient mice that not only have more γδ IELs, but these cells also migrate faster and establish greater crosstalk with epithelial cells. Moreover, loss of GPR55 fails to affect αβ IELs.[109] Thus, αβ and γδ IELs share many similarities; however, their homing and motility are differentially regulated.

The phenotype of αβ and γδ IELs is very similar, almost indistinguishable in fact.[110,111] Both αβ and γδ IELs express the semaphorin, CD100 (also called SEMA4D), which controls their proliferation.[112] Compared with other T cells, IELs exist in a constitutively active, cytolytic state with elevated effector potential. IELs constitutively express a number of cytolytic molecules, including granzymes A and B, perforin, and Fas ligand (FasL). Granzymes and perforin work together to penetrate the cell membrane of target cells and induce killing. FasL is a member of the tumor necrosis factor (TNF) ligand superfamily that binds Fas on target cells to trigger apoptosis. IELs also produce molecules normally associated with NK cells, such as 2B4/CD244, NKG2A, NKG2D, NKp46, and NK1.1. IELs uniformly express CD69, an early activation marker of TCR activation as well as a tissue-resident marker.[113,114] On the other hand, another marker of TCR stimulation, CD25, is scarcely expressed on IELs.[110] Taken together, the phenotype of IELs indicates that these cells are always alert and poised for attack.

As suggested by their phenotype, the role of IELs in the gut is to maintain homeostasis and epithelial tight junction integrity [reviewed in Refs 91,115,116]. Crosstalk between the microbiome, epithelial cells, and IELs stimulates epithelial cell proliferation and function to reinforce the barrier.[117] γδ IELs respond to microbiota through the Toll-like receptor (TLR)/MyD88 pathway that induces REGIIIγ to protect against invading bacteria.[118] In chronic inflammatory diseases of the gut, including Crohn's disease and ulcerative colitis, loss of barrier integrity is accompanied by an influx of luminal pathogens, a subsequent massive immune response, and sustained tissue damage, illustrating the importance of intestinal homeostasis for the maintenance of a healthy gut. γδ IELs play a protective role in these pathologies by suppressing Th1 type responses.[119,120] γδ T cell–deficient mice are susceptible to spontaneous colitis with age (5% of mice over 8 months old), as well as experimentally induced colitis. However, transfer of IELs into these γδ T cell–deficient mice reverses colitis, presumably through TGFβ–mediated suppression of IFNγ and TNF.[119] By contrast, overactivation of γδ IEL is thought to perturb homeostasis in the gut, such as in inflammatory bowel disease (IBD) or celiac disease where elevated IL-15 expression is observed.[121-125]

In addition to human Vγ4 cells and mouse Vγ7 cells that constitute the γδ IEL population, other γδ T cell subsets infiltrate the gut tissue (Figure 1). These cells expressing invariant or polyclonal γδTCR repertoires arrive via the blood or lymph system and land in the lamina propria.[91,126] The lamina propria is the core of the villi that contains connective tissue, blood and lymphatic vessels,

fibroblasts, extracellular matrix, and a variety of immune cells. Gut-infiltrating γδ T cells may acquire a cytotoxic, effector-like phenotype-like IELs, but other subsets express a completely different range of cytokines, such as IL-17 or IL-22. These cytokines, often in response to IL-23 and/or IL-1β, control the secretion of AMPs and tight junctions between enterocytes to sustain barrier function.[25,127,128] In the mouse, IL-17- and IL-22–producing cells express either the Vγ4 or Vγ6 TCR. The abundance of these subsets changes between different regions of the gut with the highest enrichment of Vγ4 cells in the distal colon.[25] IL-17- and IL-22–producing cells are negatively regulated by gut $CD103^{+}CD11b^{+}$ dendritic cells (DCs), since the depletion of this DC subset using *Clec4a4-DTR* mice results in higher IL-17 and IL-22 expression from γδ T cells—an affect not observed from CD4 T cells.[25] Interestingly, there is a highly unusual population of mostly Vγ6 cells co-expressing IL-17, IL-22, and IFNγ found within the gut, which are not found in other tissues. Recent data show that STAT5 signaling controls the development of these polyfunctional cells, while STAT3 signaling controls IL-17 and IL-22 expression and retinoic acid signaling controls IFNγ expression.[38] However, it is unclear why these cells are unique to gut tissue or the purpose of their existence. Additionally, human lamina propria Vδ2 cells triggered by bacterial metabolites may reinforce barrier defense by recruiting neutrophils and stimulating CD4 T cells to produce IL-22.[129,130]

4 | γδ T CELL RECEPTOR STRUCTURE, ANTIGENS, AND BINDING PARTNERS

Human TCRα (Chr14, q11.2), TCRβ (Chr7, q34), TCRγ (Chr7, p14), and TCRδ (Chr14, q11.2) are encoded in the genome by several segments for each variable (V), diversity (D), joining (J), and constant (C) segments. Human TCRγ and TCRδ contain 6 and 8 functional V segments, respectively (5 of the 8 V segments of TCRδ are shared with TCRα), while TCRα and TCRβ contain 41 and 30 V segments, respectively.[131] So, γδTCRs seem to have much smaller repertoire potential than αβTCRs.

A few studies have reported on the structure of various γδTCRs.[132-139] In comparison with the αβTCR, the V regions of the γδTCR are similar, whereas the C regions are substantially different.[134] The antigen-binging domain of γδTCR has more protrusions and clefts, exhibiting more similarity to the surface of immunoglobulin heavy chain (V_H) than to the αβTCR, which has a flat surface. The determinant for antigen binding lies within amino acid sequences separated into 3 regions called complementarity-determining regions (CDRs). CDR1 and CDR2 are germline-encoded loops derived from the V segment, and CDR3 forms loops from the recombined region around the junction of the V, D, and J segments. CDR3 forms the center of the antigen-binding site. Interestingly, the length of the CDR3 of TCRδ is as very variable and long as that of V_H,[140] indicating that the γδTCR is closer related to immunoglobulins than TCRα and thus can recognize a variety of antigen types. The more striking difference between γδTCRs and αβTCRs is observed in the C domain, where angles, glycosylation sites, and charges of residues are distinct. Given that the C domain binds CD3 subunits, these differences suggest that the association with CD3 subunits and subsequent intracellular signaling may be different to that of αβTCR. This view is supported by biochemical assays and knockout mice, which have demonstrated that most mouse γδTCRs do not associate with the CD3δε heterodimer, but with two CD3γε heterodimers.[141-143] However, thymic γδ T cells from $Cd3d^{+/-}Cd3g^{+/-}$ mice exhibit reduced γδTCR expression with dysfunctional differentiation into IFNγ–producing γδ T cells,[144] indicating that the CD3δε heterodimer is important for some γδTCRs. Human γδTCRs are entirely reliant on CD3δε and CD3γε heterodimers.[143]

Antigens and ligands for γδTCRs constitute a wide variety of unconventional molecules. An important distinction between γδTCRs and αβTCRs is that γδTCRs do not bind major histocompatibility complex (MHC) molecules, and γδ T cells rarely express CD4 or CD8 MHC co-receptors (except in the case of gut-resident γδ T cells as discussed above). This major difference between T cell populations has contributed to the poorly understood nature and biology of γδ T cells over the last decades. However, recent work is uncovering new information in this elusive area [reviewed in Ref. 145].

γδTCR antigens fall into two main categories: adaptive-like molecules and B7-like molecules with similarity to the CD28 co-stimulatory receptor. Circulating γδ T cells in both humans and mice can bind the self-stress molecule endothelial protein C receptor (EPCR),[146,147] phycoerythrin,[148] and MHC class I–like molecules, such as T10, T22, MR1, CD1c, and CD1d.[135-137,139,149-156] T10 and T22 are only expressed in mice. Human γδ T cells can also recognize annexin A2.[157] γδTCRs recognize several of these molecules independently of peptides or lipids or metabolites, except in the case of the CD1 family that presents lipids and MR1 that presents metabolites, although the requirement of lipids and metabolites for interaction between γδTCRs and CD1c/d or MR1 is unknown. It should be noted that only a small frequency of γδTCRs binds the various molecules listed above, leaving unanswered questions about what other adaptive-like molecules interact with the majority of γδTCRs. Members of the butyrophilin (BTN) and butyrophilin-like (BTNL) family make up the other category of γδTCR antigens. The BTN/BTNL family of proteins is structurally and phylogenetically related to the B7 superfamily of co-stimulatory molecules, encompassing B7.1 (CD80), B7.2 (CD86), CD28, CTLA-4, PD-L1, and PD-L2.[158,159] In humans, circulating Vγ9Vδ2 cells recognize the BTN3A1-BTN2A1 heterodimer, a receptor complex that acts as a sensor for a group of pyrophosphate-containing metabolites called phosphoantigens that may be endogenous or exogenous (ie, non-self, microbial-derived) products.[160,161] Some of these phosphoantigens include isopentenyl pyrophosphate (IPP) generated from the mevalonate pathway important for cholesterol biosynthesis [162,163] or the microbial hydroxyl-methyl-butyl-pyrophosphate (HMBPP) metabolite produced from the isoprenoid pathway.[164] BTN3A1 and BTN2A1 sense the accumulation of intracellular phosphoantigens such as IPP and HMBPP, and binding of these phosphoantigens to intracellular domains of BTN3A1 causes a conformational change in the protein, promoting recruitment of

BTN2A1 that directly binds the Vγ9Vδ2 TCR.[161,165-167] The buildup of IPP is a common occurrence in many types of cancers with dysregulated metabolism, making cancer cells amenable to Vγ9Vδ2 cell killing. The role of BTN/BTNL family members in regulating resident γδ T cell subsets is discussed below.

Pioneering work on thymic development and maturation of mouse skin-resident Vγ5 cells led to the first link between BTN/BTNL family members and γδ T cells. This discovery identified SKINT1, which is a BTN/BTNL family member specifically expressed in the skin and thymus, as a crucial protein for Vγ5 cell expansion during development and survival in skin.[168-170] More recently, mouse BTNL1 and human BTNL3 were demonstrated to be the equivalent of SKINT1 for gut Vγ7 and Vγ4Vδ1 IELs, respectively.[1] These BTNL family members are expressed solely in the SI and colon.[171] *Btnl1*-deficient mice display a marked reduction in Vγ7 cells in gut tissue. BTNL1 dimerizes with BTNL6, and BTNL3 dimerizes with BTNL8 to maintain γδ IEL survival and function in both humans and mice, indicating a highly conversed network between species. In subsequent studies, mutagenesis, computational modeling, and functional assays showed that the Ig-V regions of BTNL3 directly interact with the germline-encoded non-variable regions of the Vγ4 TCR.[172,173] These data uncover a unique ability of γδTCRs to recognize and respond to ligands via variable and non-variable sequences. Given these sophisticated properties of combining innate and adaptive traits at the cell-intrinsic level, the term "adaptate" was coined to better represent γδ T cell biology.[174,175]

5 | COLORECTAL CANCER ETIOLOGY AND MODELING IN MICE

Colorectal cancer is the third most common cancer in men and second most common in women worldwide,[176] rising from fourth and third most common in 2002,[177] respectively. CRC is estimated to kill over 881 000 people worldwide.[176] Incidence and mortality are likely to increase as life expectancy rises and developing countries become increasingly westernized. Key risk factors for CRC include increased red meat consumption, low fiber intake, and a low level of physical activity.[178-180]

The progression from adenoma to CRC is driven by the acquisition of multiple genetic aberrations. There are two types of adenomas whose genetic mutations differ that correspond with two postulated avenues to metastatic CRC. In the case of sessile serrated polyps (SSPs), mutational drivers consist of BRAF mutations, CpG island methylator phenotype (CIMP), and microsatellite instability (MSI).[181,182] In contrast, traditional serrated adenoma conversion to carcinoma is driven by mutation of the tumor suppressor protein, APC, the inactivation of which allows for stabilization and translocation of β-catenin to the nucleus where it participates in upregulation of WNT target genes.[183,184] Further mutations occur in KRAS, TGFβ signaling, and p53, leading to progression of the tumor,[185-188] and may be influenced by MSI.[189] Although significant hereditary factors are present in approximately 35% of CRC incidence,[190] driver mutations in genes causing established hereditary syndromes account for approximately 5% of CRC incidence.[191,192] The most prevalent of these hereditary syndromes is Lynch syndrome,[193,194] driven by a mutation in genes that jeopardize DNA mismatch repair (MMR), such as MLH1, MSH2, MSH6, and PMS2.[195-203] The remaining hereditary syndromes are associated with severe polyposis and subsequently an increased likelihood of progression from the polyp stage, such as Peutz-Jeghers syndrome,[204] familial adenomatous polyposis, and other adenomatous polyposis syndromes.[205]

In 2015, a consortium of CRC scientists agreed on a set of four molecular subtypes derived from gene expression data, termed consensus molecular subtypes (CMS).[206] The authors produced the CMS subtypes from over 3000 patients to determine biological characteristics associated with each CMS subtype. CMS1, the immune-related group, is characterized by MSI and high immune infiltrate, in addition to high CIMP, BRAF mutations, activation of the JAK/STAT pathway, and an intermediate overall survival. CMS2, the canonical subtype, is characterized by high DNA somatic copy number alterations (SCNA), low immune infiltrate and stromal invasion, activation of WNT signaling, and the best overall survival. CMS3 is characterized by mutations in KRAS, low immune infiltrate and stromal invasion, activation of metabolic genes, and an intermediate survival. CMS4 is characterized by high SCNA, high stromal invasion, activation of the TGFβ and VEGF pathways, and the worst overall survival. These CMS subtypes were developed from whole tumor tissue, which may be confounded by stroma and immune cell composition. Other attempts at refining transcriptional gene signatures using cancer cell–intrinsic gene expression have made valuable improvements to the CMS stratification; this approach was named CRIS for CRC intrinsic signature.[207] Incidentally, CMS4 and CRIS-B share the same TGFβ–enriched pathways, so there is a degree of overlap between methodologies. Given the difficulty in translating transcriptomics into routine pathology as well as the costly nature of generating and analyzing gene expression data for every patient with CRC, we developed a phenotypic subtyping method based on the CMS subtypes with the aim of introducing histology-based subtyping into clinical practice.[208,209] This method incorporates immune cell infiltration using the Klintrup-Mäkinen (KM) grade, proliferation of cancer cells using the Ki-67 marker, and stromal invasion using the tumor-stroma percentage.[209] These measures produce four phenotypic subtypes: immune, canonical, latent, and stromal. The phenotypic subtypes are prognostic classifiers in stage I-stage III CRC independent of TNM staging and predict recurrence and chemotherapy response.[208] Taken together, these approaches provide robust classification systems for CRC and opportunities for personalized anti-cancer therapy and immunotherapy.

In the quest to understand the etiology and biology of CRC, cancer researchers are developing increasingly accurate ways to model colorectal cancer that mirror the CMS phenotyping. Since APC alterations are the most common mutations in colorectal cancer,[188] mouse models have historically relied on APC mutations,[210] such as the $Apc^{Min/+}$ model, which carries a point mutation in *Apc*.[211] Models of this type readily develop sporadic polyps. However, there are

drawbacks to *Apc* mutant models, including their predilection for SI tumors rather than colonic tumors as seen in humans, and the low penetrance of advanced carcinomas. The development of mouse models looking beyond APC mutations in isolation, thus better reflecting the progressive series of mutations seen in CRC, and the subsequent engineering of mouse models which reflect the most up-to-date CRC subtyping method allow researchers to study CRC in a more relevant context. Some examples include advances in organoid technology, which can be transplanted into syngeneic or immunodeficient mice, and new genetically engineered mouse models that fully recapitulate CRC progression from adenoma to metastasis.[212-215] However, the use of these models either older or newer in γδ T cell biology is limited. The importance of γδ T cells in CRC is discussed next.

6 | THE ROLE OF ANTI-TUMORIGENIC γδ T CELLS IN CRC

The anti-tumorigenic function of γδ T cells in mice was first observed in 2001, showing in carcinogen-induced mouse models that cutaneous Vγ5 cells (DETCs) regulate skin cancer growth in a manner dependent on NKG2D recognition of the stress ligand, RAE-1.[216] The anti-tumorigenic role of both human and mouse γδ T cells has since been expanded to multiple cancer types [reviewed in Ref. 5]. Much of the evidence for an anti-tumorigenic role for human γδ T cells specifically in CRC is related to their functional ability to kill established CRC cell lines, taken from patients with advanced disease (discussed further below). However, there is some evidence that γδ T cells play a protective role in earlier stages of disease progression and even tumor initiation from mouse models. For example, in a mouse model of hypocholesteremia (ApoE-deficient mice) given the carcinogen, azoxymethane (AOM), tumor incidence and severity are associated with decreased numbers of γδ T cells.[217] Here, hypocholesteremia adversely impacts hematopoietic stem cells, skewing differentiation away from γδ T cell and NK cell development. Indeed, mice lacking γδ T cells exhibit greater numbers of AOM-induced gut tumors.[217,218] Together, these data suggest that γδ T cells are important in immune surveillance during the early stages of CRC disease progression. Whether thymic-derived γδ T cells or γδ IEL or both play a role in counteracting gut tumor progression is unclear.

Circulating human Vγ9Vδ2 cells have so far been the focus of research into the killing ability of γδ T cells across multiple cancer types. Transcriptomic analysis of Vγ9Vδ2 cells has shown that this subset expresses of blend of αβ T cell, NK cell, and MAIT cell gene signatures.[219,220] Interestingly, Vγ9Vδ2 cells produce higher levels of NKG2D, NKG2A, granzyme B, FasL, and several DC-related cytokines and chemokines than αβ T cells or NK cells.[219] Vγ9Vδ2 cells can kill a variety of CRC cell lines, regardless of whether these cells were isolated from the ascites of a metastatic CRC patient, the primary tumor of a CRC patient, or the peripheral blood of a healthy donor.[221] The equivalent cytotoxic capacity of Vγ9Vδ2 cells from cancer patients and healthy donors suggests that tumors fail to negatively influence the anti-tumorigenic properties of circulating Vγ9Vδ2 cells. However, when γδ T cells are cocultured with supernatants from CRC patient–derived cancer stem cells (CSCs) or cancer-associated fibroblasts (CAFs), the proliferation of γδ T cells and IFNγ expression is reduced, while IL-17 expression is increased.[222] These observations indicate that the tumor microenvironment may influence the ability of γδ T cells to recognize cancer cells. In support of this notion, the characterization of Vγ9Vδ2 cells in CRC patients with liver metastasis treated with standard of care, 5-fluorouracil/oxaliplatin (FOLFOX), has shown that absolute numbers of circulating Vγ9Vδ2 cells are reduced.[223,224] Vγ9Vδ2 cells in these patients exhibit a higher frequency of terminally differentiated and senescent phenotype with impaired effector function, when compared to Vγ9Vδ2 cells from healthy donors. In addition, the number of chemotherapy cycles is correlated with a decrease in Vγ9Vδ2 cells expressing central memory markers, and chemotherapy also skews Vγ9Vδ2 cells toward terminal differentiation.[223] However, whether this change in Vγ9Vδ2 cell effector function adversely impacts prognosis or patient survival is unknown.

As discussed above, metabolites of the mevalonate pathway such as isopentenyl pyrophosphate (IPP) activate Vγ9Vδ2 cells via recognition of the BTN3A1-BTN2A1 heterodimer on cancer cells.[160,161,165-167] Blocking the mevalonate pathway to inhibit IPP accumulation in cancer cell lines reduces Vγ9Vδ2-mediated cell lysis.[221] Conversely, the use of nitrogen-containing bisphosphonates, such as zoledronate, to increase IPP accumulation in CRC stem cells, sensitizes cancer cells to Vγ9Vδ2-induced cytotoxicity.[225,226] These data underscore the importance of IPP-stimulated BTN3A1-BTN2A1 receptors in Vγ9Vδ2 cell recognition of CRC, although it is unclear whether the mevalonate pathway and IPP accumulation are dysfunctional in every subtype of CRC.

Despite a large focus of γδ T cell research centered on Vγ9Vδ2 cells, of increasing interest are the $Vδ2^-$ subsets, particularly the Vδ1 cells. Vδ1 cells are the dominant population in human colorectal tumors,[222] and these cells display cytolytic reactivity against CRC cell lines both in vitro and in a xenograft model.[227-230] Killing of cancer cells by Vδ1 cells is independent of MHC molecule recognition.[228] Interestingly, one study reported that the cytotoxic ability of Vδ1 cells from the primary tumor of three CRC patients (one metastatic) is markedly higher against epithelial tissues than alternative tissues such as hematopoietic cancer cell lines, as quantified by percentage cell lysis and IFNγ release.[227] Thus, Vδ1 cells may be reacting to a ligand which is native to epithelial tissues by a receptor that is constitutively expressed on these cells, such as NKG2D.[114,133,231-234] In comparisons between Vδ1 cells and Vδ2 cells isolated from peripheral blood of healthy donors and CRC patients, Vδ1 express higher levels of activation markers, cytotoxicity markers, and terminal differentiation markers. This apparent difference in phenotype is also observed functionally, as Vδ1 T cells induce greater lysis of CRC cell lines than did Vδ2 T cells.[228,229] As interest in Vδ1 cells continues to grow, research is beginning to elucidate more specific details about these cells, how they differ from Vδ2 cells, and ultimately how they function. An $NKp46^+$ subpopulation of IELs that mostly

consist of Vγ4Vδ1-expressing cells has recently been characterized.[114] The abundance of these cells is less in stage III/IV than stage I/II. After coculture with the myelogenous leukemia cell line K562, the NKp46^{+} γδ T cells produce more IFNγ, granzyme B, and CD107a than the NKp46^{-} population and kill K562 cells more efficiently. Blocking NKp46 also reduces K562 killing in these cocultures.[114] Collectively, Vδ1 appear to demonstrate a stronger anti-tumorigenic potency than their Vδ2 counterparts. The anti-tumorigenic roles of γδ T cells are summarized in Figure 2.

The majority of cancer deaths are a consequence of metastasis so understanding how γδ T cells may counter the metastatic process is of great interest. In an orthotopic mouse xenograft model of luciferase-expressing HT29 cells injected into the cecum of immunodeficient mice, the administration of Vδ1 T cells reduces primary tumor growth as well as the formation of spontaneous liver and lung metastases. Similarly, Vδ1 cell immunotherapy decreases HT29 cell growth in the lung after intravenous injection.[235] Thus, Vδ1 cells counteract CRC growth regardless of a tumor's anatomical location, indicating that Vδ1 cells not only exhibit anti-tumor potential but also anti-metastatic potential.

7 | THE ROLE OF PRO-TUMORIGENIC γδ T CELLS IN CRC

The knowledge of how γδ T cells may promote CRC is still limited, but what is known is largely centered on IL-17–producing γδ T cell subsets (Figure 3). This is also true for other tumor types, where we and others showed that IL-17–producing γδ T cells drive cancer progression and metastasis.[236-240] In mouse models driven by mutant *Apc* or loss

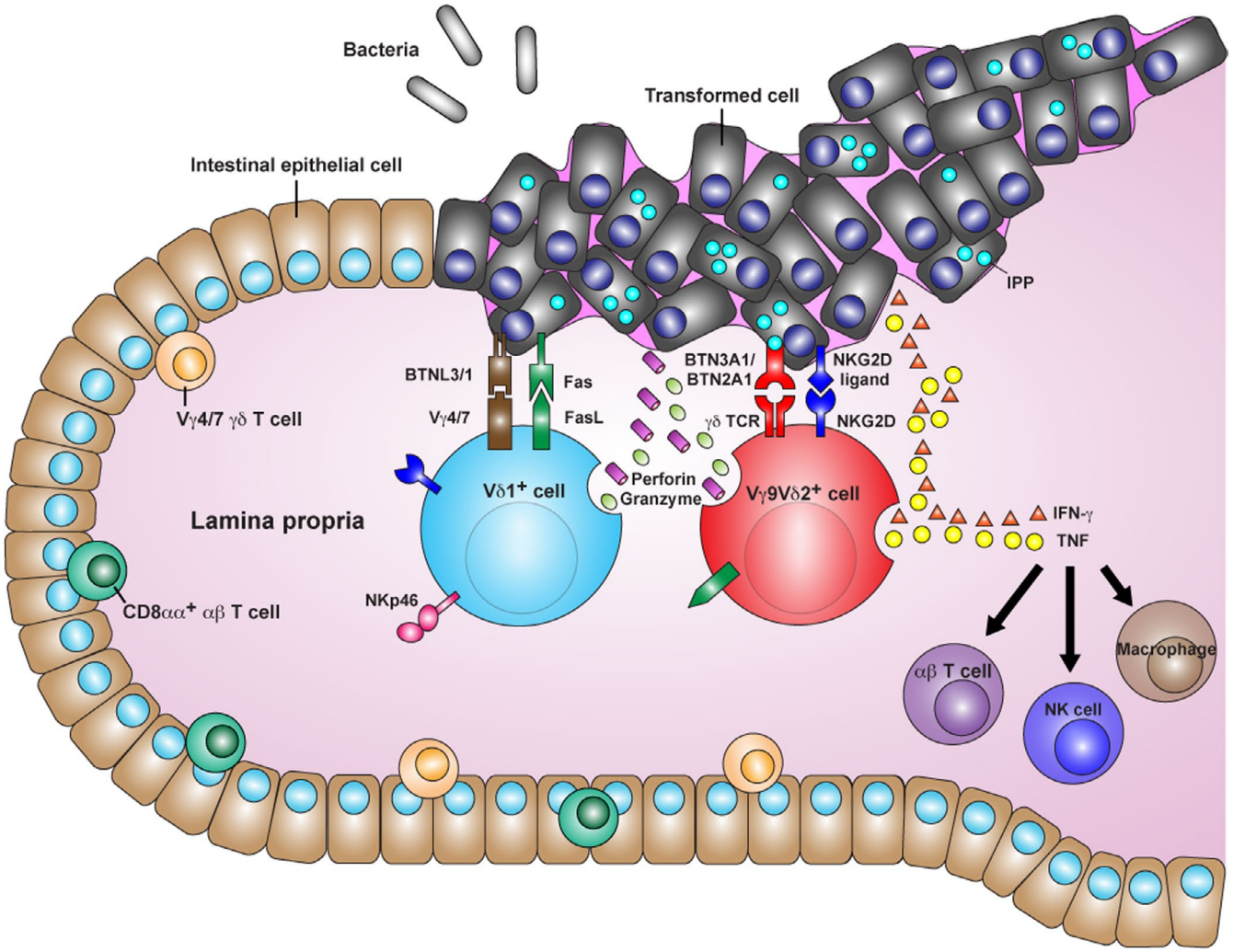

FIGURE 2 Anti-tumorigenic functions of γδ T cells in colorectal cancer. In humans, two major subsets of γδ T cells can recognize and kill cancer cells: One is the gut-resident Vδ1 cell subset and the other is the Vγ9Vδ2 cell subset that enters the gut from the circulation. Both subsets express cytotoxic molecules, such as granzyme, perforin, FasL, IFNγ, and TNF. During immunosurveillance, γδ T cells may sense abnormalities through the NKG2D receptor by stress ligands expressed on cancer cells. Vδ1 cells that express the Vγ4 chain (or Vγ7 chain in mice) and NKp46 may bind cancer cells through BTNL3 (or BTNL1 in mice). By contrast, Vγ9Vδ2 cells recognize cancer cells through BTN3A1/BTN2A1 heterodimers, which bind to the γδ T cell receptor (TCR) after activation by the IPP metabolite, a product of the mevalonate pathway

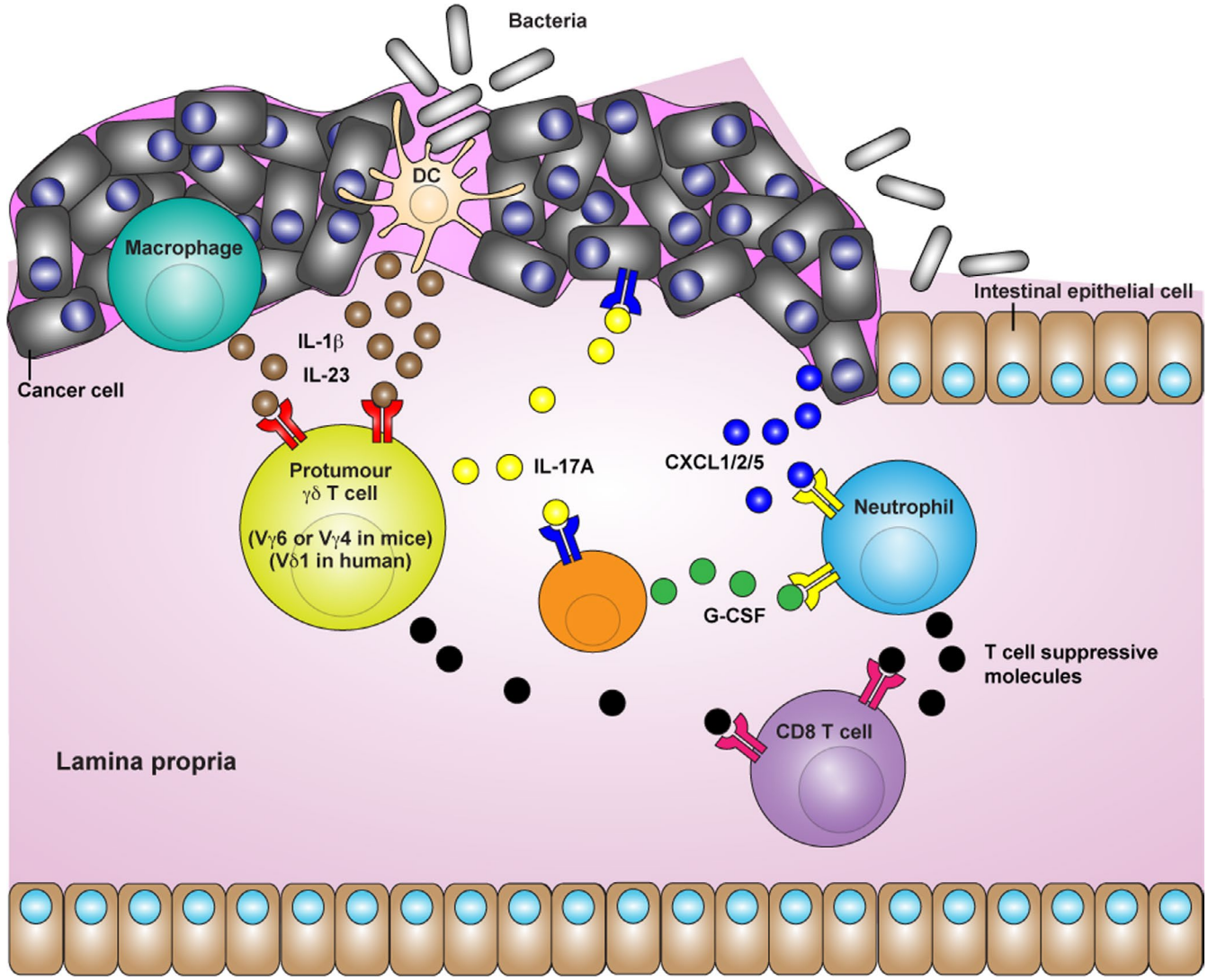

FIGURE 3 Pro-tumorigenic functions of γδ T cells in colorectal cancer. Breakdown of the epithelial barrier by the disorganization of cancer cells allows bacteria to penetrate gut tissue. These microorganisms activate dendritic cells (DC) and macrophages to secrete the cytokines, IL-1β and IL-23, which are received by γδ T cells expressing Vγ6 or Vγ4 cells (in mice or Vδ1 cells in humans). In response to this stimulus, these γδ T cell subsets release IL-17A, and IL-17A can induce proliferation of cancer cells or induce G-CSF expression by other cells. G-CSF mediates neutrophil expansion; neutrophils are drawn into the tumor microenvironment by the chemokines, CXCL1, CXCL2, or CXCL5. Neutrophils and γδ T cells can suppress the anti-tumor activity of CD8 T cells to promote cancer progression

of *Apc*, inflammation activated through the Toll-like receptor (TLR) pathway plays a central role in tumorigenesis.[241,242] When MyD88, an adapter molecule through which TLR signaling is mediated, is deleted in these *Apc* models, tumor formation and pro-inflammatory molecules including COX-2, IL-6, IL-23, and IL-1β are reduced. Microbial products exposed to myeloid cells via breakdown of the epithelial barrier trigger these pro-inflammatory molecules and potentiate cancer progression. These events converge on the upregulation of IL-17A in CD4 T cells (ie, Th17 cells) and/or γδ T cells. The importance of IL-17A signaling in *Apc* models has been shown by deleting the *Il17a* or *Il17ra* genes, which phenocopy the decrease in tumor incidence seen in MyD88-deficient mice.[242-244] IL-17A functions directly on APC-deficient enterocytes to stimulate their proliferation through activation of MAPK signal transduction pathways.[244] Genetically deleting the cellular source of IL-17A—either αβ T cells or γδ T cells—abrogates tumor formation in $Apc^{Min/+}$ mice.[245] Taken together, these studies indicate that inflammation directed through TLR-mediated activation of IL-17–producing CD4 T cells and γδ T cells promotes CRC development. These IL-17–producing γδ T cells are likely Vγ4 or Vγ6 cells, as Vγ7 IELs are not capable of making IL-17.

Like the *Apc* models of CRC, Th17 cells and IL-17–producing γδ T cells are increased in enterotoxigenic *Bacteroides fragilis* (ETBF)–induced mouse models of CRC.[246-248] Unlike the $Apc^{Min/+}$ model, however, Th17 cells and IL-17–producing γδ T cells are redundant in this context. Inhibiting the function of Th17 cells via STAT3 depletion fails to prevent tumorigenesis, because γδ T cells also express IL-17A. To prevent tumor formation in the ETBF model, both Th17 cells and γδ T cells must be ablated.[247] This redundancy is not observed in

every mouse model of cancer. In the *K14-Cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ model of breast cancer, we found that both CD4 T cells and γδ T cells increase expression of IL-17A in response to tumor-associated macrophage-derived IL-1β; however, the depletion of Th17 cells failed to reduce pro-metastatic neutrophils.[236,249] These data indicate that IL-17–producing γδ T cells are the dominant pro-tumorigenic population in this model. γδ T cells are also dominant over CD4 T cells in the *Kras*^{G12D}*;Trp53*$^{F/F}$ lung cancer model. γδ T cells, not CD4 T cells, upregulate IL-17A to drive cancer progression, after bacterial-induced IL-23 and IL-1β expression.[250] It is unclear how CD4 T cells and γδ T cells are differentially regulated in these contexts, when both cell types can be induced to make IL-17A by the same mechanisms. On a per cell basis, γδ T cells express higher levels of IL-17A than CD4 T cells. Thus, targeting IL-17A rather than Th17 cells or IL-17–producing γδ T cells may be a more viable approach to limit CRC progression, but this approach requires the identification of shared regulators of IL-17A expression in both cell types. One strategy may be the targeting of RORγt—the master transcriptional regulator of IL-17A—via manipulating its degradation. RORγt protein expression levels are controlled by ITCH-mediated ubiquitination, and *Itch* knockout mice are more susceptible to AOM/dextran sodium sulfate (DSS)–induced tumorigenesis due to increased IL-17 production by CD4 T cells and γδ T cells.[251] Small molecule inhibitors are being developed for several autoimmune disorders, such as multiple sclerosis, psoriasis, and rheumatoid arthritis, where Th17 cells and γδ T cells drive pathology that could be repurposed for anti-cancer therapy.[252]

Whether the importance of IL-17–producing γδ T cells is conserved in human cancer is controversial. Circulating Vγ9Vδ2 cells from healthy donors do not readily produce IL-17A, although they can be enticed to secrete this cytokine in vitro when given IL-23, IL-1β, TGFβ, and/or IL-7.[34,253,254] Nevertheless, IL-17–producing γδ T cells are found in human CRC tissue, and these cells are more prevalent in tumors than in normal tissue.[222,255-257] Their frequency positively correlates with tumor stage, tumor size, invasion, lymph node metastasis, vascular and lymphatic invasion, and immunosuppressive neutrophils.[255] Interestingly, IL-17–producing γδ T cells outnumber Th17 cells in at least one cohort of CRC patients.[255] Some IL-17–producing γδ T cells may have regulatory functions that suppress anti-tumor T cells and express CD39.[257]

The lack of knowledge surrounding the role of γδ T cells in CRC is partly related to the paucity of more sophisticated mouse models that incorporate other common CRC genetic mutations, such as KRAS, p53, and TGFβ signaling. New CRC models have recently been developed that should provide further insight into the pro-tumorigenic role of γδ T cells.[210] Some of these new models fully progress to the carcinoma stage and metastasize to distant organs, so they should allow researchers to dissect γδ T cell function at different stages of disease progression. A transplantation model with organoids carrying mutations in *Apc*, *Kras*, *Tgfbr2*, and *Trp53* genes demonstrated that TGFβ signaling plays a critical role in colorectal cancer metastasis.[213] In this study, combination treatment of TGFβ inhibitor and PD-L1 inhibitor improves survival of tumor-bearing mice and reduces metastasis formation. As γδ T cells can produce TGFβ and suppress cytotoxic CD8 T cells through PD-L1 expression,[258] γδ T cells might be involved in metastasis via immunosuppression in this CRC model. We reported on the ability of IL-17–producing γδ T cells to control immunosuppressive neutrophils in breast cancer and potentiate metastasis to the lung.[236,249] Therefore, γδ T cells may also control pro-metastatic neutrophils in CRC. Recently, neutrophils were shown to promote liver metastasis in a new genetically engineered mouse model whose tumors are driven by mutations in KRAS, loss of p53, and overexpression of NOTCH1.[212] From these new models, we may also learn additional mechanisms of tumor promotion independent of IL-17 expression. γδ T cells can express galectin-1 to suppress anti-tumor T cells in a Kras/p53 sarcoma model,[259] as well as IL-22 and amphiregulin (AREG) to stimulate epithelial cells in a Kras/p53 lung cancer model.[250]

8 | THE PROGNOSTIC POTENTIAL OF γδ T CELLS IN CRC

Prognostic indicators of disease progression are of key interest specifically in CRC, as the gold standard tumor burden/nodal status/metastasis or TNM staging system is not sufficiently accurate prognostic markers for stage II and stage III patients. In addition, approximately 25% of stage II and stage III patients relapse, despite the lack of evidence for residual cancer cells or distant metastasis following surgical resection.[260,261] Moreover, TMN cannot predict response to chemotherapy. Attempts to refine or replace the TNM staging system have given rise to the Immunoscore,[262] which is based on the observation that T cells have a strong, favorable prognostic role in CRC. This originated from a study in 415 CRC patients showing that increased infiltration of CD3, CD8, or CD45RO (effector/memory) T cells at the tumor center or invasive margin, but particularly when high in both, is associated with greater disease-free survival.[263] Importantly, this analysis outperformed the TNM staging system. In follow-up studies using two independent cohorts of 602 combined patients, only 4.8% of the high Immunoscore group exhibited relapse after 5 years.[264] Immunoscore can also be applied to lung and liver metastatic lesions to predict patient outcome.[265] The Immunoscore encompasses tumor-infiltrating γδ T cells due to their expression of CD3 molecules. However, these cells represent less than 5% of the total CD3 population,[222,266] so it is unclear whether their abundance actually contributes to the overall predictive power of Immunoscore in CRC. To date, a comprehensive histological analysis of the prognostic power of γδ T cells in CRC has not been reported. Given the association of high Immunoscore with favorable outcome, one may speculate that γδ T cells will correlate with good prognosis, although increased intratumoral γδ T cells in breast and gallbladder correlate with poor prognosis.[267,268]

In a study from 2015 that is often quoted by the γδ T cell community to justify the use of anti-tumorigenic γδ T cells in cancer immunotherapy, γδ T cells were the best indicator of good prognosis, among every immune cell population in multiple tumor types.[269] To arrive at this conclusion, about 18,000 human tumors including CRC

were analyzed for their composition of immune cells using a computational method called CIBERSORT that infers individual immune cell populations from bulk transcriptomic datasets. After pooling cancer types to determine global leukocyte prognostic patterns based on overall survival, this study found that myeloid cell populations were generally unfavorably prognostic, while lymphoid populations were more positive markers of patient outcome. Interestingly, γδ T cells scored highest in the favorable prognosis group across more than 20 cancer types.[269] However, the delineation of a γδ T cell gene signature from transcriptomic data by the CIBERSORT method has been challenged,[270] because there is a significant overlap between the γδ T cell gene signature and other lymphocyte subsets. CIBERSORT relies on a series of reference gene signatures derived from peripheral blood immune cells.[271] When CIBERSORT was applied to Vγ9Vδ2 cells purified from the peripheral blood of 12 healthy donors, there was significant overlap between CD8 T cells, CD4 T cells, and NK cells.[270] Therefore, the CIBERSORT γδ T cell gene signature was refined to include 375 genes, which significantly improved specific detection of Vγ9Vδ2 cells. Further analysis showed that αβ T cell abundance in CRC correlates with TCR signaling, TLR signaling, antigen processing, cytolytic activity, and interferon response pathways, whereas Vγ9Vδ2 abundance did not correlate with any of these pathways. Regardless, tumors with high infiltration of Vγ9Vδ2 cells or αβ T cells associated with good outcome of CRC patients.[270] This observation was validated in a separate cohort of CRC patients using the same methodology.[222] It would be interesting to learn the prognostic value of $V\delta2^-$ cells, particularly the gut-resident Vδ1 subset, and the IL-17–producing subsets in those patient cohorts. Independent of γδ T cells, *IL17A* expression is associated with poor survival in CRC,[222,272] but IL-17–producing γδ T cells are also associated with poor outcome in CRC.[255,257]

The simple presence of γδ T cells in CRC is not what dictates their contribution to tumorigenesis of course, but rather their functional capabilities, such as cytolytic activity. A quantitative measure of immune cell cytolytic activity (CYT) has been developed using mRNA expression levels of two genes, granzyme A and perforin.[273] In TCGA datasets, CYT is higher in normal colorectal tissue than colorectal tumor tissue with MSI expectantly exhibiting the highest CYT among all CRC subtypes.[273-275] The CYT score is a favorable prognostic factor for both overall and disease-free survival. Interestingly, γδ T cells are more abundant in the CYT high colorectal tumors together with CD4 T cells, NK cells, and anti-tumorigenic macrophages, when compared to CYT low colorectal tumors.[274,275] These γδ T cells are presumably Vγ9Vδ2 cells, since the original CIBERSORT method was used to identify them. Their association with CYT is perhaps not surprising given that Vγ9Vδ2 cells are enriched in MSI tumors such as αβ T cells.[222,276] Whether γδ T cells contribute to CYT score by directly producing granzyme A and perforin in CRC is unclear.

One area of CRC-specific oncology that needs further development is the ability of γδ T cell subsets to predict response to T cell checkpoint inhibitor immunotherapy, such as anti-CTLA4 and anti-PD-1. This is because γδ T cell subsets are biomarkers of immunotherapy response in other cancer types. In patients with melanoma that received ipilimumab (anti-CTLA4), the discernment between circulating levels of Vδ1 and Vδ2 cells is imperative, as the frequency of γδ T cells in peripheral blood of cancer patients versus healthy donors is similar. However, when melanoma patients have low levels of Vδ1 cells or high levels of Vδ2 cells, overall survival—assessed from the first dose of ipilimumab—is positive.[277] Another consideration for immunotherapy is whether γδ T cells either play a role in the immunotherapy-induced inflammatory side effects, such as colitis, or could serve as biomarkers for inflammation. A recent investigation into the mechanisms responsible for immunotherapy-driven colitis in melanoma patients showed that gut-resident γδ IELs are reduced in colitis patients when compared to patients on immunotherapy without colitis or healthy tissue from patients undergoing colonoscopies. By contrast, infiltrating γδ T cells were unaffected in immunotherapy-induced colitis.[278]

9 | THE THERAPEUTIC POTENTIAL OF γδ T CELLS

γδ T cells are of increasing interest in cancer immunotherapy due to their potent cytotoxicity and ability to recognize MHC-unrestricted antigens. For solid tumors, case studies, and phase I trials in renal cell carcinoma, metastatic breast cancer and lung cancer suggest that Vγ9Vδ2 cell immunotherapy can significantly impact cancer progression in patients.[279-282] This type of immunotherapy is also well-tolerated.[283,284] Therefore, considerable effort has been focused on preconditioning γδ T cells ex vivo to bolster their anti-tumorigenic functions before adoptive cell transfer into cancer patients. In many studies, IL-2 and zoledronate are used to expand Vγ9Vδ2 cells in vitro; however, zoledronate must be slowly diluted during expansion because continuous exposure is toxic.[280,281,285,286] Autologous Vγ9Vδ2 cells expanded in vitro with zoledronate have been given to CRC patients following surgery to remove pulmonary metastasis. These cells maintain their effector functions as determined by IFNγ production and CD107a expression during culture conditions,[287] although the efficacy of these Vγ9Vδ2 cells in controlling tumor progression was not reported. IL-23 in combination with zoledronate and IL-2 may further encourage cytotoxic functions from Vγ9Vδ2 cells during expansion.[220,288] The impact of synthetic phosphoantigens on Vγ9Vδ2 cells has also been tested in patients with solid tumors, including CRC. Bromohydrin pyrophosphate (BrHPP, IPH 1101) is one such synthetic phosphoantigen.[289] The numbers of Vγ9Vδ2 cells extracted from cancer patients treated with BrHPP show an initial increase that was over time with subsequent BrHPP/IL-2 treatments.[290] Similarly, Vγ9Vδ2 cells from the peripheral blood of CRC patients that are expanded ex vivo with BrHPP and IL-2 acquire an effector phenotype and show strong lytic activity specifically toward tumor cells in both a TCR- and NKG2D-mediated manner.[291] Blocking B7-H3 on Vγ9Vδ2 cells increases colorectal cancer cell line killing in vitro and in vivo,[224] suggesting that interfering with B7-H3 signaling may further improve cytotoxicity by these cells. Thus, Vγ9Vδ2 cells may provide a safe and effective form of

immunotherapy for CRC patients. Given that Vδ1 cells are consistently shown to be more potent responders to CRC than Vγ9Vδ2 cells, harnessing Vδ1 cells for immunotherapy in CRC and utilizing different γδ T cell subsets may also prove beneficial in patients.

Another strategy to boost Vγ9Vδ2 cell killing capacity is to increase their ability to recognize cancer cells. Given that the human Vγ9Vδ2 subset shows cytotoxicity against CRC cell lines via IPP-stimulated activation of BTN3A1,[160,221,226,292-294] inducing IPP accumulation will position BTN3A1 and BTN2A1 in the right conformational position for Vγ9Vδ2 cell recognition. Zoledronate sensitizes CRC cells to Vγ9Vδ2 cell killing.[226,295] Zoledronate allows the accumulation of IPP by blocking farnesyl pyrophosphate synthase so that IPP is not converted into cholesterol or ubiquinones. However, in order for Vγ9Vδ2 cell immunotherapy to be effective in CRC patients, the right patient population must first be selected. There are clues from the literature that p53 status may be the key to patient selection. In CRC, breast and liver cancer cells, p53 suppresses mevalonate pathway–related enzymes, whereas p53-mutant or p53-deficient cells show elevated expression of mevalonate pathway–related enzymes.[296-298] These observations would suggest that tumors with p53 mutations accumulate IPP, making them amenable to Vγ9Vδ2 cell immunotherapy. Conversely, colorectal tumors with wildtype p53 may need zoledronate treatment to build up IPP in cancer cells and expose BTN3A1-BTN2A1 to Vγ9Vδ2 cells. The class of drugs known as statins also inhibits the mevalonate pathway at the level of hydroxyl-methylglutaryl coenzyme A reductase (HMG-CoAR), an enzyme that converts HMG-CoA into mevalonate far upstream of IPP. While statins may induce apoptosis of p53-deficient CRC cells by starving cancer cells of ubiquinones,[298] CRC cells treated with statins may actually reduce Vγ9Vδ2 cell recognition as IPP production will be prevented in these cells. p53 mutations are extremely common in CRC where 34% of proximal tumors and 45% of distal colorectal tumors contain p53 abnormalities. Future studies will hopefully shed light on various ways that genetic makeup of CRC may be exploited for γδ T cell immunotherapy.

Other strategies to exploit the killing capacity of γδ T cells include bispecific antibodies, transduction of γδTCRs into αβ T cells (named T cells engineered with defined γδTCRs or TEGs),[299,300] chimeric antigen receptors (CARs),[301,302] or specific expansion protocols for Vδ1 cells (named Delta One T [DOT] cells).[303] The development of these cell-based methods for solid tumors is still in its infancy. A bispecific nanobody capable of activating Vγ9Vδ2 T cells while inhibiting the activation of EGFR has shown efficacy in CRC cell lines.[304] This type of immunotherapy may prove extremely useful in patients with KRAS-mutant tumors resistant to the EGFR-targeted antibodies cetuximab or panitumumab.[305]

10 | FUTURE PERSPECTIVES AND CONCLUSIONS

Given their prominence in gut tissue, the various subsets of γδ T cells should receive more attention in cancer research to understand their behavior during tumorigenesis and their potential for cancer immunotherapy. Recent data have shed light on these underappreciated cells, but their function in CRC is still largely unexplored. The complex nature of γδ T cell subsets includes both pro- and anti-tumorigenic roles in cancer progression, providing new opportunities for therapeutic intervention. The seminal works on the importance of BTN/BTNL proteins in the regulation of γδ T cells and the high homology between mouse and human species open up new avenues for γδ T cell biology in the context of CRC. To gain new knowledge and discover new biology, more sophisticated CRC mouse models that capture CRC development from tumor initiation to metastasis formation should be employed. Along these lines, the existing mouse models for in vivo imaging should be applied to the cancer context to determine how γδ T cells move in and around gut tumors. Organoids or tissue explants derived from human tumors that are cocultured with human γδ T cell subsets will also be valuable. Overall, the tools currently available to manipulate γδ T cell subsets are limited, but they will be necessary to fully understand their pro- and anti-tumorigenic functions. These models may generate additional data in less-studied areas of γδ T cell biology including immunometabolism. In regard to cancer immunotherapy, we must learn which γδ T cell strategy is most efficacious and which patient population to treat with γδ T cell therapy. Together, these new methodologies and insights will uncover various ways to exploit γδ T cell biology for CRC treatment.

ACKNOWLEDGMENTS

This work was supported by funding from the Cancer Research UK Glasgow Centre (A25142 to SBC); the Wellcome Trust (208990/Z/17/Z to SBC); Marie Curie European Fellowship (GDCOLCA 800112 to TS); Naito Foundation Grant for Research Abroad (to TS); and the Medical Research Council (MR/R502327/1 to SBC & JE).

CONFLICT OF INTEREST

The authors have no conficts of interest to declare.

ORCID

Joanne Edwards https://orcid.org/0000-0002-7192-6906
Seth B. Coffelt https://orcid.org/0000-0003-2257-2862

REFERENCES

1. Di Marco Barros R, Roberts NA, Dart RJ, et al. Epithelia use butyrophilin-like molecules to shape organ-specific γδ T cell compartments. *Cell*. 2016;167(1):203-18 e17.
2. Ebert LM, Meuter S, Moser B. Homing and function of human skin γδ T cells and NK cells: relevance for tumor surveillance. *J Immunol*. 2006;176(7):4331-4336.
3. Hunter S, Willcox CR, Davey MS, et al. Human liver infiltrating γδ T cells are composed of clonally expanded circulating and tissue-resident populations. *J Hepatol*. 2018;69(3):654-665.
4. Bonneville M, Janeway CA Jr, Ito K, et al. Intestinal intraepithelial lymphocytes are a distinct set of γδ T cells. *Nature*. 1988;336(6198):479-481.
5. Silva-Santos B, Mensurado S, Coffelt SB. γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer. *Nat Rev Cancer*. 2019;19(7):392-404.

6. Prinz I, Silva-Santos B, Pennington DJ. Functional development of γδ T cells. *Eur J Immunol*. 2013;43(8):1988-1994.
7. Munoz-Ruiz M, Sumaria N, Pennington DJ, Silva-Santos B. Thymic determinants of γδ T cell differentiation. *Trends Immunol*. 2017;38(5):336-344.
8. Pellicci DG, Koay HF, Berzins SP. Thymic development of unconventional T cells: how NKT cells, MAIT cells and γδ T cells emerge. *Nature Reviews Immunology*, 2020. http://dx.doi.org/10.1038/s41577-020-0345-y.
9. Carding SR, Kyes S, Jenkinson EJ, et al. Developmentally regulated fetal thymic and extrathymic T-cell receptor γδ gene expression. *Genes Dev*. 1990;4(8):1304-1315.
10. Havran WL, Allison JP. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature*. 1988;335(6189):443-445.
11. Allison JP, Havran WL. The immunobiology of T cells with invariant γδ antigen receptors. *Annu Rev Immunol*. 1991;9:679-705.
12. Medvinsky A, Rybtsov S, Taoudi S. Embryonic origin of the adult hematopoietic system: advances and questions. *Development*. 2011;138(6):1017-1031.
13. Havran WL, Allison JP. Origin of Thy-1+ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature*. 1990;344(6261):68-70.
14. Ito K, Bonneville M, Takagaki Y, et al. Different γδ T-cell receptors are expressed on thymocytes at different stages of development. *Proc Natl Acad Sci USA*. 1989;86(2):631-635.
15. Hatano S, Tun X, Noguchi N, et al. Development of a new monoclonal antibody specific to mouse Vγ6 chain. *Life Science Alliance*. 2019;2(3):e201900363. http://dx.doi.org/10.26508/lsa.201900363.
16. Payer E, Elbe A, Stingl G. Circulating CD3+/T cell receptor V gamma 3+ fetal murine thymocytes home to the skin and give rise to proliferating dendritic epidermal T cells. *J Immunol*. 1991;146(8):2536-2543.
17. Nandi D, Allison JP. Phenotypic analysis and γδ-T cell receptor repertoire of murine T cells associated with the vaginal epithelium. *J Immunol*. 1991;147(6):1773-1778.
18. Itohara S, Farr AG, Lafaille JJ, et al. Homing of a γδ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature*. 1990;343(6260):754-757.
19. Heyborne K, Fu YX, Kalataradi H, et al. Evidence that murine V gamma 5 and V gamma 6 γδ-TCR+ lymphocytes are derived from a common distinct lineage. *J Immunol*. 1993;151(9):4523-4527.
20. Carding SR, Egan PJ. γδ T cells: functional plasticity and heterogeneity. *Nat Rev Immunol*. 2002;2(5):336-345.
21. Monin L, Ushakov DS, Arnesen H, et al. γδ T cells compose a developmentally regulated intrauterine population and protect against vaginal candidiasis. *Mucosal Immunology*. 2020. http://dx.doi.org/10.1038/s41385-020-0305-7.
22. Tan L, Sandrock I, Odak I, et al. Single-cell transcriptomics identifies the adaptation of Scart1(+) Vgamma6(+) T cells to skin residency as activated effector cells. *Cell Rep*. 2019;27(12):3657-71 e4.
23. Gentek R, Ghigo C, Hoeffel G, et al. Epidermal γδ T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. *J Exp Med*. 2018;215(12):2994-3005.
24. Sandrock I, Reinhardt A, Ravens S, et al. Genetic models reveal origin, persistence and non-redundant functions of IL-17-producing γδ T cells. *J Exp Med*. 2018;215(12):3006-3018.
25. Muzaki A, Soncin I, Setiagani YA, et al. Long-lived innate IL-17-producing γ/δ T cells modulate antimicrobial epithelial host defense in the colon. *J Immunol*. 2017;199(10):3691-3699.
26. Haas JD, Ravens S, Duber S, et al. Development of interleukin-17-producing γδ T cells is restricted to a functional embryonic wave. *Immunity*. 2012;37(1):48-59.
27. Gerber DJ, Azuara V, Levraud JP, Huang SY, Lembezat MP, Pereira P. IL-4-producing γδ T cells that express a very restricted TCR repertoire are preferentially localized in liver and spleen. *J Immunol*. 1999;163(6):3076-3082.
28. Narayan K, Sylvia KE, Malhotra N, et al. Intrathymic programming of effector fates in three molecularly distinct γδ T cell subtypes. *Nat Immunol*. 2012;13(5):511-518.
29. Sagar, Pokrovskii M, Herman JS, et al. Deciphering the regulatory landscape of fetal and adult γδ T-cell development at single-cell resolution. *EMBO J*. 2020;39(13):e104159.
30. Sumaria N, Grandjean CL, Silva-Santos B, Pennington DJ. Strong TCRγδ signaling prohibits thymic development of IL-17A-secreting γδ T cells. *Cell Rep*. 2017;19(12):2469-2476.
31. Ribot JC, deBarros A, Pang DJ, et al. CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing γδ T cell subsets. *Nat Immunol*. 2009;10(4):427-436.
32. Powolny-Budnicka I, Riemann M, Tanzer S, Schmid RM, Hehlgans T, Weih F. RelA and RelB transcription factors in distinct thymocyte populations control lymphotoxin-dependent interleukin-17 production in γδ T cells. *Immunity*. 2011;34(3):364-374.
33. He YW, Malek TR. Interleukin-7 receptor alpha is essential for the development of γδ + T cells, but not natural killer cells. *J Exp Med*. 1996;184(1):289-293.
34. Michel ML, Pang DJ, Haque SF, Potocnik AJ, Pennington DJ, Hayday AC. Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing γδ cells. *Proc Natl Acad Sci USA*. 2012;109(43):17549-17554.
35. Baccala R, Witherden D, Gonzalez-Quintial R, et al. γδ T cell homeostasis is controlled by IL-7 and IL-15 together with subset-specific factors. *J Immunol*. 2005;174(8):4606-4612.
36. Nakamura M, Shibata K, Hatano S, et al. A genome-wide analysis identifies a notch-RBP-Jkappa-IL-7Ralpha axis that controls IL-17-producing γδ T cell homeostasis in mice. *J Immunol*. 2015;194(1):243-251.
37. Haas JD, Gonzalez FH, Schmitz S, et al. CCR6 and NK1.1 distinguish between IL-17A and IFN-gamma-producing γδ effector T cells. *Eur J Immunol*. 2009;39(12):3488-3497.
38. Kadekar D, Agerholm R, Rizk J, et al. The neonatal microenvironment programs innate γδ T cells through the transcription factor STAT5. *J Clin Invest*. 2020;130(5):2496-2508.
39. Zuberbuehler MK, Parker ME, Wheaton JD, et al. The transcription factor c-Maf is essential for the commitment of IL-17-producing γδ T cells. *Nat Immunol*. 2019;20(1):73-85.
40. Shibata K, Yamada H, Sato T, et al. Notch-Hes1 pathway is required for the development of IL-17-producing γδ T cells. *Blood*. 2011;118(3):586-593.
41. Gray EE, Ramirez-Valle F, Xu Y, et al. Deficiency in IL-17-committed Vgamma4(+) γδ T cells in a spontaneous Sox13-mutant CD45.1(+) congenic mouse substrain provides protection from dermatitis. *Nat Immunol*. 2013;14(6):584-592.
42. Lu Y, Cao X, Zhang X, Kovalovsky D. PLZF controls the development of fetal-derived IL-17+Vgamma6+ γδ T cells. *J Immunol*. 2015;195(9):4273-4281.
43. Buus TB, Odum N, Geisler C, Lauritsen JPH. Three distinct developmental pathways for adaptive and two IFN-gamma-producing γδ T subsets in adult thymus. *Nat Commun*. 2017;8(1):1911.
44. Krangel MS, Yssel H, Brocklehurst C, Spits H. A distinct wave of human T cell receptor γ/δ lymphocytes in the early fetal thymus: evidence for controlled gene rearrangement and cytokine production. *J Exp Med*. 1990;172(3):847-859.
45. Casorati G, De Libero G, Lanzavecchia A, Migone N. Molecular analysis of human γ/δ+ clones from thymus and peripheral blood. *J Exp Med*. 1989;170(5):1521-1535.
46. Tieppo P, Papadopoulou M, Gatti D, et al. The human fetal thymus generates invariant effector γδ T cells. *The Journal of*

Experimental Medicine. 2020;217(3). http://dx.doi.org/10.1084/jem.20190580.
47. Ribot JC, Ribeiro ST, Correia DV, Sousa AE, 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*. 2014;192(5):2237-2243.
48. Dimova T, Brouwer M, Gosselin F, et al. Effector Vgamma9Vdelta2 T cells dominate the human fetal γδ T-cell repertoire. *Proc Natl Acad Sci USA*. 2015;112(6):E556-E565.
49. Davey MS, Willcox CR, Joyce SP, et al. Clonal selection in the human Vdelta1 T cell repertoire indicates γδ TCR-dependent adaptive immune surveillance. *Nat Commun*. 2017;8:14760.
50. Kallemeijn MJ, Kavelaars FG, van der Klift MY, et al. Next-generation sequencing analysis of the human TCRγδ+ T-cell repertoire reveals shifts in Vgamma- and Vdelta-usage in memory populations upon aging. *Front Immunol*. 2018;9:448.
51. Di Lorenzo B, Ravens S, Silva-Santos B. High-throughput analysis of the human thymic Vdelta1(+) T cell receptor repertoire. *Sci Data*. 2019;6(1):115.
52. Papadopoulou M, Tieppo P, McGovern N, et al. TCR sequencing reveals the distinct development of fetal and adult human Vgamma9Vdelta2 T cells. *J Immunol*. 2019;203(6):1468-1479.
53. Parker CM, Groh V, Band H, et al. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J Exp Med*. 1990;171(5):1597-1612.
54. Morita CT, Parker CM, Brenner MB, Band H. TCR usage and functional capabilities of human γδ T cells at birth. *J Immunol*. 1994;153(9):3979-3988.
55. McVay LD, Carding SR, Bottomly K, Hayday AC. Regulated expression and structure of T cell receptor γ/δ transcripts in human thymic ontogeny. *EMBO J*. 1991;10(1):83-91.
56. Bos JD, Teunissen MB, Cairo I, et al. T-cell receptor γδ bearing cells in normal human skin. *J Invest Dermatol*. 1990;94(1):37-42.
57. Deusch K, Luling F, Reich K, Classen M, Wagner H, Pfeffer K. A major fraction of human intraepithelial lymphocytes simultaneously expresses the γ/δ T cell receptor, the CD8 accessory molecule and preferentially uses the V delta 1 gene segment. *Eur J Immunol*. 1991;21(4):1053-1059.
58. Li F, Hao X, Chen Y, et al. The microbiota maintain homeostasis of liver-resident γδT-17 cells in a lipid antigen/CD1d-dependent manner. *Nat Commun*. 2017;7:13839.
59. Goodall KJ, Nguyen A, Matsumoto A, et al. Multiple receptors converge on H2–Q10 to regulate NK and γδT-cell development. *Immunol Cell Biol*. 2019;97(3):326-339.
60. Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. *Development*. 2011;138(18):3865-3878.
61. Lin T, Matsuzaki G, Kenai H, Nakamura T, Nomoto K. Thymus influences the development of extrathymically derived intestinal intraepithelial lymphocytes. *Eur J Immunol*. 1993;23(8):1968-1974.
62. Locke NR, Stankovic S, Funda DP, Harrison LC. TCR γδ intraepithelial lymphocytes are required for self-tolerance. *J Immunol*. 2006;176(11):6553-6559.
63. Lambolez F, Arcangeli ML, Joret AM, et al. The thymus exports long-lived fully committed T cell precursors that can colonize primary lymphoid organs. *Nat Immunol*. 2006;7(1):76-82.
64. Chennupati V, Worbs T, Liu X, et al. Intra- and intercompartmental movement of γδ T cells: intestinal intraepithelial and peripheral γδ T cells represent exclusive nonoverlapping populations with distinct migration characteristics. *J Immunol*. 2010;185(9):5160-5168.
65. Zhao H, Nguyen H, Kang J. Interleukin 15 controls the generation of the restricted T cell receptor repertoire of γδ intestinal intraepithelial lymphocytes. *Nat Immunol*. 2005;6(12):1263-1271.
66. Bandeira A, Itohara S, Bonneville M, et al. Extrathymic origin of intestinal intraepithelial lymphocytes bearing T-cell antigen receptor γδ. *Proc Natl Acad Sci USA*. 1991;88(1):43-47.
67. Nonaka S, Naito T, Chen H, et al. Intestinal γδ T cells develop in mice lacking thymus, all lymph nodes, Peyer's patches, and isolated lymphoid follicles. *J Immunol*. 2005;174(4):1906-1912.
68. Guy-Grand D, Cerf-Bensussan N, Malissen B, Malassis-Seris M, Briottet C, Vassalli P. Two gut intraepithelial CD8+ lymphocyte populations with different T cell receptors: a role for the gut epithelium in T cell differentiation. *J Exp Med*. 1991;173(2):471-481.
69. Suzuki S, Sugahara S, Shimizu T, et al. Low level of mixing of partner cells seen in extrathymic T cells in the liver and intestine of parabiotic mice: its biological implication. *Eur J Immunol*. 1998;28(11):3719-3729.
70. Sugahara S, Shimizu T, Yoshida Y, et al. Extrathymic derivation of gut lymphocytes in parabiotic mice. *Immunology*. 1999;96(1):57-65.
71. Fusco A, Panico L, Gorrese M, et al. Molecular evidence for a thymus-independent partial T cell development in a FOXN1-/- athymic human fetus. *PLoS One*. 2013;8(12):e81786.
72. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007;449(7165):1003-1007.
73. Sasaki N, Sachs N, Wiebrands K, et al. Reg4+ deep crypt secretory cells function as epithelial niche for Lgr5+ stem cells in colon. *Proc Natl Acad Sci USA*. 2016;113(37):E5399-E5407.
74. Rothenberg ME, Nusse Y, Kalisky T, et al. Identification of a cKit(+) colonic crypt base secretory cell that supports Lgr5(+) stem cells in mice. *Gastroenterology*. 2012;142(5):1195-205.e6.
75. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2016;14(1):20-32.
76. Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol*. 1996;4(11):430-435.
77. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med*. 2019;25(3):377-388.
78. Edelblum KL, Shen L, Weber CR, et al. Dynamic migration of γδ intraepithelial lymphocytes requires occludin. *Proc Natl Acad Sci USA*. 2012;109(18):7097-7102.
79. Regnault A, Cumano A, Vassalli P, Guy-Grand D, Kourilsky P. Oligoclonal repertoire of the CD8 alpha alpha and the CD8 alpha beta TCR-alpha/beta murine intestinal intraepithelial T lymphocytes: evidence for the random emergence of T cells. *J Exp Med*. 1994;180(4):1345-1358.
80. Chowers Y, Holtmeier W, Harwood J, Morzycka-Wroblewska E, Kagnoff MF. The V delta 1 T cell receptor repertoire in human small intestine and colon. *J Exp Med*. 1994;180(1):183-190.
81. Takagaki Y, DeCloux A, Bonneville M, Tonegawa S. Diversity of γδ T-cell receptors on murine intestinal intra-epithelial lymphocytes. *Nature*. 1989;339(6227):712-714.
82. Asarnow DM, Goodman T, LeFrancois L, Allison JP. Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. *Nature*. 1989;341(6237):60-62.
83. Hershberg R, Eghtesady P, Sydora B, et al. Expression of the thymus leukemia antigen in mouse intestinal epithelium. *Proc Natl Acad Sci USA*. 1990;87(24):9727-9731.
84. Leishman AJ, Naidenko OV, Attinger A, et al. T cell responses modulated through interaction between CD8alphaalpha and the nonclassical MHC class I molecule, TL. *Science*. 2001;294(5548):1936-1939.
85. Olivares-Villagomez D, Van Kaer L. Intestinal intraepithelial lymphocytes: sentinels of the mucosal barrier. *Trends Immunol*. 2018;39(4):264-275.
86. Lundqvist C, Baranov V, Hammarstrom S, Athlin L, Hammarstrom ML. Intra-epithelial lymphocytes. Evidence for regional specialization and extrathymic T cell maturation in the human gut epithelium. *Int Immunol*. 1995;7(9):1473-1487.
87. Kilshaw PJ, Baker KC. A unique surface antigen on intraepithelial lymphocytes in the mouse. *Immunol Lett*. 1988;18(2):149-154.

88. Russell GJ, Parker CM, Cepek KL, et al. Distinct structural and functional epitopes of the alpha E beta 7 integrin. *Eur J Immunol*. 1994;24(11):2832-2841.
89. Wagner N, Lohler J, Kunkel EJ, et al. Critical role for beta7 integrins in formation of the gut-associated lymphoid tissue. *Nature*. 1996;382(6589):366-370.
90. Schon MP, Arya A, Murphy EA, et al. Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice. *J Immunol*. 1999;162(11):6641-6649.
91. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol*. 2011;11(7):445-456.
92. Grueter B, Petter M, Egawa T, et al. Runx3 regulates integrin alpha E/CD103 and CD4 expression during development of CD4-/CD8+ T cells. *J Immunol*. 2005;175(3):1694-1705.
93. El-Asady R, Yuan R, Liu K, et al. TGF-{beta}-dependent CD103 expression by CD8(+) T cells promotes selective destruction of the host intestinal epithelium during graft-versus-host disease. *J Exp Med*. 2005;201(10):1647-1657.
94. Ericsson A, Arya A, Agace W. CCL25 enhances CD103-mediated lymphocyte adhesion to E-cadherin. *Ann N Y Acad Sci*. 2004;1029:334-336.
95. Ericsson A, Svensson M, Arya A, Agace WW. CCL25/CCR9 promotes the induction and function of CD103 on intestinal intraepithelial lymphocytes. *Eur J Immunol*. 2004;34(10):2720-2729.
96. Wurbel MA, Philippe JM, Nguyen C, et al. The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur J Immunol*. 2000;30(1):262-271.
97. Zabel BA, Agace WW, Campbell JJ, et al. Human G protein-coupled receptor GPR-9-6/CC chemokine receptor 9 is selectively expressed on intestinal homing T lymphocytes, mucosal lymphocytes, and thymocytes and is required for thymus-expressed chemokine-mediated chemotaxis. *J Exp Med*. 1999;190(9):1241-1256.
98. Wurbel MA, Malissen M, Guy-Grand D, Malissen B, Campbell JJ. Impaired accumulation of antigen-specific CD8 lymphocytes in chemokine CCL25-deficient intestinal epithelium and lamina propria. *J Immunol*. 2007;178(12):7598-7606.
99. Wurbel MA, Malissen M, Guy-Grand D, et al. Mice lacking the CCR9 CC-chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in T-cell receptor γδ(+) gut intraepithelial lymphocytes. *Blood*. 2001;98(9):2626-2632.
100. Yu S, Bruce D, Froicu M, Weaver V, Cantorna MT. Failure of T cell homing, reduced CD4/CD8alphaalpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. *Proc Natl Acad Sci USA*. 2008;105(52):20834-20839.
101. Ma LJ, Acero LF, Zal T, Schluns KS. Trans-presentation of IL-15 by intestinal epithelial cells drives development of CD8alphaalpha IELs. *J Immunol*. 2009;183(2):1044-1054.
102. Suzuki H, Duncan GS, Takimoto H, Mak TW. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor beta chain. *J Exp Med*. 1997;185(3):499-505.
103. Lodolce JP, Boone DL, Chai S, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*. 1998;9(5):669-676.
104. Hu MD, Ethridge AD, Lipstein R, et al. Epithelial IL-15 is a critical regulator of γδ intraepithelial lymphocyte motility within the intestinal mucosa. *J Immunol*. 2018;201(2):747-756.
105. Zhu Y, Cui G, Miyauchi E, et al. Intestinal epithelial cell-derived IL-15 determines local maintenance and maturation of intra-epithelial lymphocytes in the intestine. *Int Immunol*. 2020;32(5):307-319.
106. Inagaki-Ohara K, Nishimura H, Mitani A, Yoshikai Y. Interleukin-15 preferentially promotes the growth of intestinal intraepithelial lymphocytes bearing γδ T cell receptor in mice. *Eur J Immunol*. 1997;27(11):2885-2891.
107. Wang X, Sumida H, Cyster JG. GPR18 is required for a normal CD8alphaalpha intestinal intraepithelial lymphocyte compartment. *J Exp Med*. 2014;211(12):2351-2359.
108. Becker AM, Callahan DJ, Richner JM, et al. GPR18 controls reconstitution of mouse small intestine intraepithelial lymphocytes following bone marrow transplantation. *PLoS One*. 2015;10(7):e0133854.
109. Sumida H, Lu E, Chen H, Yang Q, Mackie K, Cyster JG. GPR55 regulates intraepithelial lymphocyte migration dynamics and susceptibility to intestinal damage. *Sci Immunol*. 2017;2(18):eaao1135.
110. Shires J, Theodoridis E, Hayday AC. Biological insights into TCRγδ+ and TCRalphabeta+ intraepithelial lymphocytes provided by serial analysis of gene expression (SAGE). *Immunity*. 2001;15(3):419-434.
111. Fahrer AM, Konigshofer Y, Kerr EM, et al. Attributes of γδ intraepithelial lymphocytes as suggested by their transcriptional profile. *Proc Natl Acad Sci USA*. 2001;98(18):10261-10266.
112. Meehan TF, Witherden DA, Kim CH, et al. Protection against colitis by CD100-dependent modulation of intraepithelial γδ T lymphocyte function. *Mucosal Immunol*. 2014;7(1):134-142.
113. Cibrian D, Sanchez-Madrid F. CD69: from activation marker to metabolic gatekeeper. *Eur J Immunol*. 2017;47(6):946-953.
114. Mikulak J, Oriolo F, Bruni E, et al. NKp46-expressing human gut-resident intraepithelial Vδ1 T cell subpopulation exhibits high antitumor activity against colorectal cancer. *JCI insight*. 2019;4(24):e125884.
115. Hu MD, Jia L, Edelblum KL. Policing the intestinal epithelial barrier: Innate immune functions of intraepithelial lymphocytes. *Curr Pathobiol Rep*. 2018;6(1):35-46.
116. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol*. 2017;17(12):733-745.
117. Dalton JE, Cruickshank SM, Egan CE, et al. Intraepithelial γδ+ lymphocytes maintain the integrity of intestinal epithelial tight junctions in response to infection. *Gastroenterology*. 2006;131(3):818-829.
118. Ismail AS, Severson KM, Vaishnava S, et al. γδ intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc Natl Acad Sci USA*. 2011;108(21):8743-8748.
119. Inagaki-Ohara K, Chinen T, Matsuzaki G, et al. Mucosal T cells bearing TCRγδ play a protective role in intestinal inflammation. *J Immunol*. 2004;173(2):1390-1398.
120. Catalan-Serra I, Sandvik AK, Bruland T, Andreu-Ballester JC. γδ T cells in Crohn's disease: a new player in the disease pathogenesis? *J Crohns Colitis*. 2017;11(9):1135-1145.
121. Liu Z, Geboes K, Colpaert S, D'Haens GR, Rutgeerts P, Ceuppens JL. IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production. *J Immunol*. 2000;164(7):3608-3615.
122. Maiuri L, Ciacci C, Vacca L, et al. IL-15 drives the specific migration of CD94+ and TCR-γδ+ intraepithelial lymphocytes in organ cultures of treated celiac patients. *Am J Gastroenterol*. 2001;96(1):150-156.
123. Maiuri L, Ciacci C, Ricciardelli I, et al. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet*. 2003;362(9377):30-37.
124. Meresse B, Chen Z, Ciszewski C, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity*. 2004;21(3):357-366.
125. McVay LD, Li B, Biancaniello R, et al. Changes in human mucosal γδ T cell repertoire and function associated with the disease process in inflammatory bowel disease. *Mol Med*. 1997;3(3):183-203.

126. McCarthy NE, Eberl M. Human γδ T-cell control of mucosal immunity and inflammation. *Front Immunol*. 2018;9:985.
127. Mielke LA, Jones SA, Raverdeau M, et al. Retinoic acid expression associates with enhanced IL-22 production by γδ T cells and innate lymphoid cells and attenuation of intestinal inflammation. *J Exp Med*. 2013;210(6):1117-1124.
128. Lee JS, Tato CM, Joyce-Shaikh B, et al. Interleukin-23-independent IL-17 production regulates intestinal epithelial permeability. *Immunity*. 2015;43(4):727-738.
129. Davey MS, Lin CY, Roberts GW, et al. Human neutrophil clearance of bacterial pathogens triggers anti-microbial γδ T cell responses in early infection. *PLoS Pathog*. 2011;7(5):e1002040.
130. Tyler CJ, McCarthy NE, Lindsay JO, Stagg AJ, Moser B, Eberl M. Antigen-presenting human γδ T cells promote intestinal CD4(+) T cell expression of IL-22 and mucosal release of calprotectin. *J Immunol*. 2017;198(9):3417-3425.
131. Lefranc MP, Giudicelli V, Duroux P, et al. IMGT(R), the international ImMunoGeneTics information system(R) 25 years on. *Nucleic Acids Res*. 2015;43(Database issue):D413-D422.
132. Li H, Lebedeva MI, Llera AS, Fields BA, Brenner MB, Mariuzza RA. Structure of the Vdelta domain of a human γδ T-cell antigen receptor. *Nature*. 1998;391(6666):502-506.
133. Xu B, Pizarro JC, Holmes MA, et al. Crystal structure of a γδ T-cell receptor specific for the human MHC class I homolog MICA. *Proc Natl Acad Sci USA*. 2011;108(6):2414-2419.
134. Allison TJ, Winter CC, Fournié JJ, Bonneville M, Garboczi DN. Structure of a human γδ T-cell antigen receptor. *Nature*. 2001;411(6839):820-824.
135. Adams EJ, Chien YH, Garcia KC. Structure of a γδ T cell receptor in complex with the nonclassical MHC T22. *Science*. 2005;308(5719):227-231.
136. Uldrich AP, Le Nours J, Pellicci DG, et al. CD1d-lipid antigen recognition by the γδ TCR. *Nat Immunol*. 2013;14(11):1137-1145.
137. Luoma AM, Castro CD, Mayassi T, et al. Crystal structure of Vdelta1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human γδ T cells. *Immunity*. 2013;39(6):1032-1042.
138. Yang Y, Li L, Yuan L, et al. A structural change in butyrophilin upon phosphoantigen binding underlies phosphoantigen-mediated Vgamma9Vdelta2 T cell activation. *Immunity*. 2019;50(4):1043-53. e5.
139. Le Nours J, Gherardin NA, Ramarathinam SH, et al. A class of γδ T cell receptors recognize the underside of the antigen-presenting molecule MR1. *Science*. 2019;366(6472):1522-1527.
140. Rock EP, Sibbald PR, Davis MM, Chien YH. CDR3 length in antigen-specific immune receptors. *J Exp Med*. 1994;179(1):323-328.
141. Hayes SM, Love PE. Distinct structure and signaling potential of the γδ TCR complex. *Immunity*. 2002;16(6):827-838.
142. Hayes SM, Love PE. Stoichiometry of the murine γδ T cell receptor. *J Exp Med*. 2006;203(1):47-52.
143. Siegers GM, Swamy M, Fernandez-Malave E, et al. Different composition of the human and the mouse γδ T cell receptor explains different phenotypes of CD3gamma and CD3delta immunodeficiencies. *J Exp Med*. 2007;204(11):2537-2544.
144. Munoz-Ruiz M, Ribot JC, Grosso AR, et al. TCR signal strength controls thymic differentiation of discrete proinflammatory γδ T cell subsets. *Nat Immunol*. 2016;17(6):721-727.
145. Willcox BE, Willcox CR. γδ TCR ligands: the quest to solve a 500-million-year-old mystery. *Nat Immunol*. 2019;20(2):121-128.
146. Willcox CR, Pitard V, Netzer S, et al. Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor. *Nat Immunol*. 2012;13(9):872-879.
147. Mantri CK, St John AL. Immune synapses between mast cells and γδ T cells limit viral infection. *J Clin Invest*. 2019;129(3):1094-1108.
148. Zeng X, Wei YL, Huang J, et al. γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response. *Immunity*. 2012;37(3):524-534.
149. Ito K, Van Kaer L, Bonneville M, Hsu S, Murphy DB, Tonegawa S. Recognition of the product of a novel MHC TL region gene (27b) by a mouse γδ T cell receptor. *Cell*. 1990;62(3):549-561.
150. Weintraub BC, Jackson MR, Hedrick SM. γδ T cells can recognize nonclassical MHC in the absence of conventional antigenic peptides. *J Immunol*. 1994;153(7):3051-3058.
151. Chien YH, Meyer C, Bonneville M. γδ T cells: first line of defense and beyond. *Annu Rev Immunol*. 2014;32:121-155.
152. Russano AM, Bassotti G, Agea E, et al. CD1-restricted recognition of exogenous and self-lipid antigens by duodenal γδ+ T lymphocytes. *J Immunol*. 2007;178(6):3620-3626.
153. Ogongo P, Steyn AJ, Karim F, et al. Differential skewing of donor-unrestricted and γδ T cell repertoires in tuberculosis-infected human lungs. *J Clin Invest*. 2020;130(1):214-230.
154. Crowley MP, Fahrer AM, Baumgarth N, et al. A population of murine γδ T cells that recognize an inducible MHC class Ib molecule. *Science*. 2000;287(5451):314-316.
155. Bai L, Picard D, Anderson B, et al. The majority of CD1d-sulfatide-specific T cells in human blood use a semiinvariant Vdelta1 TCR. *Eur J Immunol*. 2012;42(9):2505-2510.
156. Roy S, Ly D, Castro CD, et al. Molecular analysis of lipid-reactive Vdelta1 γδ T cells identified by CD1c tetramers. *J Immunol*. 2016;196(4):1933-1942.
157. Marlin R, Pappalardo A, Kaminski H, et al. Sensing of cell stress by human γδ TCR-dependent recognition of annexin A2. *Proc Natl Acad Sci USA*. 2017;114(12):3163-3168.
158. Arnett HA, Viney JL. Immune modulation by butyrophilins. *Nat Rev Immunol*. 2014;14(8):559-569.
159. Afrache H, Gouret P, Ainouche S, Pontarotti P, Olive D. The butyrophilin (BTN) gene family: from milk fat to the regulation of the immune response. *Immunogenetics*. 2012;64(11):781-794.
160. Harly C, Guillaume Y, Nedellec S, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood*. 2012;120(11):2269-2279.
161. Karunakaran MM, Willcox CR, Salim M, et al. Butyrophilin-2A1 directly binds germline-encoded regions of the Vgamma9Vdelta2 TCR and is essential for phosphoantigen sensing. *Immunity*. 2020;52(3):487-98 e6.
162. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature*. 1995;375(6527):155-158.
163. Burk MR, Mori L, De Libero G. Human V gamma 9-V delta 2 cells are stimulated in a cross-reactive fashion by a variety of phosphorylated metabolites. *Eur J Immunol*. 1995;25(7):2052-2058.
164. Hintz M, Reichenberg A, Altincicek B, et al. Identification of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human γδ T cells in Escherichia coli. *FEBS Lett*. 2001;509(2):317-322.
165. Sandstrom A, Peigne CM, Leger A, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells. *Immunity*. 2014;40(4):490-500.
166. Nguyen K, Li J, Puthenveetil R, et al. The butyrophilin 3A1 intracellular domain undergoes a conformational change involving the juxtamembrane region. *FASEB J*. 2017;31(11):4697-4706.
167. Salim M, Knowles TJ, Baker AT, et al. BTN3A1 discriminates γδ T cell phosphoantigens from nonantigenic small molecules via a conformational sensor in its B30.2 domain. *ACS Chem Biol*. 2017;12(10):2631-2643.
168. Lewis JM, Girardi M, Roberts SJ, Barbee SD, Hayday AC, Tigelaar RE. Selection of the cutaneous intraepithelial γδ+ T cell repertoire by a thymic stromal determinant. *Nat Immunol*. 2006;7(8):843-850.

169. Boyden LM, Lewis JM, Barbee SD, et al. Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. *Nat Genet*. 2008;40(5):656-662.
170. Barbee SD, Woodward MJ, Turchinovich G, et al. Skint-1 is a highly specific, unique selecting component for epidermal T cells. *Proc Natl Acad Sci USA*. 2011;108(8):3330-3335.
171. Bas A, Swamy M, Abeler-Dorner L, et al. Butyrophilin-like 1 encodes an enterocyte protein that selectively regulates functional interactions with T lymphocytes. *Proc Natl Acad Sci USA*. 2011;108(11):4376-4381.
172. Melandri D, Zlatareva I, Chaleil RAG, et al. The γδTCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. *Nat Immunol*. 2018;19(12):1352-1365.
173. Willcox CR, Vantourout P, Salim M, et al. Butyrophilin-like 3 directly binds a human Vgamma4(+) T cell receptor using a modality distinct from clonally-restricted antigen. *Immunity*. 2019;51:813-825.e4.
174. Hayday AC. γδ T cell update: adaptate orchestrators of immune surveillance. *J Immunol*. 2019;203(2):311-320.
175. Hayday AC, Vantourout P. The innate biologies of adaptive antigen receptors. *Annu Rev Immunol*. 2020;38:487-510.
176. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424.
177. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55(2):74-108.
178. Fidler MM, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. *Int J Cancer*. 2016;139(11):2436-2446.
179. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin*. 2009;59(6):366-378.
180. Johnson CM, Wei C, Ensor JE, et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control*. 2013;24(6):1207-1222.
181. Park S-J, Rashid A, Lee J-H, Kim SG, Hamilton SR, Wu T-T. Frequent CpG island methylation in serrated adenomas of the colorectum. *Am J Pathol*. 2003;162(3):815-822.
182. Hawkins N, Norrie M, Cheong K, et al. CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability. *Gastroenterology*. 2002;122(5):1376-1387.
183. Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science*. 1997;275(5307):1787-1790.
184. Sansom OJ, Reed KR, Hayes AJ, et al. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev*. 2004;18(12):1385-1390.
185. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. *Nature*. 1987;327(6120):293-297.
186. Naguib A, Cooke JC, Happerfield L, et al. Alterations in PTEN and PIK3CA in colorectal cancers in the EPIC Norfolk study: associations with clinicopathological and dietary factors. *BMC Cancer*. 2011;11(1):123.
187. Fearon ER, Cho KR, Nigro JM, et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science*. 1990;247(4938):49-56.
188. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. *Nature*. 2013;502(7471):333-339.
189. Iino H, Simms L, Young J, et al. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. *Gut*. 2000;47(1):37-42.
190. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer — analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. 2000;343(2):78-85.
191. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet*. 1999;36(11):801.
192. Salovaara R, Loukola A, Kristo P, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol*. 2000;18(11):2193-2200.
193. Boland C, Troncale FJ. FAmilial colonic cancer without antecedent polyposis. *Ann Int Med*. 1984;100(5):700-701.
194. Lynch HT, Shaw MW, Magnuson CW, Larsen AL, Krush AJ. Hereditary factors in cancer: study of two large midwestern kindreds. *Arch Intern Med*. 1966;117(2):206-212.
195. Ligtenberg MJL, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. *Nat Genet*. 2008;41:112.
196. Fishel R, Lescoe MK, Rao MRS, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*. 1993;75(5):1027-1038.
197. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell*. 1993;75(6):1215-1225.
198. Miyaki M, Konishi M, Tanaka K, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. *Nat Genet*. 1997;17:271.
199. Akiyama Y, Sato H, Yamada T, et al. Germ-line mutation of the %3cem%3ehMSH6/GTBP%3c/em%3e gene in an atypical hereditary nonpolyposis colorectal cancer kindred. *Cancer Res*. 1997;57(18):3920.
200. Peltomäki P, Vasen H. Mutations associated with HNPCC predisposition – update of ICG-HNPCC/INSiGHT mutation database. *Dis Markers*. 2004;20(4–5):269-276.
201. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med*. 2006;354(26):2751-2763.
202. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH 1 is associated with hereditary non-polyposis colon cancer. *Nature*. 1994;368:258.
203. ten Broeke SW, van der Klift HM, Tops CMJ, et al. Cancer risks for PMS2-associated lynch syndrome. *J Clin Oncol*. 2018;36(29):2961-2968.
204. Beggs AD, Latchford AR, Vasen HFA, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut*. 2010;59(7):975.
205. Talseth-Palmer BA. The genetic basis of colonic adenomatous polyposis syndromes. *Hered Cancer Clin Pract*. 2017;15:5.
206. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21(11):1350-1356.
207. Dunne PD, Alderdice M, O'Reilly PG, et al. Cancer-cell intrinsic gene expression signatures overcome intratumoural heterogeneity bias in colorectal cancer patient classification. *Nat Commun*. 2017;8:15657.
208. Roseweir AK, Park JH, Hoorn ST, et al. Histological phenotypic subtypes predict recurrence risk and response to adjuvant chemotherapy in patients with stage III colorectal cancer. *The Journal of Pathology: Clinical Research*. 2020. http://dx.doi.org/10.1002/cjp2.171.
209. Roseweir AK, McMillan DC, Horgan PG, Edwards J. Colorectal cancer subtypes: translation to routine clinical pathology. *Cancer Treat Rev*. 2017;57:1-7.
210. Jackstadt R, Sansom OJ. Mouse models of intestinal cancer. *J Pathol*. 2016;238(2):141-151.
211. Moser AR, Luongo C, Gould KA, McNeley MK, Shoemaker AR, Dove WF. ApcMin: a mouse model for intestinal and mammary tumorigenesis. *Eur J Cancer*. 1995;31(7):1061-1064.
212. Jackstadt R, van Hooff SR, Leach JD, et al. Epithelial NOTCH signaling rewires the tumor microenvironment of colorectal cancer

to drive poor-prognosis subtypes and metastasis. *Cancer Cell*. 2019;36(3):319-36 e7.
213. Tauriello DVF, Palomo-Ponce S, Stork D, et al. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538-543.
214. Fumagalli A, Drost J, Suijkerbuijk SJ, et al. Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc Natl Acad Sci USA*. 2017;114(12):E2357-E2364.
215. Drost J, van Boxtel R, Blokzijl F, et al. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. *Science*. 2017;358(6360):234-238.
216. Girardi M, Oppenheim DE, Steele CR, et al. Regulation of cutaneous malignancy by γδ T cells. *Science*. 2001;294(5542):605.
217. Tie G, Yan J, Khair L, et al. Hypercholesterolemia increases colorectal cancer incidence by reducing production of NKT and γδ T cells from hematopoietic stem cells. *Cancer Res*. 2017;77(9):2351.
218. Matsuda S, Kudoh S, Katayama S. Enhanced formation of azoxymethane-induced colorectal adenocarcinoma in γδ T lymphocyte-deficient mice. *Jpn J Cancer Res*. 2001;92(8):880-885.
219. Pont F, Familiades J, Dejean S, et al. The gene expression profile of phosphoantigen-specific human γδ T lymphocytes is a blend of alpha-beta T-cell and NK-cell signatures. *Eur J Immunol*. 2012;42(1):228-240.
220. Wragg KM, Tan HX, Kristensen AB, et al. High CD26 and low CD94 expression identifies an IL-23 responsive Vdelta2(+) T cell subset with a MAIT cell-like transcriptional profile. *Cell Rep*. 2020;31(11):107773.
221. Corvaisier M, Moreau-Aubry A, Diez E, et al. Vγ9Vδ2 T cell response to colon carcinoma cells. *J Immunol*. 2005;175(8):5481.
222. Meraviglia S, Lo Presti E, Tosolini M, et al. Distinctive features of tumor-infiltrating γδ T lymphocytes in human colorectal cancer. *Oncoimmunology*. 2017;6(10):e1347742.
223. Bruni E, Cazzetta V, Donadon M, et al. Chemotherapy accelerates immune-senescence and functional impairments of Vdelta2(pos) T cells in elderly patients affected by liver metastatic colorectal cancer. *J Immunother Cancer*. 2019;7(1):347.
224. Lu H, Shi T, Wang M, et al. B7–H3 inhibits the IFN-gamma-dependent cytotoxicity of Vgamma9Vdelta2 T cells against colon cancer cells. *Oncoimmunology*. 2020;9(1):1748991.
225. Thompson K, Roelofs AJ, Jauhiainen M, et al. Activation of γδ T cells by bisphosphonates. *Osteoimmunology*. 2010;658:11–20.
226. Todaro M, Asaro M, Caccamo N, et al. Efficient killing of human colon cancer stem cells by γδ T lymphocytes. *J Immunol*. 2009;182(11):7287-7296.
227. Maeurer MJ, Martin D, Walter W, et al. Human intestinal Vdelta1+ lymphocytes recognize tumor cells of epithelial origin. *J Exp Med*. 1996;183(4):1681-1696.
228. Halary F, Pitard V, Dlubek D, et al. Shared reactivity of Vδ2neg γδ T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells. *J Exp Med*. 2005;201(10):1567-1578.
229. Wu D, Wu P, Wu X, et al. Ex vivo expanded human circulating Vδ1 γδT cells exhibit favorable therapeutic potential for colon cancer. *Oncoimmunology*. 2015;4(3):e992749-e.
230. Devaud C, Bilhere E, Loizon S, et al. Antitumor activity of γδ T cells reactive against cytomegalovirus-infected cells in a mouse xenograft tumor model. *Cancer Res*. 2009;69(9):3971-3978.
231. Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T. Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. *Proc Natl Acad Sci USA*. 1999;96(12):6879-6884.
232. Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. *Science*. 1998;279(5357):1737.
233. Rincon-Orozco B, Kunzmann V, Wrobel P, Kabelitz D, Steinle A, Herrmann T. Activation of Vγ9Vδ2 T cells by NKG2D. *J Immunol*. 2005;175(4):2144-2151.
234. Wu J, Groh V, Spies T. T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial γδ T cells. *J Immunol*. 2002;169(3):1236-1240.
235. Devaud C, Rousseau B, Netzer S, et al. Anti-metastatic potential of human Vδ1+ γδ T cells in an orthotopic mouse xenograft model of colon carcinoma. *Cancer Immunol Immunother*. 2013;62(7):1199-1210.
236. Coffelt SB, Kersten K, Doornebal CW, et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature*. 2015;522(7556):345-348.
237. Wakita D, Sumida K, Iwakura Y, et al. Tumor-infiltrating IL-17-producing γδ T cells support the progression of tumor by promoting angiogenesis. *Eur J Immunol*. 2010;40(7):1927-1937.
238. Rei M, Goncalves-Sousa N, Lanca T, et al. Murine CD27(-) Vgamma6(+) γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. *Proc Natl Acad Sci USA*. 2014;111(34):E3562-E3570.
239. Van Hede D, Polese B, Humblet C, et al. Human papillomavirus oncoproteins induce a reorganization of epithelial-associated γδ T cells promoting tumor formation. *Proc Natl Acad Sci USA*. 2017;114(43):E9056-E9065.
240. Ma S, Cheng Q, Cai Y, et al. IL-17A produced by γδ T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res*. 2014;74(7):1969-1982.
241. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science*. 2007;317(5834):124-127.
242. Grivennikov SI, Wang K, Mucida D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature*. 2012;491(7423):254-258.
243. Chae WJ, Gibson TF, Zelterman D, Hao L, Henegariu O, Bothwell AL. Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. *Proc Natl Acad Sci USA*. 2010;107(12):5540-5544.
244. Wang K, Kim MK, Di Caro G, et al. Interleukin-17 receptor a signaling in transformed enterocytes promotes early colorectal tumorigenesis. *Immunity*. 2014;41(6):1052-1063.
245. Marsh L, Coletta PL, Hull MA, Selby PJ, Carding SR. Altered intestinal epithelium-associated lymphocyte repertoires and function in ApcMin/+ mice. *Int J Oncol*. 2012;40(1):243-250.
246. Wu S, Rhee K-J, Albesiano E, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med*. 2009;15(9):1016-1022.
247. Housseau F, Wu S, Wick EC, et al. Redundant innate and adaptive sources of IL17 production drive colon tumorigenesis. *Cancer Res*. 2016;76(8):2115-2124.
248. Dejea CM, Fathi P, Craig JM, et al. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science*. 2018;359(6375):592-597.
249. Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*. 2019;572(7770):538-542.
250. Jin C, Lagoudas GK, Zhao C, et al. Commensal microbiota promote lung cancer development via γδ T cells. *Cell*. 2019;176(5):998-1013 e16.
251. Kathania M, Khare P, Zeng M, et al. Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-γt ubiquitination. *Nat Immunol*. 2016;17(8):997-1004.
252. Zhong C, Zhu J. Small-molecule RORgammat antagonists: one stone kills two birds. *Trends Immunol*. 2017;38(4):229-231.
253. Ness-Schwickerath KJ, Jin C, Morita CT. Cytokine requirements for the differentiation and expansion of IL-17A- and IL-22-producing human Vgamma2Vdelta2 T cells. *J Immunol*. 2010;184(12):7268-7280.
254. Caccamo N, La Mendola C, Orlando V, et al. Differentiation, phenotype, and function of interleukin-17-producing human Vgamma9Vdelta2 T cells. *Blood*. 2011;118(1):129-138.

255. Wu P, Wu D, Ni C, et al. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity*. 2014;40(5):785-800.
256. Amicarella F, Muraro MG, Hirt C, et al. Dual role of tumour-infiltrating T helper 17 cells in human colorectal cancer. *Gut*. 2017;66(4):692-704.
257. Hu G, Wu P, Cheng P, et al. Tumor-infiltrating CD39(+)γδTregs are novel immunosuppressive T cells in human colorectal cancer. *Oncoimmunology*. 2017;6(2):e1277305.
258. Daley D, Zambirinis CP, Seifert L, et al. γδ T cells support pancreatic oncogenesis by restraining alphabeta T cell activation. *Cell*. 2016;166(6):1485-99 e15.
259. Rutkowski MR, Stephen TL, Svoronos N, et al. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. *Cancer Cell*. 2015;27(1):27-40.
260. Li J, Yi C-H, Hu Y-T, et al. TNM staging of colorectal cancer should be reconsidered according to weighting of the T stage: verification based on a 25-year follow-up. *Medicine (Baltimore)*. 2016;95(6):e2711-e.
261. Nagtegaal ID, Quirke P, Schmoll HJ. Has the new TNM classification for colorectal cancer improved care? *Nat Rev Clin Oncol*. 2011;9(2):119-123.
262. Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *J Pathol*. 2014;232(2):199-209.
263. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313(5795):1960-1964.
264. Pages F, Kirilovsky A, Mlecnik B, et al. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol*. 2009;27(35):5944-5951.
265. Van den Eynde M, Mlecnik B, Bindea G, et al. The link between the multiverse of immune microenvironments in metastases and the survival of colorectal cancer patients. *Cancer Cell*. 2018;34(6):1012-26 e3.
266. Eugene J, Jouand N, Ducoin K, et al. The inhibitory receptor CD94/NKG2A on CD8(+) tumor-infiltrating lymphocytes in colorectal cancer: a promising new druggable immune checkpoint in the context of HLAE/beta2m overexpression. *Mod Pathol*. 2020;33(3):468-482.
267. Ma C, Zhang Q, Ye J, et al. Tumor-infiltrating γδ T lymphocytes predict clinical outcome in human breast cancer. *J Immunol*. 2012;189(10):5029-5036.
268. Patil RS, Shah SU, Shrikhande SV, Goel M, Dikshit RP, Chiplunkar SV. IL17 producing γδT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. *Int J Cancer*. 2016;139(4):869-881.
269. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. 2015;21(8):938-945.
270. Tosolini M, Pont F, Poupot M, et al. Assessment of tumor-infiltrating TCRVgamma9Vdelta2 γδ lymphocyte abundance by deconvolution of human cancers microarrays. *Oncoimmunology*. 2017;6(3):e1284723.
271. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12(5):453-457.
272. Tosolini M, Kirilovsky A, Mlecnik B, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. *Cancer Res*. 2011;71(4):1263.
273. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell*. 2015;160(1–2):48-61.
274. Narayanan S, Kawaguchi T, Yan L, Peng X, Qi Q, Takabe K. Cytolytic activity score to assess anticancer immunity in colorectal cancer. *Ann Surg Oncol*. 2018;25(8):2323-2331.
275. Narayanan S, Kawaguchi T, Peng X, et al. Tumor infiltrating lymphocytes and macrophages improve survival in microsatellite unstable colorectal cancer. *Sci Rep*. 2019;9(1):13455.
276. de Vries NL, van Unen V, Ijsselsteijn ME, et al. High-dimensional cytometric analysis of colorectal cancer reveals novel mediators of antitumour immunity. *Gut*. 2020;69(4):691-703.
277. Wistuba-Hamprecht K, Martens A, Haehnel 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*. 2016;64:116-126.
278. Luoma AM, Suo S, Williams HL, et al. Molecular Pathways of Colon Inflammation Induced by Cancer Immunotherapy. *Cell*. 2020. http://dx.doi.org/10.1016/j.cell.2020.06.001.
279. Kobayashi H, Tanaka Y, Shimmura H, Minato N, Tanabe K. Complete remission of lung metastasis following adoptive immunotherapy using activated autologous γδ T-cells in a patient with renal cell carcinoma. *Anticancer Res*. 2010;30(2):575-579.
280. Nicol AJ, Tokuyama H, Mattarollo SR, et al. Clinical evaluation of autologous γδ T cell-based immunotherapy for metastatic solid tumours. *Br J Cancer*. 2011;105(6):778-786.
281. Kakimi K, Matsushita H, Murakawa T, Nakajima J. γδ T cell therapy for the treatment of non-small cell lung cancer. *Transl Lung Cancer Res*. 2014;3(1):23-33.
282. Kondo M, Kondo M, Sakuta K, et al. Zoledronate facilitates large-scale ex vivo expansion of functional γδ T cells from cancer patients for use in adoptive immunotherapy. *Cytotherapy*. 2008;10(8):842-856.
283. Nakajima J, Murakawa T, Fukami T, et al. A phase I study of adoptive immunotherapy for recurrent non-small-cell lung cancer patients with autologous γδ T cells☆, ☆☆. *Eur J Cardiothorac Surg*. 2010;37(5):1191-1197.
284. Sakamoto M, Nakajima J, Murakawa T, et al. Adoptive immunotherapy for advanced non-small cell lung cancer using zoledronate-expanded γδ T cells: a phase I clinical study. *J Immunother*. 2011;34(2):202-211.
285. Wang H, Sarikonda G, Puan KJ, et al. Indirect stimulation of human Vgamma2Vdelta2 T cells through alterations in isoprenoid metabolism. *J Immunol*. 2011;187(10):5099-5113.
286. Nada MH, Wang H, Workalemahu G, Tanaka Y, Morita CT. Enhancing adoptive cancer immunotherapy with Vgamma2Vdelta2 T cells through pulse zoledronate stimulation. *J Immunother Cancer*. 2017;5:9.
287. Izumi T, Kondo M, Takahashi T, et al. Ex vivo characterization of γδ T-cell repertoire in patients after adoptive transfer of Vgamma9Vdelta2 T cells expressing the interleukin-2 receptor beta-chain and the common gamma-chain. *Cytotherapy*. 2013;15(4):481-491.
288. Zocchi MR, Costa D, Vene R, et al. Zoledronate can induce colorectal cancer microenvironment expressing BTN3A1 to stimulate effector γδ T cells with antitumor activity. *Oncoimmunology*. 2017;6(3):e1278099.
289. Espinosa E, Belmant C, Pont F, et al. Chemical synthesis and biological activity of bromohydrin pyrophosphate, a potent stimulator of human γδ T cells. *J Biol Chem*. 2001;276(21):18337-18344.
290. Bennouna J, Levy V, Sicard H, et al. Phase I study of bromohydrin pyrophosphate (BrHPP, IPH 1101), a Vγ9Vδ2 T lymphocyte agonist in patients with solid tumors. *Cancer Immunol Immunother*. 2010;59(10):1521-1530.
291. Bouet-Toussaint F, Cabillic F, Toutirais O, et al. Vγ9Vδ2 T cell-mediated recognition of human solid tumors. Potential for immunotherapy of hepatocellular and colorectal carcinomas. *Cancer Immunol Immunother*. 2008;57(4):531-539.

292. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature*. 1995;375(6527):155-158.
293. Bürk MR, Mori L, de Libero G. Human Vγ9-Vδ2 cells are stimulated in a crossreactive fashion by a variety of phosphorylated metabolites. *Eur J Immunol*. 1995;25(7):2052-2058.
294. Alexander AAZ, Maniar A, Cummings J-S, et al. Isopentenyl pyrophosphate-activated CD56+ γδ T lymphocytes display potent antitumor activity toward human squamous cell carcinoma. *Clin Cancer Res*. 2008;14(13):4232-4240.
295. Mattarollo SR, Kenna T, Nieda M, Nicol AJ. Chemotherapy and zoledronate sensitize solid tumour cells to Vγ9Vδ2 T cell cytotoxicity. *Cancer Immunol Immunother*. 2007;56(8):1285-1297.
296. Freed-Pastor WA, Mizuno H, Zhao X, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell*. 2012;148(1–2):244-258.
297. Moon SH, Huang CH, Houlihan SL, et al. p53 represses the mevalonate pathway to mediate tumor suppression. *Cell*. 2019;176(3):564-80 e19.
298. Kaymak I, Maier CR, Schmitz W, et al. Mevalonate pathway provides ubiquinone to maintain pyrimidine synthesis and survival in p53-deficient cancer cells exposed to metabolic stress. *Cancer Res*. 2020;80(2):189-203.
299. Grunder C, van Dorp S, Hol S, et al. gamma9 and delta2CDR3 domains regulate functional avidity of T cells harboring gamma9delta2TCRs. *Blood*. 2012;120(26):5153-5162.
300. Marcu-Malina V, Heijhuurs S, van Buuren M, et al. Redirecting alphabeta T cells against cancer cells by transfer of a broadly tumor-reactive γδT-cell receptor. *Blood*. 2011;118(1):50-59.
301. Mirzaei HR, Mirzaei H, Lee SY, Hadjati J, Till BG. Prospects for chimeric antigen receptor (CAR) γδ T cells: a potential game changer for adoptive T cell cancer immunotherapy. *Cancer Lett*. 2016;380(2):413-423.
302. Deniger DC, Switzer K, Mi T, et al. Bispecific T-cells expressing polyclonal repertoire of endogenous γδ T-cell receptors and introduced CD19-specific chimeric antigen receptor. *Mol Ther*. 2013;21(3):638-647.
303. Almeida AR, Correia DV, Fernandes-Platzgummer A, et al. Delta one T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept. *Clin Cancer Res*. 2016;22(23):5795-5804.
304. de Bruin RCG, Veluchamy JP, Lougheed SM, et al. A bispecific nanobody approach to leverage the potent and widely applicable tumor cytolytic capacity of Vγ9Vδ2-T cells. *Oncoimmunology*. 2017;7(1):e1375641-e.
305. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov*. 2014;4(11):1269-1280.

How to cite this article: Suzuki T, Hayman L, Kilbey A, Edwards J, Coffelt SB. Gut γδ T cells as guardians, disruptors, and instigators of cancer. *Immunol Rev*. 2020;298:198–217. https://doi.org/10.1111/imr.12916