Trends in Microbiology

CellPress OPEN ACCESS

Review

The microbiota shapes the life trajectory of mucosal-associated invariant T cells

Yan-Ruide Li [1,2,8,*], Xinyuan Shen [1,2,8], Yichen Zhu [1,2], Zibai Lyu [1,2], and Lili Yang [1,2,3,4,5,6,7,*]

Mucosal-associated invariant T (MAIT) cells are innate-like T cells predominantly located in barrier tissues such as the lung, liver, skin, and colon. These cells recognize metabolites derived from the riboflavin biosynthetic pathway, which can rapidly traverse epithelial barriers and be presented during MAIT cell differentiation in the thymus and maturation in peripheral tissues. Furthermore, microbial metabolites significantly influence MAIT cell functions in various conditions, including cancer. This review summarizes how the microbiota shapes the life trajectory of MAIT cells and their antitumor reactivity. Additionally, we discuss the therapeutic implications of manipulating the microbiota as a 'bug-drug' strategy to enhance MAIT cell antitumor immunity, particularly in mucosal cancers, while emphasizing challenges and future directions for integrating microbiota considerations into MAIT cell-based therapies.

Highlights

The microbiota significantly influences the life trajectory of mucosal-associated invariant T (MAIT) cells, affecting their development from hematopoietic stem cells, peripheral migration, and functional roles as mature cells.

The microbiota impacts the antitumor reactivity of MAIT cells, particularly regarding their capacity to target liver and colon cancers. This relationship involves a complex interplay among the microbiota, MAIT cells, and cancer cells.

The microbiota has the potential to be utilized as a 'bug-drug' to enhance the antitumor functions of MAIT cells, presenting a promising strategy for improving cancer immunotherapy.

The microbiota influences the development, function, and antitumor immunity of MAIT cells

MAIT cells represent a distinct subset of innate-like unconventional T cells that are evolutionarily conserved. Unlike invariant natural killer T (iNKT) cells, which recognize lipid antigens presented by CD1d, and gamma delta (γδ) T cells, which recognize stress-induced ligands or phosphoantigens via butyrophilin (BTN) proteins, MAIT cells are specialized in recognizing bacterial metabolism-derived antigens, positioning them as key players in antimicrobial defense at barrier sites [1]. Specifically, MAIT cells recognize derivatives of vitamin B2 precursor metabolites, which are presented by the major histocompatibility complex class Ib molecule, also known as MHC class I-related protein 1 (MR1) [2–4]. These metabolites are synthesized by a majority of bacteria and yeasts but are absent in animal cells [5–7]. MAIT cells feature a semi-invariant αβ T cell receptor (TCR), commonly characterized by an α chain composed of TRAV1-2 paired with TRAJ33, TRAJ12, or TRAJ20, and exhibit a limited array of β chains, including TRBV20 or TRBV6 in humans and TRBV19 or TRBV13 in mice [8–10]. In humans, MAIT cells are found in significant quantities within various tissues, accounting for approximately 5% in the lungs, 20–40% in the liver, 2–7% in the colon, and about 2% in the oral mucosa [8,11]. In peripheral circulation, their levels range from 1–10%. By contrast, MAIT cell populations are markedly lower in mice. During their development in the thymus, MAIT cells undergo a specific differentiation program that equips them for various effector functions. These include type-1 immunity, characterized by the secretion of interferon-gamma (IFN-γ) and robust cytotoxic activity, as well as type-17 responses involved in tissue repair, which include the production of IL-17, IL-22, and **amphiregulin** (see Glossary) [12–15]. In mice, different subsets of MAIT cells (MAIT1 and MAIT17) mediate these effector functions, while human MAIT cells display a more uniform population that can facilitate both type-1 and type-17 immune responses.

To date, two specific natural antigens derived from the **riboflavin** biosynthetic pathway have been identified as potent activators of MAIT cells via their TCRs, including

[1]Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA
[2]Department of Bioengineering, University of California, Los Angeles, Los Angeles, CA 90095, USA
[3]Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA
[4]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA 90095, USA
[5]Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
[6]Parker Institute for Cancer Immunotherapy, University of California, Los Angeles, Los Angeles, CA 90095, USA
[7]Goodman-Luskin Microbiome Center, University of California, Los Angeles, Los Angeles, CA 90095, USA
[8]These authors contributed equally.

*Correspondence:
charlie.li@ucla.edu (Y.-R. Li) and
liliyang@ucla.edu (L. Yang).

5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) [16]. Recent studies have identified cholic acid 7-sulfate (CA7S), a sulfated bile acid, as a gut microbiota-derived metabolite capable of activating MAIT cells in an MR1-dependent manner [17,18]. Upon bacterial exposure, myeloid cells process and present these microbial antigens derived from the riboflavin biosynthetic pathway via the MR1 molecule, while simultaneously producing cytokines that shape the differentiation and effector functions of MAIT cell subsets [13]. Thus, MAIT cells can be activated in a TCR-independent, innate-like manner by inflammatory cytokines such as IL-12 and IL-18 [10,19,20]. These cells play significant roles not only in the defense against bacterial infections but also in combating other conditions, including viral infections, autoimmune diseases, and cancers [19,21–24].

Microbiota-derived metabolites of vitamin B2 circulate within the body and play a crucial role in the thymic development of MAIT cells [5,25]. These microbial ligands may also be involved in maintaining epithelial homeostasis, as MAIT cells are known to facilitate tissue repair upon TCR activation [26]. Consequently, MAIT cells offer the mammalian immune system a direct mechanism to detect microbial presence and maintain mucosal barrier integrity. **Dysbiosis** significantly affects both the population and function of MAIT cells [27,28]. These cells have the ability to differentiate a range of bacteria, exhibiting a robust TCR response to those with an enhanced riboflavin synthesis pathway [29]. Moreover, MAIT cells can discriminate and categorize complex human microbiota by interpreting TCR signals based on the antigen load and the nature of presenting cells, allowing them to finely tune their functional responses [28,29].

MAIT cells are implicated in providing protective functions across a range of conditions, including autoimmune diseases, tissue repair, infections, skin wound healing, and cancers [1,8,30–33]. Owing to their high abundance and residency in human mucosal tissues, particularly in the liver and colon, MAIT cells are believed to play significant roles in liver and colon cancer. However, their function in cancer progression is controversial. This arises from the opposing protumoral effects of MAIT cells within the **tumor microenvironment** (**TME**) and their inherent cytotoxic potential, which is indicated by elevated expressions of TLRs (e.g., TLR1, TLR2, TLR6), natural killer (NK) cell activating receptors (e.g., CD161, NKG2D, DNAM-1, NKp33, NKp40), and cytotoxic molecules (e.g., Perforin and Granzyme B) [34–37]. For example, in patients with hepatocellular carcinoma (HCC), MAIT cells located within tumors have shown increased expression of inhibitory immune checkpoints (e.g., PD-1, CTLA-4, and TIM-3) and have produced lower levels of effector molecules (e.g., IFN-γ, Granzyme B, and Perforin) [38]. This immunosuppressive phenotype correlates with poor prognosis in HCC patients exhibiting high MAIT cell infiltration into solid tumors [38]. The contrasting roles of MAIT cells suggest a manipulable plasticity exploited by extrinsic tumor signaling. Notably, the microbial metabolite 5-OP-RU has been shown to significantly enhance MAIT cell cytotoxicity across various cancers, while also augmenting their production of proinflammatory cytokines, such as IFN-γ [39,40]. Therefore, leveraging microbial 'bug-drug' strategies, such as the application of 5-OP-RU, represents a promising approach to enhancing the cytotoxic efficacy of MAIT cells and overcoming their immunosuppressive properties in cancer therapy.

In this review, we explore the complex interplay between the microbiota, MAIT cells, and antitumor immunity. We highlight the role of the microbiota in shaping the life trajectory of MAIT cells, including their differentiation, maturation, and functional responses, as well as their reactivity against tumors. Additionally, we discuss prospective strategies for manipulating microbiota to augment MAIT cell-based immunotherapies for cancer, stressing the necessity of incorporating microbiota factors into cancer treatment paradigms.

Glossary

Actinobacteria: a phylum of Gram-positive bacteria known for their high G+C content in DNA, which includes many important species involved in natural antibiotic production, soil health, and the maintenance of human gut microbiota.
Amphiregulin: a growth factor belonging to the epidermal growth factor family that plays a crucial role in tissue repair, cell proliferation, and modulation of immune responses, particularly within the context of inflammation and cancer.
Bacteroidetes: a phylum of Gram-negative bacteria that play a significant role in the gut microbiota, contributing to the fermentation of complex carbohydrates and influencing host metabolism and immune function.
CpG: a synthetic oligonucleotide sequence that acts as a Toll-like receptor 9 (TLR9) agonist.
Crohn's disease: a chronic inflammatory bowel disease characterized by inflammation of the gastrointestinal tract, particularly affecting the ileum and colon, and is associated with symptoms such as abdominal pain, diarrhea, weight loss, and fatigue, often leading to complications like strictures and fistulas.
Dysbiosis: imbalance in the microbial communities within a particular environment, such as the gut, characterized by a reduction in microbial diversity and an abnormal composition that can lead to negative health outcomes and contribute to various diseases.
Firmicutes: a phylum of Gram-positive bacteria characterized by a thick cell wall, encompassing a diverse group of species that play essential roles in fermentation, digestion, and the maintenance of gut health in humans and other animals.
Immune checkpoint blockade (ICB): a form of cancer immunotherapy that inhibits immune checkpoint proteins, such as PD-1/PD-L1 and CTLA-4, thereby enhancing the immune system's ability to recognize and destroy cancer cells.
Invariant natural killer T (iNKT): a specialized subset of T cells that recognize lipid antigens presented by the CD1d molecule and play a crucial role in bridging the innate and adaptive immune responses.
Multiple sclerosis: a chronic neurological disorder characterized by

The microbiota affects the development of MAIT cells from stem cells to functionally mature cells

The development of mouse and human MAIT cells has been characterized by distinct stages, transcription factor controls, and phenotypic changes (Box 1). It has been demonstrated that the microbiota influences MAIT cell development at multiple stages, including the differentiation of hematopoietic stem cells (HSCs) within the thymus (Figure 1A) and the maturation of MAIT cells in peripheral tissues (Figure 1B).

The microbiota affects MAIT cell differentiation in the thymus

Previous studies have demonstrated that germ-free (GF) mice exhibit a significantly reduced population of mouse MAIT cells compared to specific pathogen-free (SPF) mice [41,42]. This finding suggests that the microbiota has a specific influence on the development of MAIT cells, as other T cell subsets, such as **invariant natural killer T (iNKT)** cells, do not show a similar reduction in GF mice [43]. Notably, GF mice retain a small residual population of thymic MAIT cells, whereas MR1-deficient mice completely lack MAIT cells [15]. This emphasizes the critical role of MR1 in the positive selection of MAIT cells, as their differentiation necessitates the presence of MR1-expressing $CD4^+CD8^+$ double-positive thymocytes [44].

Additionally, another murine study has shown that metabolites of vitamin B2 (riboflavin) are directly transported to the thymus, where they are presented to thymocytes, thereby promoting the intrathymic expansion of RORγt$^+$ MAIT cells [25]. This result underscores the pivotal role of commensal bacteria in maintaining barrier homeostasis by modulating the thymic production of T cells, particularly those that are targeted towards mucosal surfaces. In this investigation, adult GF mice were cohoused with SPF mice, resulting in microbial colonization. Following this colonization, MAIT cell frequencies increased initially in the thymus, followed by increases in the spleen and lungs, although these levels did not reach those seen in mice that were colonized at birth [25].

The increase in mature thymic MAIT cell numbers was primarily attributed to the expansion of MAIT17 cells, while the frequencies of MAIT1 cells remained unchanged [25]. This illustrates the necessity of commensal microbes for the complete maturation of MAIT17 cells. Intriguingly, the development of MAIT cells seems to be directly dependent on the production of the riboflavin metabolite 5-OP-RU by commensal bacteria. To control for microbial factors unrelated to 5-OP-RU synthesis, the study used genetically modified strains of *Escherichia coli* with deletions in vitamin B2 biosynthesis enzymes, specifically upstream (ΔRibD) or downstream (ΔRibE) of 5-OP-RU production [25]. Mice colonized with ΔRibD bacteria displayed persistently low MAIT cell frequencies in the thymus and lungs, whereas those colonized with ΔRibE bacteria exhibited the development of MAIT cells and their migration to the lungs [25]. This indicates that the production of 5-OP-RU by commensal bacteria is essential for the full maturation of thymic MAIT cells.

Following either topical or oral administration, 5-OP-RU rapidly translocates from mucosal surfaces (skin and stomach) to the thymus, where it engages with MR1. This interaction stimulates an increase in the population of early MAIT cell precursors, promoting the expansion of more mature RORγt$^+$ MAIT17 cells [9]. Thus, microbiota-derived metabolites, particularly 5-OP-RU, play a fundamental role in regulating the development of mucosally targeted T cells, effectively blurring the distinctions between exogenous antigens and self-antigens in the context of immune system education and functionality.

The microbiota affects MAIT cell maturation in the peripheral tissues

In addition to affecting thymic MAIT cells, GF mice display significantly reduced populations of MAIT cells in peripheral tissues, including the spleen and lungs [9]. Following microbial

the immune-mediated destruction of myelin sheaths surrounding nerve fibers in the central nervous system, leading to symptoms such as fatigue, visual disturbances, motor dysfunction, and cognitive impairment, with disease progression often resulting in varying degrees of neurological disability.

Proteobacteria: a phylum of Gram-negative bacteria that includes a wide variety of pathogens and non-pathogenic species, playing crucial roles in ecological processes and human health, particularly in the gut microbiome.

Psoriasis: a chronic autoimmune skin condition characterized by the rapid proliferation of skin cells, resulting in the formation of thick, red, scaly patches that can be itchy and painful, and may also be associated with arthritis in the form of psoriatic arthritis, affecting the joints.

Rheumatoid arthritis: a chronic autoimmune disorder characterized by inflammation of the synovial joints, leading to pain, swelling, and potential destruction of joint tissues, often accompanied by systemic symptoms such as fatigue and malaise.

Riboflavin: a crucial vitamin that serves as a precursor for the production of the metabolite 5-OP-RU, which is essential for the activation and development of MAIT cells.

Tumor microenvironment (TME): the complex environment surrounding a tumor, including immune cells, stromal cells, extracellular matrix, and signaling molecules, that influences tumor growth and response to therapy.

Box 1. MAIT cell development from HSCs

The differentiation of MAIT cells from HSCs has been elucidated in murine models [12,42,96,97] (Figure IA). MAIT cells are selected from double-positive thymocytes in the thymus that express MR1, allowing them to present commensal antigens or self-ligands to other cells. This interaction leads to positive selection and intrathymic expansion of MAIT cells, with SLAM engagement being a critical requirement for this positive selection process. Subsequently, MAIT cell precursors express PLZF and differentiate into either MAIT1 cells, which express T-bet, or MAIT17 cells, which express RORγt. An additional step involves the presentation of microbiota-derived metabolites by thymic cells, promoting the proliferation of MAIT17 cells before their exit from the thymus. The expression of distinct homing receptors in each subset directs MAIT1 cells preferentially to the spleen and liver, while MAIT17 cells migrate to peripheral tissues such as the lungs, skin, and gut lamina propria. During the development of MAIT cells, the expression profiles of CD24 and CD44 are used to define various stages of maturation.

In humans, the development of MAIT cells exhibits some notable differences (Figure IB). First, there is no divergence between MAIT1 and MAIT17 cells in humans; instead, all generated MAIT cells exhibit a more uniform phenotype combining both Th1 and Th17 characteristics, with co-expression of RORγt and T-bet [98–100]. Second, CD27 and CD161 serve as markers for defining each developmental stage, in contrast to CD24 and CD44 in mice. Third, while human MAIT cells express PLZF and display intermediate levels of both RORγt and T-bet in the thymus, they remain as CD45RA+ naïve cells in both the thymus and cord blood. Following birth, MAIT cells recognize bacterial MR1 ligand presentations by APCs, which leads to an increase in CD45RO expression, the acquisition of a memory phenotype, and subsequent proliferation in peripheral tissues.

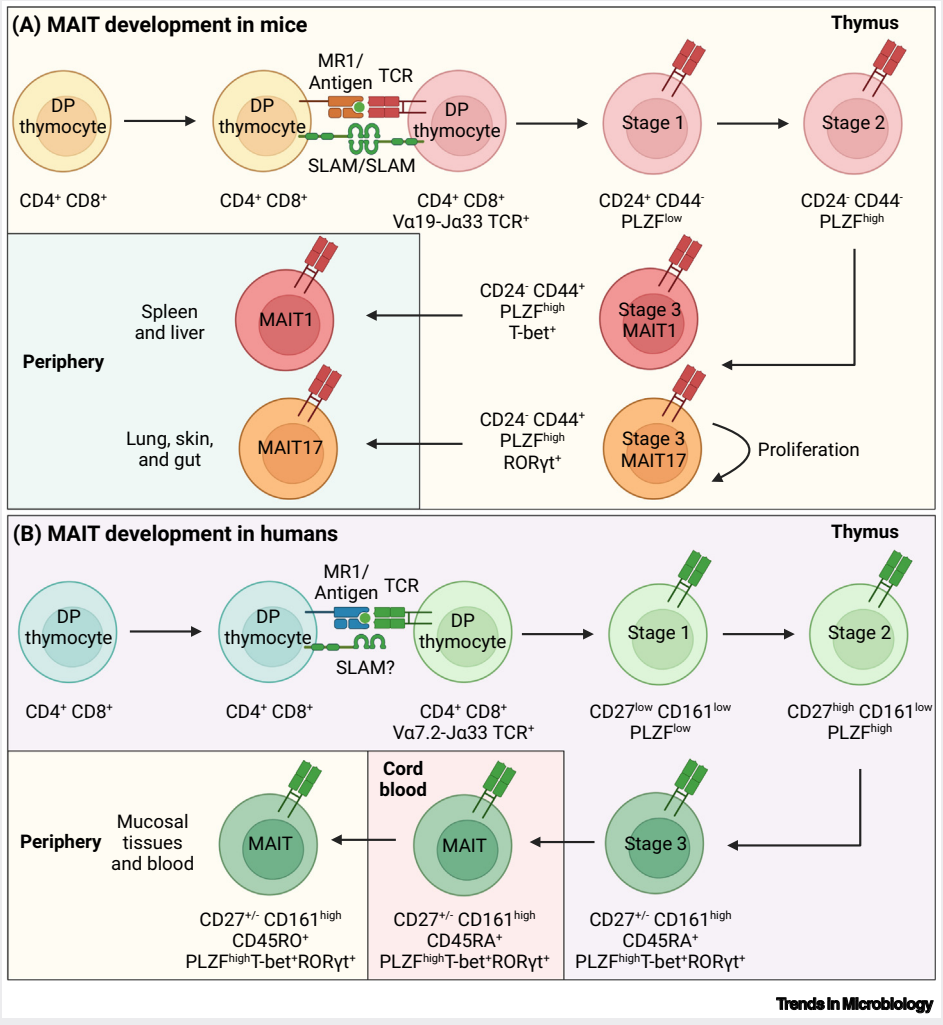

Figure I. Development of mucosal-associated invariant T (MAIT) cells in mice and humans.

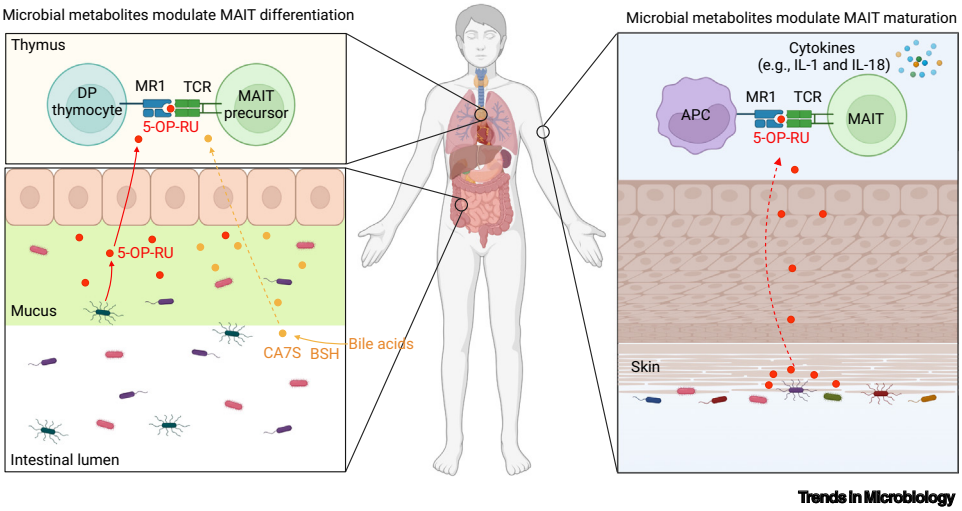

Figure 1. The microbiota influences mucosal-associated invariant T (MAIT) cell differentiation in the thymus and maturation in the peripheral tissues. During the differentiation of MAIT cells from hematopoietic stem cells (HSCs) in the thymus, microbial metabolites traverse epithelial barriers to reach the thymus, where they are presented by double-positive thymocytes to MAIT cell precursors, facilitating their successful differentiation. In addition to 5-A-RU-derived ligands, bile acid metabolites, primarily produced by bacterial bile salt hydrolases (BSH), are also present in the thymus and can influence the development of MAIT cells. During the maturation of MAIT cells in peripheral tissues, a high abundance of riboflavin-synthesizing commensal bacteria promotes the establishment of a substantial population of tissue-resident MAIT cells in adults. Following their development in response to early-life commensals, MAIT cells emerge as a predominant type-17 effector subset in the skin. Furthermore, cutaneous MAIT cells exhibit a distinct transcriptional program associated with tissue repair, highlighting their role in maintaining skin integrity. The models have been well studied in mice and are likely applicable to humans, although additional investigations are necessary to substantiate this applicability.

colonization, while MAIT cell levels normalize in the thymus and spleen, they remain significantly diminished in the lungs [9]. This observation suggests that microbial recolonization alone is insufficient to replenish MAIT cell populations in peripheral tissues, indicating that the microbiota plays a critical role in regulating MAIT cell maturation in these tissues.

Recent research has highlighted the abundance of MAIT cells in the skin, where they are present in both human and murine models [45]. In mice skin, MAIT cells can comprise up to 40% of αβ T cells, and they are likewise enriched in human skin, constituting about 2% of $CD3^+$ lymphocytes compared to roughly 1% in peripheral blood [26,46]. Notably, genetically identical mice housed in separate cages exhibited marked variability in MAIT cell proportions, whereas those housed in the same environment demonstrated similar frequencies, suggesting that these differences are attributable to variations in microbiota [26].

A critical neonatal window exists during the first 3 weeks of mouse life, during which recolonization of GF mice can restore MAIT cell populations. Isolation of early-life intestinal commensals and subsequent colonization of neonatal GF mice with defined bacterial strains have been shown to induce MAIT cell development [26]. Conversely, colonization initiated later in life fails to promote the maturation of MAIT cells within peripheral tissues, including the lungs, spleen, and skin [26]. This underscores the importance of microbial exposure during early life in establishing and maintaining MAIT cell abundance over time. Furthermore, topical application of 5-OP-RU in one-week-old mice effectively increased MAIT cell numbers in the skin without promoting the accumulation of other T cell subsets; however, this treatment was ineffective in inducing MAIT cells in adult GF mice, highlighting a specific developmental window for microbial influence on MAIT cell maturation in peripheral tissues [26].

Following their development in response to early-life commensals, MAIT cells become a predominant MAIT17 subset in the skin and exhibit a distinct transcriptional profile associated with tissue repair [26]. These MAIT cells express type 17-associated transcripts, including *Il22* and *Rorc*, the latter encoding the transcription factor RORγt. Additionally, they express *Il1r1* and *Il23r*, which encode the receptors for IL-1 and IL-23, respectively [26]. The recognition of skin commensals by MAIT cells is facilitated by MR1-mediated presentation of riboflavin metabolites, which further enhances the tissue-repair functions of these lymphocytes. Within the skin, MAIT cells are localized at the interface between the dermis and epidermis, closely associated with the basal layer [47]. Topical application of a riboflavin derivative selectively boosts MAIT cell populations in the skin and significantly facilitates cutaneous wound healing, underscoring the contribution of MAIT cells to skin physiology [26].

It is important to note, however, that all of the aforementioned studies were conducted in murine models. The extent to which microbiota-derived riboflavin intermediates contribute to the final maturation and long-term tissue imprinting of MAIT cells in humans, both before and after birth, warrants further investigation. Nevertheless, human T cells begin their development in utero [48], lending credence to the hypothesis that early MAIT cell imprinting may occur prior to birth. Functionally mature MAIT cells have been identified in second-trimester human fetuses, suggesting that early differentiation is likely in preparation for postnatal immune challenges [49].

Dysbiosis impairs MAIT cell populations

Microbial diversity is tissue-specific and exhibits significant variation between healthy and diseased states, often characterized by dysbiosis that results in reduced microbial diversity. Given the strong association between MAIT cells and the microbiota, dysbiosis can substantially impair both the populations and functionality of MAIT cells. Dysfunction of MAIT cells is frequently observed in immune-mediated diseases, which can be driven by chronic infections, autoimmune, atopic, and metabolic processes. This impairment in MAIT cell function may contribute to the pathogenesis of these conditions, highlighting the importance of microbiota composition in regulating MAIT cell activity and overall immune homeostasis.

Multiple observations suggest that MAIT cells may play a significant role in modulating immune responses to microbiota in both the human gut and skin, particularly during chronic inflammatory diseases. For instance, the frequency of MAIT cells is often decreased in the bloodstream of patients suffering from inflammatory conditions such as **Crohn's disease**, **multiple sclerosis**, and **rheumatoid arthritis**; conversely, their levels are elevated in inflamed tissues compared to healthy controls, indicating active migration to sites of inflammation [31,50,51]. Additionally, the composition of the local microbiota, which can influence the activation and recruitment of MAIT cells, alters in the inflamed tissues of individuals with Crohn's disease and **psoriasis** [52–55]. Furthermore, reduced MAIT cell numbers have been observed in the blood of HIV-infected individuals, both adults and children, who experience chronic inflammation partly attributable to disruptions in gut mucosal immunity and alterations in the microbiota [56–59].

Consequently, MAIT cells may detect alterations in microbial ecology, with changes in their blood frequency serving as an indicator of dysbiosis, which is commonly observed in these diseases. These findings underscore the integral role of MAIT cells in the immune response associated with microbial interactions, highlighting their potential involvement in the pathophysiology of chronic inflammatory diseases.

Microbial diversity affects MAIT cell responses via riboflavin pathway metabolites

MAIT cells exhibit the capability to recognize and differentiate among various microbial species present in the microbiota. These cells selectively respond to a diverse range of microorganisms

that possess the biosynthetic pathway for riboflavin metabolism. This selective recognition allows MAIT cells to mount an immune response to specific microbial antigens, thereby influencing the overall immune response and shaping interactions between the host immune system and the microbial community.

In a recent study, Tastan developed an *in vitro* functional assay utilizing human T cells engineered to express MAIT TCRs, stimulating these cells with MR1-expressing antigen-presenting cells (APCs). This study screened 47 microbiota-associated bacterial species from various phyla to evaluate their capacity to stimulate MAIT TCRs, measuring T cell activation markers such as CD25 and CD69, as well as the production of pro-inflammatory cytokines and cytotoxic molecules, including IFN-γ, Perforin, and Granzyme B [29]. The findings provide a comprehensive resource detailing which microbiota can be recognized by human MAIT cells and have the potential to activate them effectively (Box 2).

Only bacterial species that encode components of the riboflavin biosynthetic pathway were found to stimulate MAIT TCRs. Notably, the most potent stimulators predominantly belonged to the phyla **Bacteroidetes** and **Proteobacteria**, while low or non-stimulatory species were primarily from the phyla **Actinobacteria** or **Firmicutes** [29]. This dichotomy is particularly interesting, as the distribution and proportions of these bacterial species within the human microbiota fluctuate throughout an individual's life and in different disease states, which may correlate with variations in MAIT cell numbers and activity [29]. For instance, the ratio of Bacteroidetes to Firmicutes is significantly higher in the gut microbiota of infants compared to adults, shifting towards an adult-like composition within the first few years of life [60]. Moreover, the study noted that *Cloacibacterium* spp., which displayed the highest stimulatory capacity in their assays, have also been detected in the breast milk of healthy women [61], highlighting a mechanism through which Bacteroidetes and other microbiota are established in the intestinal flora of infants. These observations suggest a potential correlation between specific microbiota species and the populations of MAIT cells in humans, influenced by variations in age, activity levels, and disease stages.

Furthermore, *E. coli* strains have demonstrated a considerable capacity to stimulate MAIT TCRs, suggesting that manipulation of *E. coli* could be a viable approach for enhancing MAIT cell activity across various applications [29,62]. These findings underscore the importance of designing effective microbial 'bug-drugs' that can be utilized to enhance MAIT cell therapy, improving their recognition of $MR1^+$ APCs and $MR1^+$ tumor cells. Bacterial strains exhibiting strong riboflavin biosynthesis, along with feasibility and safety for *in vivo* use, may serve as potent MAIT cell boosters, thereby supporting MAIT cell-based immunotherapy, particularly in the context of cancer treatment.

Impact of the microbiota on MAIT cell function in tumor modulation

Microbial metabolites potentiate MAIT cell antitumor immunity

Intratumoral microbial compositions differ between tumor types, with correlations between microbial metabolic pathways and clinical features. Tumor-related microbiota plays an important

Box 2. Microbiota species recognized and discriminated by MAIT cells

MAIT cells have been reported to correlate with microbiota, and dysbiosis has been shown to impair both the populations and functions of MAIT cells. However, it is less understood whether MAIT cells respond to commensal human microbiota residing in mucosal tissues or the skin, as current knowledge primarily identifies selective and largely pathogenic bacterial species that specifically stimulate MAIT cells. A recent study screened 47 microbiota-associated bacterial species from various phyla to evaluate their capacity to stimulate MAIT TCRs and ranked these species based on their MAIT TCR activation (Table I) [29]. This information is crucial as it directs researchers toward potential commensal species that may be involved in MAIT cell activation and contributes to a better understanding of the role of the microbiota in immune regulation and homeostasis, ultimately enhancing the approaches to therapeutic interventions targeting MAIT cells in various diseases.

Table I. Bacterial species that stimulate MAIT TCRs

Bacterial species ranked by their stimulation of MAIT TCRs from strongest to weakest (top to bottom)	Bacterial phylum	Bacterial tissue distribution
Cloacibacterium normanense	Bacteroidetes	Gut
Bergeyella zoohelcum	Bacteroidetes	Gut
Elizabethkingia meningoseptica	Bacteroidetes	Spinal fluid
Riemerella anatipestifer	Bacteroidetes	Unknown
Myroides odoratimimus	Bacteroidetes	Skin and mucosae
Escherichia coli	Proteobacteria	Gut
Pedobacter heparinus	Bacteroidetes	Unknown
Acidovorax temperans	Proteobacteria	Oral cavity
Prevotella melaninogenica	Bacteroidetes	Sputum
Corynebacterium simulans	Actinobacteria	Axillar lymph node
Staphylococcus hominis	Firmicutes	Skin
Klebsiella pneumoniae	Proteobacteria	Gut
Mycobacterium smegmatis	Actinobacteria	Skin
Corynebacterium diphtheriae	Actinobacteria	Skin
Pseudomonas aeruginosa	Proteobacteria	Gut
Diaphorobacter polyhydroxybutyrativorans	Proteobacteria	Unknown
Kocuria rosea	Actinobacteria	Skin
Micrococcus luteus	Actinobacteria	Skin
Porphyromonas asaccharolytica	Bacteroidetes	Gut
Salmonella enterica serovar Typhimurium	Proteobacteria	Gut
Alkalihalobacillus clausii	Firmicutes	Gut
Proteus mirabilis	Proteobacteria	Gut
Bacillus atrophaeus	Firmicutes	Unknown
Bacteroides thetaiotaomicron	Bacteroidetes	Gut
Enterococcus casseliflavus	Firmicutes	Blood
Staphylococcus epidermidis	Firmicutes	Skin
Bacillus subtilis	Firmicutes	Gut
Bacillus amyloliquefaciens	Firmicutes	Unknown
Mycobacterium phlei	Actinobacteria	Unknown
Anaerococcus prevotii	Firmicutes	Skin
Geobacillus stearothermophilus	Firmicutes	Unknown
Propionibacterium acnes	Actinobacteria	Skin
Staphylococcus lugdunensis	Firmicutes	Skin
Veillonella parvula	Firmicutes	Oral cavity
Streptococcus agalactiae	Firmicutes	Gut
Streptomyces griseus	Actinobacteria	Unknown
Actinomyces naeslundii	Actinobacteria	Skin
Staphylococcus saprophyticus	Firmicutes	Skin
Streptococcus pneumoniae	Firmicutes	Respiratory system
Lactococcus lactis	Firmicutes	Human milk

role in the TME, especially in cancers arising from mucosal sites, including the skin, lung, liver, and gastrointestinal tract. The species of bacteria residing within tumors have been identified as tumor-type specific and associated with tumor progression [63], although the concept of intratumor bacteria has recently become a topic of controversy. Riboflavin producing bacteria species, for example Proteobacteria, Bacteroidetes, and Fusobacteria, are present in colorectal cancer, esophageal cancer, lung cancer, and breast cancer [64]. MAIT cells modulate their functional responses based on antigen load and APC interactions, responding to variations in microbiota composition through TCR signaling driven by riboflavin metabolites [9]. Commensal bacteria species in mucosal area that encoded the riboflavin pathway have been proven to activate MAIT through TCR (Figure 2A) [9,29]. With engineered MAIT-TCR presenting T cells, commensal bacteria species that lacked riboflavin pathway was confirmed to have no stimulatory effect for MAIT-TCRs, while riboflavin producing species, especially Bacteroidetes and Proteobacteria, could stimulate MAIT-TCR in a riboflavin-dependent manner [29]. Macrophage and human T cell subsets were found to present riboflavin metabolites to MAIT cells in a MR1-restricted way, resulting in production of IFN-γ, TNF-α and Granzyme B in MAIT cells [29].

In preclinical studies, evidence suggests that synthetic bacterial riboflavin synthesis pathway-derived antigen 5-OP-RU was able to induce broad antitumoral response of MAIT cells, underscoring the importance of bacteria presence in MAIT-mediated antitumor response (Figure 2B) [40,65]. 5-OP-RU with **CpG** co-stimulation was found to assist systemic MAIT cell expansion and activation in mouse models, indicating the important role of APCs like dendritic cells, B cells, and macrophages [40]. MAIT cells activated by this approach expressed high levels of CD69, effector memory markers ($CD44^+CD62L^-$), and cytotoxic molecules (IFN-γ, Granzyme B, and Perforin). Significantly, tumor inhibition was observed in multiple murine tumor models, including liver metastases, hepatocellular carcinoma, lung metastases, and subcutaneous tumors [40]. In MAIT-deficient $Mr1^{-/-}$ mice, the absence of tumor suppression confirmed the antitumor effects of MAIT cells [40]. Intriguingly, CRISPR/Cas9 knockout of MR1 in tumor cells did not reduce MAIT-mediated tumor suppression, suggesting the antitumor efficacy was independent of the engagement of MAIT TCR and MR1 on tumor cells [40].

Studies have revealed that microbial metabolites have a pivotal role in potentiating MAIT cells to modulate NK cell-mediated tumor immunity [66]. The researchers found that in the absence of MAIT cells, NK cells exhibited enhanced tumor control, suggesting an inhibitory role for MAIT cells under steady-state conditions. However, administration of 5-OP-RU dramatically shifted this paradigm by activating and expanding MAIT cells, which in turn enhanced NK cell function and antitumor immunity in both B16F10 melanoma and E0771 breast tumor models [66]. 5-OP-RU-pulsed tumor cells also showed reduced metastatic potential that is correlated with both MAIT and NK cell activation. Mechanistically, 5-OP-RU-mediated MAIT cell activation led to increased IFN-γ production, which was crucial for priming NK cells and promoting their cytotoxicity towards tumor cells [66]. This effect was further validated in human samples, where 5-OP-RU stimulation enhanced NK cell function, increasing cytokine production and cytotoxic potential [66]. Overall, these findings underscore the therapeutic potential of 5-OP-RU in reprogramming the MAIT-NK axis to improve antitumor immunity.

In another study, using *Mr1* deficient mice, the researchers observed a significant reduction in tumor burden, which was dependent on NK cells and IFN-γ. Adoptive transfer of MAIT cells into *Mr1* deficient mice reversed this protective effect, confirming their tumor-promoting function [67]. The study highlights the role of MR1-expressing tumor cells in activating MAIT cells, leading to the suppression of NK-cell effector functions, partially mediated by IL-17. Additionally, pre-treatment of tumor cells with the MAIT cell activator 5-OP-RU enhanced metastasis, whereas

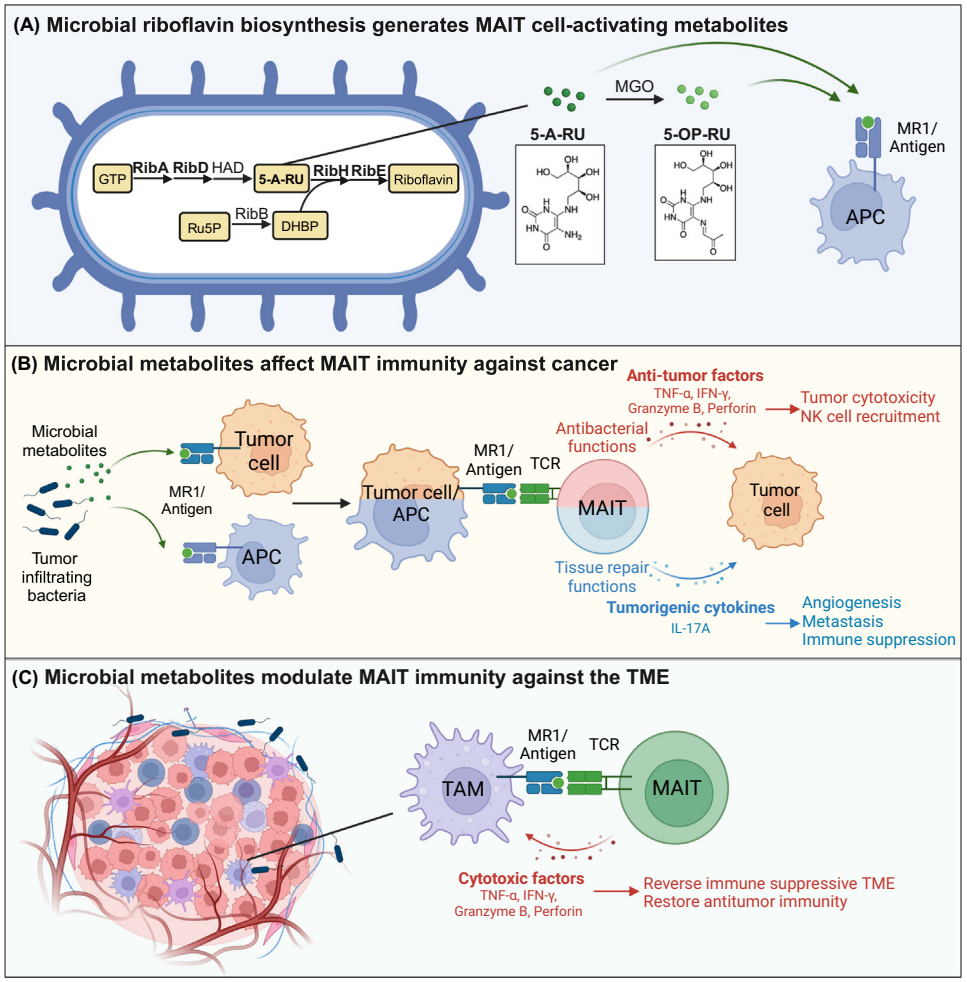

Figure 2. Microbial metabolites affect mucosal-associated invariant T (MAIT) cell immunity against cancer. (A) MAIT-activating metabolites 5-A-RU/5-OP-RU are produced in bacteria through the riboflavin synthesis. Riboflavin in bacteria is made through an enzymatic cascade starting from GTP and ribulose-5-phosphate (RU5P) molecules. 5-OP-RU is generated from 5-A-RU by a non-enzymatic reaction with methylglyoxal (MGO). Both 5-A-RU and 5-OP-RU have the capability to load on MR1, but 5-OP-RU is the most potent molecule in activating MAIT cells. (B) Tumor-associated bacteria produce microbial metabolites, which are presented by MR1 on antigen-presenting cells (APCs) and tumor cells, leading to MAIT cell activation via T cell receptor (TCR) engagement. Upon activation, MAIT cells exert dual functional roles in tumor progression: (i) antitumor effects, characterized by the production of TNF-α, IFN-γ, Granzyme B, and Perforin, which enhance tumor cytotoxicity and NK cell recruitment; (ii) pro-tumor effects, driven by the secretion of IL-17A, which promotes angiogenesis, metastasis, and immune suppression. (C) In the tumor microenvironment (TME), MAIT cells can deplete immune suppressive cells like tumor-associated macrophages (TAMs) presenting microbial metabolites thus reverse the immunosuppressive TME and restore antitumor immunity.

MR1 blockade or deletion in tumor cells led to reduced tumor spread [67]. These findings challenge the conventional view of MAIT cells as antitumor effectors and suggest that targeting MR1-MAIT cell interactions could be a novel therapeutic strategy for cancer immunotherapy.

Impact of the microbiota on tumor infiltrating MAIT cells in a context-dependent manner

Although *in vitro* studies have confirmed the antitumor potential of MAIT cells upon activation by microbial metabolites, their role in actual cancer settings remains complex and context-

dependent, influenced by the heterogeneity of the TME and malignancy types. The impact of tumor-associated microbiota on tumor-infiltrating MAIT cells remains debatable. As innate-like T cells, MAIT cells recognize microbial-derived metabolic antigens and play dual roles in antimicrobial defense and tissue repair by secreting cytokines like IFN-γ, TNF-α, and IL-17. Within the TME, their functional responses can lead to divergent immunological outcomes, shaping both tumor progression and immune regulation [2].

Several studies have shown that in cancer patients, MAIT cells exhibit an exhausted phenotype, with bacterial exposure capable of inducing their activation but resulting in limited antitumor efficacy. Upon microbial-metabolites activation, MAIT cells initiate antibacterial immune responses, which can contribute to antitumor effects, while simultaneously promoting tissue repair functions that may support tumor progression [26,68]. In colorectal cancer, high bacterial load and enrichment of bacterial strains such as Proteobacteria, *Bacteroides*, and *Fusobacterium* could potentially contribute to MAIT cell activation. In a recent study, *Fusobacterium nucleatum* metabolite was found to be capable of activating MAIT cells in a TCR-dependent manner [69]. Tumor-infiltrating MAIT cells exhibit upregulated exhaustion markers, including PD-1 and CD39 [69]. Additionally, a distinct $CD4^+FOXP3^+$ MAIT cell subset has been identified. Functional analyses indicate that these $CD4^+FOXP3^+$ MAIT cells possess the ability to produce the pro-inflammatory cytokine TNF-α, distinguishing them from conventional regulatory T cells (Tregs) [69]. Notably, *Fusobacterium* has been identified as a bacterium associated with colorectal carcinoma, contributing to tumor cell proliferation and growth [70]. This effect is mediated by *Fusobacterium nucleatum* adhesin molecules, which interact with specific surface motifs on cancer cells or immune cells, subsequently activating downstream oncogenic and immunosuppressive signaling pathways [71].

Contrarily, in the situation of breast cancer, researchers found that MAIT cells within breast duct epithelial tissue exhibited a distinct Th17-skewed cytokine profile, differing from peripheral blood MAIT cells which primarily produced IFN-γ and TNF-α [72]. Upon exposure to *E. coli*-treated breast carcinoma cells, breast duct MAIT cells were activated in an MR1-dependent manner, but instead of producing IFN-γ, they predominantly secreted IL-17, suggesting a potential pro-tumorigenic role [72]. Additionally, they found that breast carcinoma cells upregulated NKG2D ligands, and NKG2D receptor engagement contributed to MAIT cell activation [72].

In other cancers that may be colonized by bacteria, the effector functions of MAIT cells have been found to be impaired. For instance, in patients with non-small cell lung cancer (NSCLC), MAIT cells are enriched in tumor tissues through the CCR6-CCL20 axis [73]. Tumor-infiltrating MAIT cells exhibited a $PD1^+Tim3^+IL\text{-}17^+$ exhausted phenotype and produced less IFN-γ [73]. This exhaustion of MAIT cells is associated with diminished effector function and poorer prognostic outcomes, which can be partially alleviated through anti-PD-1 therapy [73].

The crosstalk between MAIT cells and other immune cells, particularly innate immune populations, plays a crucial role in shaping their overall impact on mucosal tumors. Macrophages and dendritic cells, upon activation by bacterial-derived pathogen-associated molecular patterns (PAMPs) such as *Fusobacterium* in colorectal cancer, can produce IL-12 and IL-18, thereby enhancing MAIT cell activation [74,75]. Additionally, certain innate immune cells respond to IL-17, a cytokine that can be produced by MAIT17 cells. IL-17 has been implicated in inflammation, tissue repair, and tumor progression by promoting the recruitment of myeloid-derived suppressor cells (MDSCs) and facilitating angiogenesis [76,77]. This suggests that IL-17-producing MAIT17 cells may interact with other immunosuppressive cell populations, contributing to a more immunosuppressive TME. Ultimately, the overall effect of MAIT cell interactions with innate immune cells in

mucosal tumors is likely dictated by the balance between activating and suppressive signals within the TME. Strategies that leverage microbial-derived adjuvants such as 5-OP-RU, in combination with cytokine modulation, may provide a means to shift this balance toward a more potent antitumor immune response.

Microbial metabolites regulate MAIT cells to remodel the TME

In the TME, immune suppressive cells like tumor-associated macrophages (TAMs) and MDSCs promote cancer progression and hinder antitumor immune responses [78–80]. Tumor microbial metabolites can be presented on these immune suppressive cells and lead to their depletion by tumor-infiltrating MAIT cells [2,39]. Within the immunosuppressive TME, M2-polarized TAMs contribute to tumor progression by promoting immune evasion and dampening cytotoxic immune responses. Upon microbial metabolite stimuli, human MAIT cells can directly kill M2-polarized macrophages in an MR1-dependent manner, reducing their immunosuppressive impact [39]. In an *ex vivo* 3D tumor/TAM/MAIT-cell organoid culture, human CAR-engineered MAIT cells effectively reversed TAM-mediated immune suppression. Notably, MAIT cells activated with 5-OP-RU exhibited potent cytotoxicity against TAMs, leading to the restoration of antitumor immune activity [39]. This suggests that MAIT cells not only exert direct antitumor effects but also remodel the TME by depleting TAMs, thereby enhancing overall immune reactivity (Figure 2C).

The interplay between tumor microbiota and MAIT cells in tumor immunity is complex and context-dependent, significantly influencing tumor progression and immune regulation. The role of MAIT cells within the TME is highly dependent on tumor types, microbial compositions, and local immune environments. Further studies are needed to dissect the mechanisms governing MAIT cell plasticity, including their interaction with microbial metabolites, their metabolic adaptations within the TME, and their crosstalk with NK cells and other immune subsets.

Therapeutic strategies to leverage the microbiota for enhancing MAIT cell antitumor immunity

The interplay between the gut microbiota and MAIT cell development presents an exciting opportunity to leverage microbial-based strategies to enhance MAIT cell-mediated cancer immunotherapy [25,26]. MAIT cells are uniquely equipped to recognize microbial-derived riboflavin metabolites presented by the MR1, making them strong candidates for cancer immunotherapy, particularly in targeting MR1-expressing tumors [30,39,81]. However, the functionality of MAIT cells is highly dependent on microbial-derived cues, highlighting the potential for microbiota modulation to optimize their antitumor responses.

Emerging evidence underscores the critical role of commensal bacteria in shaping MAIT cell function [29]. Dysbiosis of the gut microbiota has been implicated in the progression of several cancers, including liver cancer, where shifts in microbial composition contribute to immune dysfunction [82,83]. Consequently, strategies aimed at restoring or enhancing beneficial microbial populations that produce elevated levels of riboflavin biosynthesis products, such as 5-OP-RU, may improve MAIT cell-mediated antitumor immunity. Probiotic supplementation with bacterial strains capable of producing MAIT cell-activating metabolites offers a promising strategy to bolster MAIT cell function. Notably, *E. coli* has emerged as a particularly compelling candidate due to its robust capacity to stimulate MAIT TCRs through an intact riboflavin biosynthetic pathway [25,29]. Studies have demonstrated that *E. coli* can effectively activate MAIT cells, promoting the release of cytotoxic effector molecules such as IFN-γ, Perforin, and Granzyme B [29]. The ability of *E. coli* or other commensal bacteria to activate MAIT cells through MR1 presentation positions these bacteria as promising ‘bug drugs’ for cancer immunotherapy (Box 2).

In addition, advances in synthetic biology have enabled the development of genetically engineered bacterial therapies for cancer treatment. Clinical trials have explored tumor-targeting bacteria as immunotherapeutics, such as an attenuated *Salmonella typhimurium* strain expressing IL-2, which enhanced immune responses in liver cancer (NCT01099631). Additionally, engineered bacteria secreting immunomodulatory cytokines or stimulating localized immune responses have shown promise in supporting conventional immunotherapies, such as **immune checkpoint blockade (ICB)** [84–87]. Recent studies have further validated the therapeutic potential of engineered microbes in cancer treatment. One notable example involves engineering probiotic bacteria for the intratumoral release of nanobodies targeting PD-L1 and CTLA-4 through a stabilized lysis mechanism, demonstrating superior efficacy compared to clinically relevant antibodies and highlighting the utility of bacteria for localized immunotherapy delivery [88]. Another study developed a non-pathogenic *E. coli* strain programmed to lyse within the TME and release an encoded nanobody antagonist of CD47, thereby enhancing tumor-infiltrating T cell activation and promoting rapid tumor regression [89].

Building on this foundation, an emerging strategy involves genetically engineering bacteria to overexpress enzymes in the riboflavin biosynthesis pathway, specifically to enhance MAIT cell activation. Given that MAIT cells recognize microbial-derived metabolites presented by MR1, engineering bacterial strains to overproduce riboflavin intermediates, particularly 5-OP-RU, could effectively boost MAIT cell-mediated immune responses. Bacterial strains such as *E. coli* are particularly well-suited for this approach due to their established safety profiles, robust capacity for MAIT cell activation, and suitability for genetic modification. For example, previous studies have engineered *E. coli* strains to knockout vitamin B2 biosynthesis enzymes either upstream (ΔRibD) or downstream (ΔRibE) of 5-A-RU production [25,65]. The findings revealed that the ΔRibD knockout significantly affected MAIT cell differentiation, whereas the ΔRibE knockout, or ΔRibD knockout supplemented with 5-OP-RU, did not impact MAIT cell differentiation [25]. Therefore, leveraging genetic engineering of the riboflavin biosynthesis pathway to stimulate 5-OP-RU production could activate MAIT cells and enhance their functionality, including anti-cancer immunity. This strategy could pave the way for next-generation microbial-based immunotherapies designed to selectively enhance MAIT cell responses, particularly in solid tumors with immunosuppressive microenvironments.

Microbiota-based approaches, such as screening for bacteria with potent riboflavin biosynthesis and MAIT cell activation capabilities, along with engineering microbial therapeutics to boost their MAIT cell activation potential, offer innovative strategies for enhancing MAIT cell-driven immunotherapy, especially in mucosal cancers such as liver and colorectal cancers (Figure 3). Moreover, modulation of the microbiota could also have therapeutic implications in inflammatory diseases such as Crohn's disease, multiple sclerosis, and rheumatoid arthritis [90], where downregulation of aberrant MAIT cell activation may mitigate disease progression. Despite the significant promise of microbial-based therapies, several challenges remain, including ensuring bacterial safety, optimizing colonization efficiency, and maintaining microbial persistence *in vivo*.

A recent study utilizing a physiologically relevant intestinal organoid model demonstrated that *E. coli* stimulation can enhance the proinflammatory and cytolytic functions of MAIT cells, potentially leading to tissue damage [91]. Specifically, MAIT cells were observed to migrate toward *E. coli*-infected appendix-derived organoids, where they mediated MR1-dependent organoid cell death and structural destruction through robust degranulation, as indicated by CD107a expression [91]. These findings highlight a critical safety consideration for MAIT cell-based immunotherapy, particularly in the context of microbial 'bug-drug' strategies designed to enhance MAIT cell activity. However, this tissue-destructive capacity following *E. coli* stimulation may also have therapeutic potential in eradicating solid mucosal tumors, presenting a possible benefit for solid tumor immunotherapy.

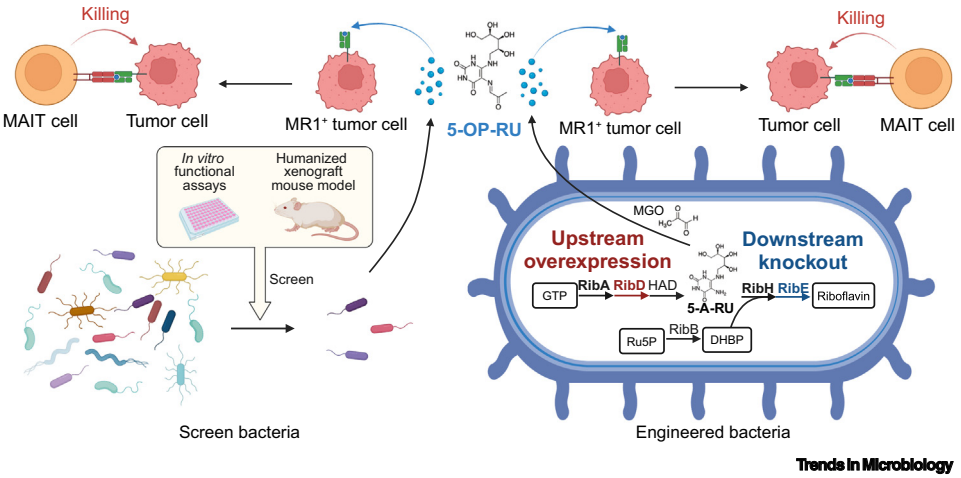

Figure 3. Therapeutic strategies to leverage microbiota to boost mucosal-associated invariant T (MAIT) cell antitumor immunity. Several microbiota-driven strategies can be used as MAIT cell boosters to enhance MAIT cell-driven immunotherapy, particularly in mucosal cancers such as liver and colorectal cancers. These strategies include screening for bacteria with potent riboflavin biosynthesis and MAIT cell activation capabilities (left panel), as well as engineering bacteria to augment their riboflavin biosynthesis in order to increase their MAIT cell activation potential (right panel).

As our understanding of the microbiota-immune axis deepens, integrating microbial-based strategies with MAIT cell-targeted therapies holds significant potential for advancing precision medicine in oncology and immunology. Future research should focus on refining bacterial engineering strategies, improving delivery systems, and maximizing therapeutic efficacy while minimizing adverse effects. Such advancements could ultimately lead to groundbreaking immunotherapies that fully harness the power of the microbiota-MAIT cell axis to combat cancer and inflammatory diseases effectively.

Concluding remarks and future perspectives

Recent advances in understanding microbiota-MAIT cell interactions have highlighted their potential roles in immune surveillance and tumor immunity. MAIT cells, known for their ability to recognize microbial-derived riboflavin metabolites via MR1-restricted antigen presentation, exhibit functional plasticity depending on antigen load, APCs, and the surrounding microenvironment [2–4]. Evidence suggests that tumor microbiota composition is tumor-type specific and significantly influences immune responses, including MAIT cell activation [64]. Riboflavin-producing bacteria such as Proteobacteria, Bacteroidetes, and Fusobacteria have been implicated in colorectal, esophageal, lung, and breast cancers, supporting the notion that microbial-derived metabolites can shape MAIT cell function within the TME [64]. The presence of such bacteria has been linked to both proinflammatory and regulatory immune responses, indicating a complex interplay between microbial metabolism, MAIT cell activation, and tumor progression.

Despite these promising insights, several gaps remain in our understanding of microbiota-MAIT cell interactions, particularly within the context of the TME (see Outstanding questions). While preclinical studies have demonstrated that synthetic bacterial-derived 5-OP-RU antigens can promote robust MAIT cell-mediated antitumor responses [40], the exact mechanisms governing MAIT cell activation and function in human cancers remain unclear. Notably, the impact of tumor microbiota on tumor-infiltrating MAIT cells is still debated. For instance, in colorectal cancer, the high bacterial load and enrichment of strains such as *E. coli*, *Bacteroides*, and *Fusobacterium nucleatum* have been associated with MAIT cell activation, with *F. nucleatum* metabolites directly stimulating MAIT cells in a TCR-dependent manner [69]. However, the functional state of these

Outstanding questions

How do the interactions between MAIT cells and other immune cell types, influenced by the microbiota, shape the tumor microenvironment?

What specific signaling pathways are involved in the interaction between MAIT cells and microbial signals that influence tumor immunity?

What specific bacterial species are most effective at stimulating MAIT cell responses, and how can this knowledge be leveraged for cancer treatment?

How do the functional characteristics of MAIT cells in humans differ from those in mice in the context of microbiota interaction and antitumor immunity?

What are the long-term effects of microbiota modulation on MAIT cell memory and durability of antitumor responses?

How do environmental factors, such as diet and lifestyle, impact the composition of the microbiota and subsequently affect MAIT cell function in cancer settings?

What specific metabolites produced by the microbiota are most influential in modulating MAIT cell responses?

Are there specific markers associated with effective MAIT cell responses to tumor-associated antigens that could serve as predictive biomarkers for treatment success?

How can the temporal dynamics of microbiota composition influence the timing and effectiveness of MAIT cell-mediated antitumor immunity?

What are the implications of MAIT cell heterogeneity in terms of their differentiation states and functional capacities when exposed to different microbial stimuli?

How do immune checkpoint molecules expressed on MAIT cells affect their functionality and antitumor activity in the presence of microbiota?

Which bacterial strains are most effective as MAIT cell boosters, considering their safety for use in cancer therapy in humans?

tumor-infiltrating MAIT cells often exhibits features of exhaustion, characterized by PD-1 and CD39 expression [38,69], raising questions about their sustained efficacy in the TME.

In other tumor types, MAIT cell function appears to vary significantly based on local microbiota composition and tumor-intrinsic factors. For example, in breast cancer, MAIT cells within epithelial ducts are enriched for IL-17-producing subsets, and *E. coli*-induced MAIT cell activation in an MR1-dependent manner skews responses toward IL-17 production, potentially contributing to proinflammatory signaling [72]. Additionally, NKG2D expression has been linked to increased TNF-α production via MAIT cells, underscoring their potential role in modulating immune responses in epithelial cancers [72]. Contrastingly, in non-small cell lung cancer and other solid tumors, MAIT cells exhibit divergent phenotypes, ranging from proinflammatory to immunosuppressive, depending on microbial composition and immune contexture [73,92,93].

Several directions should be focused on in future research to refine our understanding of microbiota-MAIT cell interactions and their therapeutic implications. First, explaining the specific metabolic pathways through which tumor-resident bacteria modulate MAIT cell activity is essential for using their potential in cancer immunotherapy. The development of microbiota-targeting strategies, such as probiotic supplementation, microbial metabolite-based therapies, or microbiome-engineered antigen presentation, could offer novel avenues for modulating MAIT cell function in tumors [26,94,95]. Second, investigating the impact of tumor microbiota variability on MAIT cell exhaustion and dysfunction will be crucial for optimizing therapeutic interventions. Strategies aimed at reinvigorating MAIT cells, including checkpoint blockade, metabolic reprogramming, or engineered MAIT TCR approaches, need further exploration.

Furthermore, the role of APCs in shaping MAIT cell responses should be a focus of future studies. Preclinical evidence suggests that dendritic cells, macrophages, and B cells can present riboflavin metabolites to MAIT cells, influencing their activation state. Understanding how these APCs contribute to MAIT cell-mediated tumor immunity may provide new opportunities for combinatorial therapies that enhance both innate and adaptive immune responses. Finally, the development of MAIT cell-based immunotherapies, such as adoptive cell transfer or vaccine strategies utilizing microbial metabolites such as 5-OP-RU for co-stimulation, presents a promising opportunity for cancer treatment. The observation that MAIT cells can suppress tumors independently of MR1 expression on tumor cells highlights the need for further investigation into MR1-independent pathways that govern their antitumor activity.

In conclusion, a deeper understanding of the interplay between microbial metabolism, MAIT cell activation, and cancer modulation will be critical for designing effective MAIT cell-targeted therapies. Future research should aim to translate these insights into clinically viable strategies, ultimately exploiting the power of microbiota-MAIT cell interactions for improved cancer immunotherapy.

What genetic engineering strategies can be used in bacteria to enhance their riboflavin production, thereby potentially improving MAIT cell therapy for cancer?

Can CAR-engineered MAIT cells combined with microbial 'bug-drug' approaches further enhance their antitumor functions?

Acknowledgments

This work was supported by a UCLA BSCRC Innovation Award and an Ablon Scholars Award (to L.Y.). L.Y. is also supported by UCLA Parker Institute for Cancer Immunotherapy (PICI). Y.-R.L. is a postdoctoral fellow supported by a UCLA MIMG M. John Pickett Post-Doctoral Fellow Award, a CIRM-BSCRC Postdoctoral Fellowship, a UCLA Sydney Finegold Postdoctoral Award, a UCLA Chancellor's Award for Postdoctoral Research, and a Goodman-Luskin Microbiome Center Collaborative Research Fellowship award. Figures were created with BioRender (biorender.com).

Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences, and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to this study. The other authors declare no competing interests.

References

1. Li, Y.-R. *et al.* (2024) Breaking the mold: unconventional T cells in cancer therapy. *Cancer Cell* 43, 317–322
2. McWilliam, H.E.G. and Villadangos, J.A. (2024) MR1 antigen presentation to MAIT cells and other MR1-restricted T cells. *Nat. Rev. Immunol.* 24, 178–192
3. Koay, H.-F. *et al.* (2018) Development of mucosal-associated invariant T cells. *Immunol. Cell Biol.* 96, 598–606
4. Godfrey, D.I. *et al.* (2019) The biology and functional importance of MAIT cells. *Nat. Immunol.* 20, 1110–1128
5. Kjer-Nielsen, L. *et al.* (2012) MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 491, 717–723
6. Franciszkiewicz, K. *et al.* (2016) MHC class I-related molecule, MR1, and mucosal-associated invariant T cells. *Immunol. Rev.* 272, 120–138
7. Mondot, S. *et al.* (2016) MAIT, MR1, microbes and riboflavin: a paradigm for the co-evolution of invariant TCRs and restricting MHCI-like molecules? *Immunogenetics* 68, 537–548
8. Li, Y.-R. *et al.* (2022) Mucosal-associated invariant T cells for cancer immunotherapy. *Mol. Ther.* 31, 631–646
9. Legoux, F. *et al.* (2020) MAIT cell development and functions: the microbial connection. *Immunity* 53, 710–723
10. Hinks, T.S.C. and Zhang, X.-W. (2020) MAIT cell activation and functions. *Front. Immunol.* 11, 1014
11. Kammann, T. *et al.* (2024) MAIT cell heterogeneity across paired human tissues reveals specialization of distinct regulatory and enhanced effector profiles. *Sci. Immunol.* 9, eadn2362
12. Koay, H.-F. *et al.* (2019) A divergent transcriptional landscape underpins the development and functional branching of MAIT cells. *Sci. Immunol.* 4, eaay6039
13. Karnaukhov, V.K. *et al.* (2024) Innate-like T cell subset commitment in the murine thymus is independent of TCR characteristics and occurs during proliferation. *Proc. Natl. Acad. Sci. U. S. A.* 121, e2311348121
14. Chandra, S. *et al.* (2023) Transcriptomes and metabolism define mouse and human MAIT cell populations. *Sci. Immunol.* 8, eabn8531
15. Legoux, F. *et al.* (2019) Molecular mechanisms of lineage decisions in metabolite-specific T cells. *Nat. Immunol.* 20, 1244–1255
16. Corbett, A.J. *et al.* (2014) T-cell activation by transitory neoantigens derived from distinct microbial pathways. *Nature* 509, 361–365
17. Ito, E. *et al.* (2024) Sulfated bile acid is a host-derived ligand for MAIT cells. *Sci. Immunol.* 9, eade6924
18. Ito, E. and Yamasaki, S. (2024) Regulation of MAIT cells through host-derived antigens. *Front. Immunol.* 15, 1424987
19. van Wilgenburg, B. *et al.* (2016) MAIT cells are activated during human viral infections. *Nat. Commun.* 7, 11653
20. Ussher, J.E. *et al.* (2014) CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *Eur. J. Immunol.* 44, 195–203
21. Lin, X. *et al.* (2024) Mucosal-associated invariant T cells in infectious diseases of respiratory system: recent advancements and applications. *J. Inflamm.* 21, 6
22. Provine, N.M. and Klenerman, P. (2020) MAIT cells in health and disease. *Annu. Rev. Immunol.* 38, 203–228
23. Toubal, A. *et al.* (2019) Mucosal-associated invariant T cells and disease. *Nat. Rev. Immunol.* 19, 643–657
24. Leeansyah, E. *et al.* (2021) Emerging role for MAIT cells in control of antimicrobial resistance. *Trends Microbiol.* 29, 504–516
25. Legoux, F. *et al.* (2019) Microbial metabolites control the thymic development of mucosal-associated invariant T cells. *Science* 366, 494–499
26. Constantinides, M.G. *et al.* (2019) MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science* 366, eaax6624
27. El Morr, Y. *et al.* (2024) MAIT cells monitor intestinal dysbiosis and contribute to host protection during colitis. *Sci. Immunol.* 9, eadi8954
28. Jabeen, M.F. and Hinks, T.S.C. (2023) MAIT cells and the microbiome. *Front. Immunol.* 14, 1127588
29. Tastan, C. *et al.* (2018) Tuning of human MAIT cell activation by commensal bacteria species and MR1-dependent T-cell presentation. *Mucosal Immunol.* 11, 1591–1605
30. Gnirck, A.-C. *et al.* (2023) Mucosal-associated invariant T cells contribute to suppression of inflammatory myeloid cells in immune-mediated kidney disease. *Nat. Commun.* 14, 7372
31. Cho, Y.-N. *et al.* (2014) Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. *J. Immunol.* 193, 3891–3901
32. Walkenhorst, M. *et al.* (2024) Protective effect of TCR-mediated MAIT cell activation during experimental autoimmune encephalomyelitis. *Nat. Commun.* 15, 9287
33. Crowther, M.D. and Sewell, A.K. (2021) The burgeoning role of MR1-restricted T-cells in infection, cancer and autoimmune disease. *Curr. Opin. Immunol.* 69, 10–17
34. Lamichhane, R. *et al.* (2019) TCR- or cytokine-activated CD8(+) mucosal-associated invariant T cells are rapid polyfunctional effectors that can coordinate immune responses. *Cell Rep.* 28, 3061–3076.e5
35. Chen, Z. *et al.* (2017) Mucosal-associated invariant T-cell activation and accumulation after in vivo infection depends on microbial riboflavin synthesis and co-stimulatory signals. *Mucosal Immunol.* 10, 58–68
36. Ussher, J.E. *et al.* (2016) TLR signaling in human antigen-presenting cells regulates MR1-dependent activation of MAIT cells. *Eur. J. Immunol.* 46, 1600–1614
37. Salio, M. *et al.* (2017) Activation of human mucosal-associated invariant T cells induces CD40L-dependent maturation of monocyte-derived and primary dendritic cells. *J. Immunol.* 199, 2631–2638
38. Ruf, B. *et al.* (2023) Tumor-associated macrophages trigger MAIT cell dysfunction at the HCC invasive margin. *Cell* 186, 3686–3705.e32
39. Li, Y.-R. *et al.* (2022) Targeting immunosuppressive tumor-associated macrophages using innate T cells for enhanced antitumor reactivity. *Cancers* 14, 2749
40. Ruf, B. *et al.* (2021) Activating mucosal-associated invariant T cells induces a broad antitumor response. *Cancer Immunol. Res.* 9, 1024–1034
41. Treiner, E. *et al.* (2003) Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 422, 164–169
42. Koay, H.-F. *et al.* (2016) A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nat. Immunol.* 17, 1300–1311
43. Park, S.H. *et al.* (2000) Unaltered phenotype, tissue distribution and function of Valpha14(+) NKT cells in germ-free mice. *Eur. J. Immunol.* 30, 620–625
44. Seach, N. *et al.* (2013) Double-positive thymocytes select mucosal-associated invariant T cells. *J. Immunol.* 191, 6002–6009
45. Rahimpour, A. *et al.* (2015) Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. *J. Exp. Med.* 212, 1095–1108
46. Trompette, A. and Ubags, N.D. (2023) Skin barrier immunology from early life to adulthood. *Mucosal Immunol.* 16, 194–207
47. Nel, I. *et al.* (2021) MAIT cells, guardians of skin and mucosa? *Mucosal Immunol.* 14, 803–814
48. Michaëlsson, J. *et al.* (2006) Regulation of T cell responses in the developing human fetus. *J. Immunol.* 176, 5741–5748
49. Leeansyah, E. *et al.* (2014) Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nat. Commun.* 5, 3143
50. Serriari, N.-E. *et al.* (2014) Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clin. Exp. Immunol.* 176, 266–274
51. Illés, Z. *et al.* (2004) Accumulation of Valpha7.2-Jalpha33 invariant T cells in human autoimmune inflammatory lesions in the nervous system. *Int. Immunol.* 16, 223–230
52. Wright, E.K. *et al.* (2015) Recent advances in characterizing the gastrointestinal microbiome in Crohn's disease: a systematic review. *Inflamm. Bowel Dis.* 21, 1219–1228
53. Walker, A.W. *et al.* (2011) High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. *BMC Microbiol.* 11, 7

54. Andoh, A. *et al.* (2011) Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. *J. Gastroenterol.* 46, 479–486
55. Drago, L. *et al.* (2016) Skin microbiota of first cousins affected by psoriasis and atopic dermatitis. *Clin. Mol. Allergy* 14, 2
56. Cosgrove, C. *et al.* (2013) Early and nonreversible decrease of CD161++/MAIT cells in HIV infection. *Blood* 121, 951–961
57. Wong, E.B. *et al.* (2013) Low levels of peripheral CD161++CD8+ mucosal associated invariant T (MAIT) cells are found in HIV and HIV/TB co-infection. *PLoS One* 8, e83474
58. Fernandez, C.S. *et al.* (2015) MAIT cells are depleted early but retain functional cytokine expression in HIV infection. *Immunol. Cell Biol.* 93, 177–188
59. Leeansyah, E. *et al.* (2013) Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. *Blood* 121, 1124–1135
60. Mariat, D. *et al.* (2009) The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol.* 9, 123
61. Soto, A. *et al.* (2014) Lactobacilli and bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. *J. Pediatr. Gastroenterol. Nutr.* 59, 78–88
62. Dias, J. *et al.* (2016) Human MAIT-cell responses to *Escherichia coli*: activation, cytokine production, proliferation, and cytotoxicity. *J. Leukoc. Biol.* 100, 233–240
63. Wong-Rolle, A. *et al.* (2021) Unexpected guests in the tumor microenvironment: microbiome in cancer. *Protein Cell* 12, 426–435
64. Nejman, D. *et al.* (2020) The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* 368, 973–980
65. Soudais, C. *et al.* (2015) *In vitro* and *in vivo* analysis of the gram-negative bacteria-derived riboflavin precursor derivatives activating mouse MAIT cells. *J. Immunol.* 194, 4641–4649
66. Petley, E.V. *et al.* (2021) MAIT cells regulate NK cell-mediated tumor immunity. *Nat. Commun.* 12, 4746
67. Yan, J. *et al.* (2020) MAIT cells promote tumor initiation, growth, and metastases via tumor MR1. *Cancer Discov.* 10, 124–141
68. Gherardin, N.A. *et al.* (2016) Diversity of T cells restricted by the MHC class I-related molecule MR1 facilitates differential antigen recognition. *Immunity* 44, 32–45
69. Li, S. *et al.* (2020) Human tumor-infiltrating MAIT cells display hallmarks of bacterial antigen recognition in colorectal cancer. *Cell Rep. Med.* 1, 100039
70. Kostic, A.D. *et al.* (2013) *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14, 207–215
71. Bullman, S. *et al.* (2017) Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science* 358, 1443–1448
72. Zumwalde, N.A. *et al.* (2018) Mucosal associated invariant T cells from human breast ducts mediate a Th17-skewed response to bacterially exposed breast carcinoma cells. *Breast Cancer Res.* 20, 111
73. Shi, L. *et al.* (2023) Clinicopathological and predictive value of MAIT cells in non-small cell lung cancer for immunotherapy. *J. Immunother. Cancer* 11, e005902
74. Pignatelli, P. *et al.* (2023) The role of *Fusobacterium nucleatum* in oral and colorectal carcinogenesis. *Microorganisms* 11, 2358
75. Gao, R. *et al.* (2017) Gut microbiota and colorectal cancer. *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol.* 36, 757–769
76. He, D. *et al.* (2010) IL-17 promotes tumor development through the induction of tumor promoting microenvironments at tumor sites and myeloid-derived suppressor cells. *J. Immunol.* 184, 2281–2288
77. Zhang, X. *et al.* (2024) The role of interleukin-17 in inflammation-related cancers. *Front. Immunol.* 15, 1479505
78. Li, Y.-R. *et al.* (2022) Target tumor microenvironment by innate T cells. *Front. Immunol.* 13, 999549
79. Li, Y.-R. *et al.* (2023) Exploring the dynamic interplay between cancer stem cells and the tumor microenvironment: implications for novel therapeutic strategies. *J. Transl. Med.* 21, 686
80. Veglia, F. *et al.* (2021) Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat. Rev. Immunol.* 21, 485–498
81. Dogan, M. *et al.* (2022) Engineering human MAIT cells with chimeric antigen receptors for cancer Immunotherapy. *J. Immunol.* 209, 1523–1531
82. Zhang, N. *et al.* (2021) Dysbiosis of gut microbiota promotes hepatocellular carcinoma progression by regulating the immune response. *J Immunol Res* 2021, 4973589
83. Hou, X. *et al.* (2022) Effects of gut microbiota on immune responses and immunotherapy in colorectal cancer. *Front. Immunol.* 13, 1030745
84. Low, K.B. *et al.* (1999) Lipid A mutant *Salmonella* with suppressed virulence and TNFalpha induction retain tumor-targeting in vivo. *Nat. Biotechnol.* 17, 37–41
85. Nemunaitis, J. *et al.* (2003) Pilot trial of genetically modified, attenuated *Salmonella* expressing the *E. coli* cytosine deaminase gene in refractory cancer patients. *Cancer Gene Ther.* 10, 737–744
86. Le, D.T. *et al.* (2015) Safety and survival with GVAX pancreas prime and *Listeria monocytogenes*-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 33, 1325–1333
87. Janku, F. *et al.* (2021) Intratumoral injection of *Clostridium novyi*-NT spores in patients with treatment-refractory advanced solid tumors. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 27, 96–106
88. Gurbatri, C.R. *et al.* (2020) Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *Sci. Transl. Med.* 12, eaax0876
89. Chowdhury, S. *et al.* (2019) Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat. Med.* 25, 1057–1063
90. Ugwu, O.P.-C. *et al.* (2024) Mechanisms of microbiota modulation: Implications for health, disease, and therapeutic interventions. *Medicine (Baltimore)* 103, e38088
91. Zheng, Y. *et al.* (2024) MAIT cell activation and recruitment in inflammation and tissue damage in acute appendicitis. *Sci. Adv.* 10, eadn6331
92. Ouyang, L. *et al.* (2022) Mucosal-associated invariant T cells reduce and display tissue-resident phenotype with elevated IL-17 producing capacity in non-small cell lung cancer. *Int. Immunopharmacol.* 113, 109461
93. Qu, J. *et al.* (2024) CXCR6-positive circulating mucosal-associated invariant T cells can identify patients with non-small cell lung cancer responding to anti-PD-1 immunotherapy. *J. Exp. Clin. Cancer Res.* 43, 134
94. Chen, P. *et al.* (2024) Modulation of gut microbiota by probiotics to improve the efficacy of immunotherapy in hepatocellular carcinoma. *Front. Immunol.* 15, 1504948
95. Cassidy, F.C. *et al.* (2023) Glycogen-fuelled metabolism supports rapid mucosal-associated invariant T cell responses. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2300566120
96. Martin, E. *et al.* (2009) Stepwise development of MAIT cells in mouse and human. *PLoS Biol.* 7, e54
97. Pellicci, D.G. *et al.* (2020) Thymic development of unconventional T cells: how NKT cells, MAIT cells and γδ T cells emerge. *Nat. Rev. Immunol.* 20, 756–770
98. Gherardin, N.A. *et al.* (2018) Human blood MAIT cell subsets defined using MR1 tetramers. *Immunol. Cell Biol.* 96, 507–525
99. Leeansyah, E. *et al.* (2015) Arming of MAIT cell cytolytic antimicrobial activity is induced by IL-7 and defective in HIV-1 infection. *PLoS Pathog.* 11, e1005072
100. Bugaut, H. *et al.* (2024) A conserved transcriptional program for MAIT cells across mammalian evolution. *J. Exp. Med.* 221, e20231487