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Dysfunctional states of unconventional T-cell subsets in cancer

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Abstract

Unconventional T cells represent a promising therapeutic agent to overcome the current limitations of immunotherapies due to their universal T-cell receptors, ability to respond directly to cytokine stimulation, and capacity to recruit and modulate conventional immune cells in the tumor microenvironment. Like conventional T cells, unconventional T cells can enter a dysfunctional state, and the functional differences associated with this state may provide insight into the discrepancies observed in their role in antitumor immunity in various cancers. The exhaustive signature of unconventional T cells differs from conventional αβ T cells, and understanding the differences in the mechanisms underlying exhaustive differentiation in these cell types may aid in the discovery of new treatments to improve sustained antitumor responses. Ongoing clinical trials investigating therapies that leverage unconventional T-cell populations have shown success in treating hematologic malignancies and reducing the immunosuppressive tumor environment. However, several hurdles remain to extend these promising results into solid tumors. Here we discuss the current knowledge on unconventional T-cell function/dysfunction and consider how the incorporation of therapies that modulate unconventional T-cell exhaustion may aid in overcoming the current limitations of immunotherapy. Additionally, we discuss how components of the tumor microenvironment alter the functions of unconventional T cells and how these changes can affect tumor infiltration by lymphocytes and alter conventional T-cell responses.

Keywords

cancer; dysfunction; exhaustion; immunotherapy; unconventional T cells

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1. Introduction

Immune cell exhaustion represents a complex set of cellular changes that are currently defined as a state in which a cell has impaired functionality and undergoes epigenetic changes as a result of a prolonged response to antigen stimulation.[1] Recent efforts to understand how cellular exhaustion contributes to immunotherapy resistance have primarily focused on conventional $CD8^+$ T cells and do not consider how unconventional T-cell (UCT) exhaustion contributes to treatment resistance. Additionally, mechanisms of exhaustion in UCT populations have not been widely studied in cancer but have been characterized in chronic viral infections such as HIV and hepatitis B virus (HBV). As with prior studies in conventional T cells, these viral-based exhaustion models provide important insight into the distinct transcriptional activation profiles of UCTs and could be translated into cancer research. Studying differences in exhaustion states as well as functional differences between conventional and unconventional T cells could have a profound impact on the future of immunotherapy research through the identification of novel therapeutic strategies and mechanisms of resistance.

UCTs are defined as cells that express invariant or semi-invariant T-cell receptors (TCRs) and do not recognize peptide antigens. Instead, they respond to a diverse range of nonpeptide antigens presented by nonpolymorphic antigen-presenting and B7-like molecules such as butyrophilins, CD1, and MR1.[2] The primary subtypes of UCTs include γδ T cells, invariant natural killer T (iNKT) cells, and mucosal-associated invariant T (MAIT) cells. Together, these cells make up approximately 7% of circulating T cells at steady state in humans and constitute the majority of T cells in many mucosal tissues.[3] Due to their unique localization and ability to rapidly respond to cytokine stimulation and nonpeptide antigens, UCTs bridge innate and adaptive immune responses.[4] Their unique function may act to promote or inhibit antitumor immunity as UCTs are easily influenced by the tumor microenvironment (TME) or by cancer-related changes to the microbiome and epigenome. UCTs have contradicting roles in cancer immunology, and their presence in the TME has been associated with both protumor and antitumor functionality and varying clinical outcomes in different cancers. Since these cells have the potential to rapidly respond to nonpeptide antigens, release cytokines, and regulate other cellular responses,[5] implementing therapies that mitigate or overcome UCT exhaustion may improve responses to immunotherapy. In this review, we summarize the current state of the field with regard to UCT function/dysfunction in cancer, focusing on translationally relevant animal models and clinical studies, to discuss how mitigating UCT exhaustion may influence the efficacy of current and future immunotherapies. Additionally, we examine how the TME impacts UCT function and how functional impairment of these cells affects lymphocyte infiltration and alters the response of conventional T cells in cancer.

2. Markers of exhaustive differentiation in unconventional T cells

While T-cell exhaustion is complex, and a definitive set of validated biological markers remains to be fully elucidated, conventional T cells that are classified as exhausted typically co-express a combination of inhibitory receptors such as PD-1, CTLA4, Lag3, TIGIT, TIM3, and FoxP3 while exhibiting cell cycle arrest and decreased cytolytic activity.[1,6–8]

Exhausted T cells can be further classified along a differentiation trajectory, between progenitor exhausted (PD-1^{+}TCF1^{+}CD8^{+}) and terminally exhausted (PD-1^{+}TCF1^{-}CD8^{+}) T cells.[9] Progenitor exhausted T cells are able to respond to immunotherapy, and their dysfunctional properties can be reversed while terminally exhausted T cells are in a permanent state of exhaustion that cannot be mitigated by currently known mechanisms.[9,10] Although UCTs express many of the same inhibitory receptors as conventional T cells, defining an exhausted phenotype for these cells is complex as some of these exhaustion markers (e.g. PD-1) are constitutively expressed, and the pathways involved in their function have not been entirely elucidated.[11] Furthermore, each unconventional cell type is characterized by expression of different levels of exhaustive markers, adding to the complexity of this topic.

2.1 MAIT cells

MAIT cells express a semi-invariant TCR with the α chain comprising TRAV1–2 TRAJ33/20/12 paired most commonly with members of the TRBV6 family and TRBV20–1 TCRβ chain in humans.[12] In mice, MAIT cells utilize the orthologous TRAV1-TRAJ33 TCRa chain paired frequently with a TRBV19 or TRBV13 β chain.[12–14] MAIT cells are able to recognize small metabolite products of bacterial and yeast riboflavin biosynthesis when presented by the nonpolymorphic major histocompatibility complex (MHC) class I–like molecule MR1.[13] Upon activation, MAIT cells produce a variety of cytokines, including IFNγ, TNFα, and IL-17, as well as cytolytic and other effector molecules, including perforin, granulysin, and granzymes (GZM) A, GZM B, GZM K, GZM M, and GZM H.[13,15] Through the upregulation of CD40L following MR1-dependent activation, MAIT cells are able to induce the maturation of primary and monocyte-derived dendritic cells (DCs).[16] MAIT cells are also able to promote proliferation and induce the priming of CD8^{+} T cells, although these mechanisms are less described.[17]

The role of MAIT cells in cancer is poorly understood due to their heterogeneity both in the peripheral blood and in the tumor. The frequency of these cells in the circulation and the TME is associated with different prognoses depending on the type of cancer. A potential explanation for the differences observed in MAIT cell function in various cancers is that altered gene expression due to differences in epigenetic states that result from the TME and tissue-specific microenvironments may result in variations in chemokine expression, thus affecting chemotaxis and the recruitment of other lymphocyte populations. These differences may also explain why different cancers have contrasting levels of MAIT cells in the peripheral blood compared to the tumor. Another possible explanation for this discrepancy is that certain cancers may have a greater predisposition for MAIT cell exhaustion. Currently, MAIT cell exhaustion is poorly understood and has only been specifically studied in the context of viral infections such as HIV and in colon cancer.[18,19] It has been established that terminally exhausted MAIT cells, currently defined as PD-1^{high}Tim-3^{+}CD39^{+}, have been shown to accumulate in severe viral infections and colon cancer (Fig. 1, Table 1). For example, MAIT cells from the peripheral blood of patients with HIV displayed increased PD-1 expression and decreased frequency that did not recover after initiation of treatment.[20] In patients with chronic HBV infection, MAIT cells with increased PD-1 expression produced lower levels of granzyme B and IFNγ after stimulation compared

to healthy controls.[21] In colorectal cancer, an increased infiltration of MAIT cells has been associated with poor clinical outcomes.[18] The ability of MAIT cells from colon tumors to produce IFNγ is significantly impaired compared to MAIT cells from normal colonic mucosa.[18] Furthermore, inhibiting PD-L1/PD-1 signaling in colorectal patients using immune checkpoint inhibitors (ICIs) resulted in increased expression of CD25 and coexpression of HLA-DR and CD38, potentially suggesting that these cells are progenitor exhausted T cells.[18]

Dysfunctional MAIT cells, which are typically characterized by lack of effector function and a similar PD-1^{high}Tim-$3^{+}$$CD39^{+}$ profile, have also been shown to occur in cancers such as epithelial ovarian cancer, melanoma, hepatocellular carcinoma (HCC), non–small cell lung carcinoma (NSCLC), and esophageal adenocarcinoma (ESCA) (Table 1).[18,22,24,34] Similar trends of functional impairment in MAIT cells can be observed across all of these malignancies. In NSCLC, MAIT cells with a PD-$1^{+}$$TIM3^{+}$$CD39^{+}$ profile exhibited decreased IFNγ production and an increase in IL-17A and IL-8 secretion.[25] In ovarian cancer elicited ascites, MAIT cells were shown to lose their IL-17A and IFNγ production capacity as the tumor progressed.[22] Tumor-infiltrating MAIT cells from hepatocellular carcinoma that displayed a $CCR7^{-}$$CD45RA^{-}$$CD45RO^{+}$$CD95^{+}$ effector memory phenotype also had diminished effector capabilities and decreased IL-17, IFNγ, GZM B, and perforin production while increasing IL-8 production and upregulating inhibitory molecules.[24] Perhaps due to the unique composition of the TME in these cancers, MAIT cells in these patients tend to lose their effector function and undergo functional impairment.

Contrary to the observations of reduced MAIT cell function in ovarian cancer, HCC, and NSCLC, in certain cancers such as melanoma and ESCA, lower MAIT cell infiltration is typically associated with a worse response to immunotherapy in patients.[23,35] In the presence of ESCA tumor conditioned media, MAIT cells reduced expression of IFNγ and TNFα, but ESCA cell line viability was significantly reduced when exposed to expanded MAIT cells, suggesting that MAIT cellular therapy may be beneficial for these patients.[23] In patients with melanoma undergoing anti–PD-1 therapy, patients who responded to therapy were found to have a higher frequency of activated and proliferating MAIT cells throughout treatment that produced more granzyme B.[35] These findings emphasize the need to examine differences in MAIT cell populations between responders and nonresponders to therapy as the expansion of MAIT cells or the infusion of MAIT cells from healthy donors may help promote responses to therapy in certain patients.

Many cancers express MR1 transcript and protein either on the tumor or in the TME, which could be contributing to MAIT cell exhaustion through overstimulation. High levels of MR1 expression in colorectal cancer, head and neck cancer, pancreatic cancer, stomach cancer, and testicular cancer correlated with a worse outcome for patients, while in lung and renal cancer, high levels of MR1 correlated with a favorable prognosis.[36] In glioblastoma, colorectal, pancreatic, stomach, and testicular cancer, a positive correlation was recently discovered between MAIT cell activation, MR1 upregulation, and the recruitment of tumor-associated neutrophils (TANs) and myeloid-derived suppressor cells (MDSCs) to the TME, possibly through IL-17 secretion and chemokine recruitment.[36] In glioblastoma, MR1 upregulation as well as the presence of MAIT cells in a sample correlated with

high activation of TANs/MDSCs compared to samples that did not have MAIT cells, suggesting that MAIT cell impairment in certain cancers may be responsible for promoting an immunosuppressive environment.[37] It is also possible that the reverse of this scenario is true and that TANs/MDSCs influence MAIT cell dysregulation in cancer. More studies are needed to understand the mechanism of MAIT cell dysfunction and the effect it has on other immunosuppressive cell populations in cancer. Understanding this dynamic and the underlying mechanisms will help to identify MAIT-based immunotherapies.

2.2 iNKT cells

Although there are different populations of NKT cells in humans, this section primarily focuses on iNKT cells, which use the invariant TRAV10-TRAJ18 gene-encoded TCRα chain paired most frequently with the TCRβ chain encoded by the TRBV25 gene.[38] iNKT cells can further be classified by their cytokine profiles and grouped into Th1-like, Th2-like, and Th17-like cells.[39] In healthy individuals, iNKT subsets are present at low levels in peripheral blood as well as in many organs. The highest frequency of iNKT cells can be found in the liver, colon, kidneys, and omentum.[40] These cells are CD1d restricted and respond to stimulation from α-galactosyl ceramide (αGalCer) and other microbial glycolipid antigens. They are also able to respond to cytokines and TLR signaling. Upon activation, they secrete immunoregulatory cytokines such as IFNγ, TNFα, and IL-4.[41] The role of iNKT cells in cancer remains unclear as they exhibit both antitumor properties and immunosuppressive functions.[42] Recent studies have demonstrated the potent antitumor properties iNKT cells have through the direct killing of CD1d-expressing cancer cells, the activation of NK and $CD8^+$ T cells, DC maturation, and the inhibition of MDSC/TAM immunosuppressive functions.[41,42] Prior studies have also demonstrated that mice with TP53 mutations lacking iNKT cells or CD1d are more susceptible to tumorigenesis than wild-type littermates.[43] P53 tumor suppressor mutations are among the most common mutation in cancer, and in $P53^{+/-}$ mice, this alteration leads to the development of sarcomas, carcinomas, and hematopoietic tumors.[43] αGalCer and TNF-related apoptosis ligand, which is upregulated on activated iNKT cells, has been shown to suppress tumor growth in P53 mutant mice, suggesting that these cells may have antitumor ability in human tumors with P53 mutations as well.[43,44] iNKT cells' potential as tumor-suppressive cells, as well as the recent preliminary clinical success of chimeric antigen receptor (CAR)-iNKT cells, has piqued the interest of many researchers, but more studies are needed to understand how to harness their potential for effective immunotherapy.

Several cancers such as anaplastic thyroid carcinoma and chronic lymphocytic leukemia overexpress CD1d.[45,46] Patients with higher CD1d expression had lower iNKT cell numbers and exhibited a lower IL-7 receptor a chain (CD127) expression on iNKT cells, indicating that tumor CD1d overexpression may be contributing to iNKT cell exhaustion in these cancers.[45,46] In cancers displaying decreased CD1d expression such as glioblastoma and melanoma, increased recognition of CD1d lead to improved iNKT cell-mediated responses.[47,48] Another successful strategy to improve iNKT cell function in murine models of cancer has been the incorporation of αGalCer-based immunotherapy, which has been shown to significantly improve iNKT cell proliferation and cytokine production.[49] Experiments involving the activation of iNKT cells in B16F10 OVA-bearing mice showed

that activation of iNKT via αGalCer restores the effector function of iNKT cells via iNKT-induced IL-2 and IL-12 production.[50] Additional studies have shown that tumor-localized administration of αGalCer improved tumor suppression by enhanced recruitment of iNKT cells into solid tumors.[51] The administration route of αGalCer is important for this therapy to be effective as certain conditions and the delivery of free αGalCer into the intraperitoneal cavity of mice have been shown to promote Treg expansion.[49,52] Conversely, the delivery of dendritic cell–derived exosomes loaded with αGalCer could effectively suppress B16 tumor growth in mice.[49,52] These studies show that iNKT cells, particularly in an environment with chronic inflammation, can display altered phenotypic characteristics that, when mitigated, may improve responses to immunotherapy.

Although little is currently known about iNKT cell exhaustion in cancer, recent studies have shown an altered iNKT phenotype in other inflammatory conditions such as sarcoidosis and demonstrated that iNKT function in these conditions can be restored with anti–PD-1 therapy.[53] Exhausted iNKT cells exhibit diminished cytokine production and lose their polyfunctionality. Specifically, double-negative (DN) iNKT cells had higher percentages of the activation markers CD69 and CD56, lower secretion of IFNγ, and impaired ability to function as dual secreting $IFN\gamma^{+}TNF\alpha^{+}$ compared to healthy controls (Fig. 1, Table 1).[28] The patients who had impaired dual functionality were also found to have higher PD-1 levels compared to both healthy controls and patients with sarcoidosis who still retained normal iNKT function, suggesting that these patients may benefit from anti–PD-1 therapy.[28] In murine cancer models, peroxisome proliferator-activated receptor gamma (PPARγ) has been shown to improve iNKT cell antitumor capacity by increasing IFNγ synthesis.[54,55] PPARγ agonists have shown promise when used in combination with conventional immunotherapy as they reduce the production of inflammatory cytokines and sensitize cells to therapy, but their direct effect on iNKT cell function remains to be investigated.[56] iNKT cell exhaustion has also been described in HIV infection. Patients with HIV experienced decreased CD1d expression, and $CD4^{+}$ and $CD4^{-}$ iNKT cells from these patients were shown to inhibit IFNγ, TNFα, and IL-4 secretion in following αGalCer/IL-2/phorbol myristate acetate(PMA) stimulation.[29] The functions of these cells were not able to be restored after highly active retroviral therapy, suggesting a permanently exhaustive phenotype. Other viral infections such as lymphocytic choriomeningitis virus and herpes simplex virus 1 have also been shown to cause functional impairment of iNKT cells through the inhibition of CD1d antigen presentation by reducing surface CD1d on DCs and macrophages and suppressing CD1d recycling on the cell surface.[30]

2.3 γδ T cells

Compared to αβ T cells, γδ T cells have a limited number of variable g and d gene segments for γδ T-cell receptor rearrangement.[57] These cells recognize phosphoantigens, small peptides, MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB), mycobacterial heatshock proteins, MR1, CD1d, and butyrophilins, and upon activation, they produce Th1 cytokines such as IFNγ and TNFα.[58,59] γδ T cells also express receptors such as FcγRIIIa (CD16a), DNAM-1, and NKG2D, which participate in tumor recognition.[60] Allogeneic γδ T-cell therapy has shown remarkable results in the treatment of hematologic malignancies. Recently, γδ T-cell therapy has shown durable

complete responses in acute myeloid leukemia (AML) in a phase I trial, and other clinical trials are now recruiting patients with AML who have a high risk of relapse following allogeneic hematopoietic stem cell transplantation to determine the maximum tolerated dose and the efficacy of artificial antigen-presenting cell–expanded donor γδ T cells.[61] These studies have not investigated the long-term efficacy of adoptively transferring ex vivo expanded γδ T cells, but the limited off-target effects and the objective responses thus far have made this approach a feasible option for patients.

Although γδ T-cell therapy has shown great promise in treating hematologic malignancies, its efficacy in treating solid tumors is limited by a multitude of factors, one of which is cellular exhaustion. γδ T cells in tumors display a variable exhaustive phenotype, but typically, γδ T cells with an increased exhaustive state are thought to coexpress PD-1, TIM3, CD39, and TIGIT (Fig. 1, Table 1).[31,32] Unlike conventional αβ T cells, high PD-1 expression on γδ T cells is not always indicative of cellular exhaustion as there are tissue-resident populations of PD-1^{high} γδ T cells, and PD-1 expression has been shown to not be a determinant of functional impairment.[31] Furthermore, it has been shown that high TIM3, but not PD-1 expression, correlates with impaired IFNγ production in γδ T cells.[62,63] Another recent study examining the similarities between markers of exhaustion in conventional $CD8^+$ T cells and γδ T cells in renal cell carcinoma showed that $V\delta2^-$ cells, which are predicted to be the main population of γδ T cells that express PD-1, TIGIT, and TIM3, do not exhibit decreased effector functions even when expressing markers related to a conventional exhaustive phenotype.[33] Interestingly, these $PD\text{-}1^+TIGIT^+TIM3^+V\delta2^-$ γδ T cells were present only in the tumor and not in healthy tissue and showed an increase in PD-1 and 4–1BB expression.[33] This brings up many questions regarding how γδ T cells respond differently to immunotherapy compared to conventional T cells and how the expansion of γδ T cells in patients may be useful for mitigating T-cell exhaustion and improving responses to immunotherapy. These unique properties of γδ T cells also make them promising candidates for tumor-infiltrating lymphocyte (TIL) therapy, particularly in cancers such as renal cell carcinoma (RCC), where responses to traditional immunotherapies are often low.

Vγ9 Vδ2T cells are enriched in TILs and are able to directly kill tumor cells independent of HLA presentation through upregulating cytotoxic molecules such as granzyme and perforin, making them excellent targets for immunotherapy.[57,60] γδ T cells can also function as antigen-presenting cells (γδ-APCs) and regulate $CD4^+$ or $CD8^+$ T-cell function, stimulate cytotoxicity of natural killer cells through CD137L, and inhibit DC maturation and impair αβ T-cell activation through the TLR8 signaling pathway.[59,64,65] The immunosuppressive functions of γδ T cells are also important to consider as PD-L1–expressing γδ T cells suppress cytotoxic αβ $CD8^+$ T cells and are able to promote tumor metastasis through the expression of RORγt and STAT3 to increase IL-17 production, which has been linked to tumor cell proliferation and metastasis.[57] One way that γδ T cells become activated is through the binding of pathogen-associated molecular patterns to a TLR, which causes activation through the myeloid differentiation factor (Myd-88) pathway.[64,66] γδ T cells have been shown to respond favorably to TLR agonists by improving cytotoxicity and decreasing exhaustion risk. TLR2 and TLR8/9 activation both led to improved γδ T-cell IFNγ production and the polarization of DCs into an IL-12p70–producing phenotype that

promotes TC1-polarized immunity.[67] Thus, the incorporation of specific TLR agonists in combination with immunotherapy may limit the immunosuppressive functions of γδ T cells in some cancers and result in better outcomes by improving conventional T-cell responses.

2.4 Microbiome

Cancer is known to significantly influence the microbiome in both the tumor and the body by altering gene expression and changing the secretion patterns of small-molecule metabolites that have a profound effect on energy production, metabolism, biosynthesis, and immune responses.[68] Because MAIT cells, γδ T cells, and iNKT cells are able to recognize small-molecule metabolites/microbial antigens and are concentrated primarily in mucosal surfaces, where there is the most diversity in the microbiome, it is important to understand what specific changes in the microbiome occur in association with cancer in order to limit resulting immunosuppression.

Various components of the microbiome, including commensal bacterial populations and microbial metabolite production, can alter cellular function and drug metabolism. Most of the current research that has specifically investigated how the tumor microbiome affects UCT function has focused on γδ T cells and MAIT cells. A recent study has shown that the TME has a profound impact on γδ T-cell function in both murine and human colorectal cancer (CRC) and that the γδ T cells that were present in the tumor contributed to tumor growth. On the other hand, tumor-adjacent tissue-resident γδ T cells could effectively eliminate tumors in mouse models, suggesting that the TME influences the cytotoxic function of these cells.[69] This was in part due to an enrichment of IL-17–producing γδ T cell genes in the tumors while γδ T cells in the adjacent nontumor areas maintained a cytotoxic profile.[69] It was further confirmed in mice that this effect was due to the microbiome composition within the gut as mice treated with a broad-spectrum antibiotic cocktail had a reduced frequency of IL-17– and PD-1–producing γδ T cells.[69] $V\gamma6^+$ cells were a substantial source of IL-17 in that model and have been shown to respond to microbiota signaling.[69] Studies examining the phenotype of γδ T cells confirmed their tendency to differentiate into γδ T-17 cells in many cancers, including squamous cell carcinoma, lung cancer, breast cancer, and CRC.[66] In lung adenocarcinoma, commensal bacteria were shown to stimulate Myd-88–dependent IL-1β and IL-23 myeloid cell production, which induced the proliferation of $V\gamma6^+V\delta1^+$ γδ T cells that produce IL-17, thus further promoting tumor development.[70] Increased secretion of IL-17 by γδ T cells has been correlated with increased expression of granulocyte-macrophage colony stimulating factor, which has been linked to the accumulation of PMN-MDSCs and TANs in tumors.[64] Similar Th-17 polarization tendencies have been observed in MAIT and iNKT cells in CRC, breast cancer, and lung adenocarcinoma, further emphasizing the importance of the organ-specific microbiome composition in the function of immune cells and cancer progression.[71]

MAIT cells are highly sensitive to the signals from the host microbiota, and even small changes in microbiome composition have a significant effect on MAIT cell function and expansion.[72] In both CRC and melanoma, the abundance of MAIT cells in the circulation and the TME correlated with response to immunotherapy.[35,72] In CRC, certain pathogenic

bacterium such *Parvimonas* and *Bilophila* were negatively correlated with MAIT cell frequency, and the presence of this bacterium led to poorer responses to immunotherapy.[72] Bacteriotherapy such as fecal microbiome transplants may be beneficial in certain cancers to restore microbiome diversity and improve responses to immunotherapy. A recent study has shown that fecal microbiome transplantation (FMT) combined with ipilimumab and nivolumab can improve human MAIT cell function in metastatic renal cell carcinoma.[73] MAIT cells following FMT upregulated CD69, downregulated PD-1, and had an improved TNFα response when stimulated with IL-12 and IL-18 ex vivo. This study also found that reduced γδ T-cell frequencies in the peripheral blood of patients with RCC were restored with fecal microbiome transplant while the percentage of iNKT cells in the peripheral blood remained elevated regardless of if a transplant was performed.[73] These results warrant further investigation into how microbiome composition affects UCT functions and how changes in functionality may affect tumor growth as well as responses to immunotherapy.

3. Epigenetic contributors to T-cell exhaustion

3.1 Transcription factors

Epigenetic modifications have a profound impact on T-cell function and contribute to the inability of exhausted T cells to be reinvigorated from the PD-1 blockade. Epigenetic changes contributing to cellular exhaustion as a result of cancer or infection in UCT populations have not been widely studied. Due to the important role of UCTs in cancer immunity, comparing differences in the transcriptional profiles of exhausted UCT and conventional T cells may help identify new targets to combat cellular exhaustion. Current markers of epigenetic alterations in exhausted T cells include the upregulation of transcription factors such as nuclear factor for activated T cells (NFAT), interferon regulatory factor 4 (IRF4), basic leucine zipper ATF-like transcription factor (BATF), T box transcription factor (T-bet), eomesodermin (Eomes), B lymphocyte-induced maturation protein 1 (BLIMP1), and thymocyte selection-associated HMG box (Tox).[74,75] NFAT has been shown to promote $CD8^+$ T-cell exhaustion by binding to sites that do not cooperate with activator protein 1 (AP1), thereby preventing the induction of genes involved in cytokine production and activation.[76] Defective NFAT1 signaling is also predicted to be responsible for the impaired ability of MAIT cells to produce IFNγ in patients with multiple sclerosis.[77] NFAT is important for iNKT and γδ T-cell differentiation and thus may also be an important factor in the regulation of cellular exhaustion in these cells.[78] IRF4 is responsible for the differentiation of T cells and immunosuppressive cell populations such as Tregs and MDSCs and contributes to the cellular exhaustion of $CD8^+$ T cells through the increased formation of IRF4/AP1 complexes.[75] In T cells, the binding of IRF4 to AICE motifs requires AP1 transcription factors such as BATF, which then drive the expression of IL-17, IL-10, and CTLA4.[75] Depending on the microenvironment, BATF/IRF4 has been shown to promote an exhausted or an effector phenotype in cancer.[79] In HIV, the prolonged expression of IRF4 is associated with a decrease in MAIT cells and a subsequent increase in the frequency of MAIT cells that express PD-1.[80] Interestingly, iNKT cells and γδ T cells are not dependent upon IRF4 for IL-17 production, suggesting that there may be key transcriptional differences in the development of cellular exhaustion in these cells compared to conventional T cells.[81] BLIMP1 and TOX are also key regulators of T-cell

exhaustion, but their contribution to inducing an exhaustive phenotype in UCT subtypes remains largely unexplored. Elevated levels of the transcription factors Tbet and Eomes have been shown to regulate $CD8^+$ T-cell exhaustion during infection and cancer.[82] Alterations in Tbet and Eomes were correlated with increased PD-1 and the inability to adequately produce cytokines by MAIT cells and γδ T cells.[83,84] Little is known about how Tbet and Eomes regulate iNKT cell exhaustion, but previous studies comparing iNKT cells and NK cells, which become exhausted as Tbet and Eomes expression decreases, suggest that iNKT cells may not become exhausted with increased Tbet/Eomes expression like other T cells.[85] iNKT cells have also been shown to be resistant to hydrogen peroxide produced by $CD15^+$ MDSCs, which have been shown to impair conventional T-cell responses and proliferation.[44] Their resistance to several pathways of conventional T-cell exhaustion as well as their ability to directly target CD1d-presenting cells makes these cells a promising target for immunotherapy, particularly in older populations.[44] Currently, more studies are needed to determine if selective expansion of iNKT cells will contribute to improved therapeutic outcomes in patients.

The transcription factor T cell factor 1 (TCF-1) has been shown to be important for the generation of memory $CD8^+$ T-cell responses as well as the development and the maintenance of progenitor exhausted $CD8^+$ T cells in cancer and infection.[86] Importantly, $TCF\text{-}1^+$ $CD8^+$ stem-like T cells have previously been shown to be crucial for responses to immune checkpoint blockade (ICB), especially in poorly immunogenic tumors.[9,86–88] Currently, no studies have compared TCF-1 expression in UCTs and conventional T cells to understand how UCTs may be impacting responses to the ICB blockade. There is evidence that populations of $TCF\text{-}1^+$ UCT cells exist as TCF7 gene expression, which encodes TCF-1, is significantly decreased in MAIT cells from bronchoalveolar lavages of pediatric patients with community-acquired pneumonia when compared to matched patient blood.[89] MAIT cells collected form bronchoalveolar lavages from these patients also upregulated genes associated with exhaustion such as PDCD1, LAG3, and CTLA4.[89] This may indicate the presence of terminally exhausted MAIT cells.[89] Additionally, TCF-1 is necessary for the formation of effector functions in γδ T cells as well as the development of all 3 unconventional T-cell subtypes.[90] Therefore, it is of interest to study $TCF\text{-}1^+$ UCT populations to determine how they may be assisting in controlling infection and contributing to responses to ICB therapy.

3.2 DNA methyltransferase inhibitors

As approaches to immunotherapy evolve, novel therapeutic combinations involving DNA methyltransferase inhibitors (DNMTis) such as decitabine and azacytidine in combination with ICIs have shown great potential in recent years and are now being tested in multiple cancers. Aberrations in DNA methyltransferases (DNMT) have been linked with the hypermethylation of tumor suppressor genes and the promotion of tumorigenesis.[81,82] Inhibition of DNMTs has been shown to improve patient responses to immunotherapy by priming and boosting immune responses, limiting immunosuppression, and reversing T-cell exhaustion. DNMTis improve γδ T-cell function through the upregulation of surface molecules related to γδ T-cell activation such as MICA, MICB, and the upregulation of general adhesion molecules such as ICAM-1, which has been linked to improved γδ

cytotoxicity in in vitro lung cancer models.[91] Decitabine, a type of DNMTi, has also been shown to upregulate CD1d mRNA and protein expression in some NSCLC cell lines, thus promoting iNKT cell activation and increasing cancer cell susceptibility to iNKT cell cytotoxicity.[85] MR1 upregulation by decitabine is also being explored in melanoma models to see if MAIT cell function and killing capacity can be improved following DNMTi treatment.

Many cancers alter the expression of classical and nonclassical MHC class I molecules as a mechanism to escape immunosurveillance (Fig. 2). The downregulation of MHC-associated genes such as BTN3A1–3, MR1, and CD1d significantly impact UCT activation and adaptive immune responses.[26,36,92] Examining which genes are downregulated in certain cancers could help to identify how UCT-based therapies may be used to improve responses. For example, MR1 is downregulated in ESCA, hepatocellular carcinoma, prostate cancer, and colon adenocarcinoma and rectum adenocarcinoma. MAIT cells in these cancers show signs of significant functional impairment, suggesting that upregulating MR1 expression using therapies such as DNMTis while mitigating MAIT cell exhaustion may help improve patient outcomes.[18,23,93] Because The Cancer Genome Atlas (TCGA) data analyzed in Fig. 2 are from bulk RNA sequencing of whole tumors, it is not possible to determine if the expression of these genes is from tumor cells or the immune/stromal microenvironment. More research is needed to understand how alterations in MHC-associated genes contribute to UCT exhaustion and how the restoration of expression impacts immune responses.

4. Concluding remarks

Although UCT exhaustion has received far less attention than conventional $CD8^+$ T cells, functional UCT cells are crucial in effective tumor immunity and hold great potential to help improve the efficacy of immunotherapy. UTCs have potential as excellent targets for immunotherapy due to their lack of donor restriction and their ability to directly target cancer cells, elicit strong cytokine responses, and recruit lymphocyte infiltration into the tumor. Additionally, the prospect of “off-the-shelf” immunotherapies is promising and may work to lower cost and manufacturing time of TIL and CAR-T cell therapies since these cells are not donor restricted. In order to effectively use UCT subtypes for therapy, many questions need to be addressed to understand how the functionality of these cells changes in cancer and how additional factors such as age and the TME contribute to the development of an exhaustive phenotype.

It is a well-known fact that aging is associated with changes to immune responses as well as increased susceptibility to cancer and other diseases. Immunosenescence, thymic involution, cellular exhaustion, epigenetic changes, and possible loss of TCR specificity are all responsible for the decrease in functional T cells associated with aging.[94,95] The UCT population is no exception, and aging results in a decreased frequency of γδ T cells, MAITs, and, to some extent, iNKT cells as well as altered phenotypic profiles of these cells.[95–97] It is important to investigate age-related functional changes in UCT populations as most types of cancer become more common with increasing age. Increased cellular exhaustion due to aging is an important factor to consider when treating patients with immunotherapy as additional therapeutic interventions may be required to allow these patients to respond.

Furthermore, determining if certain populations of cells such as iNKT cells may be less susceptible to cellular exhaustion in older patients and investigating the expansion of such populations may prove to be beneficial for therapeutic outcomes.

Overcoming cellular exhaustion remains a challenge in improving immunotherapies. The antitumor potential of UCTs makes them a valuable therapeutic agent for cancer immunotherapy. Understanding the mechanisms that contribute to UCT exhaustion will greatly aid in improving responses to current immunotherapy and allow for the identification of novel targets for future therapies.

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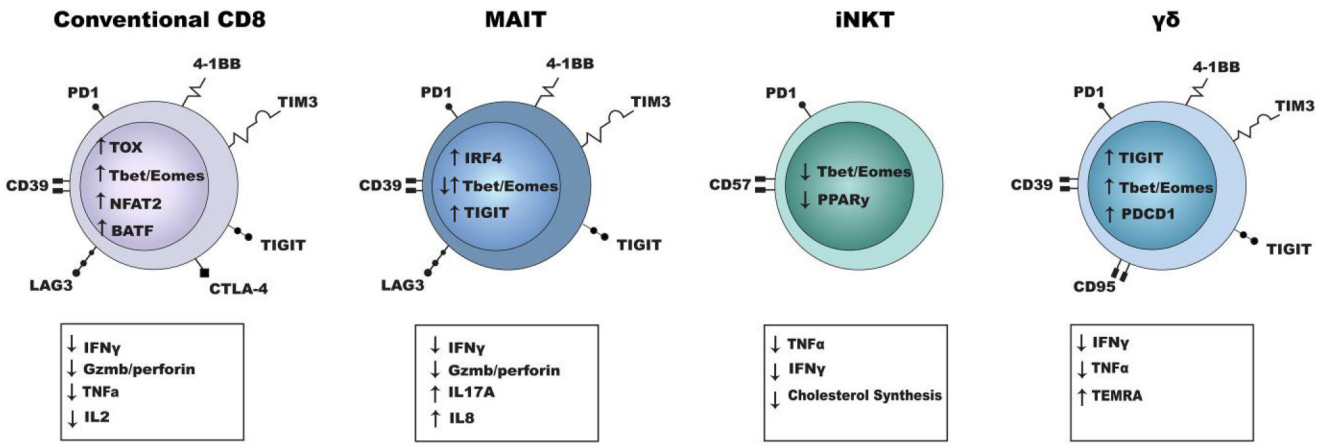

Fig. 1.

Summary of commonly known markers of exhaustive differentiation of conventional $CD8^+$ T cells and UCTs in cancer and viral infections.

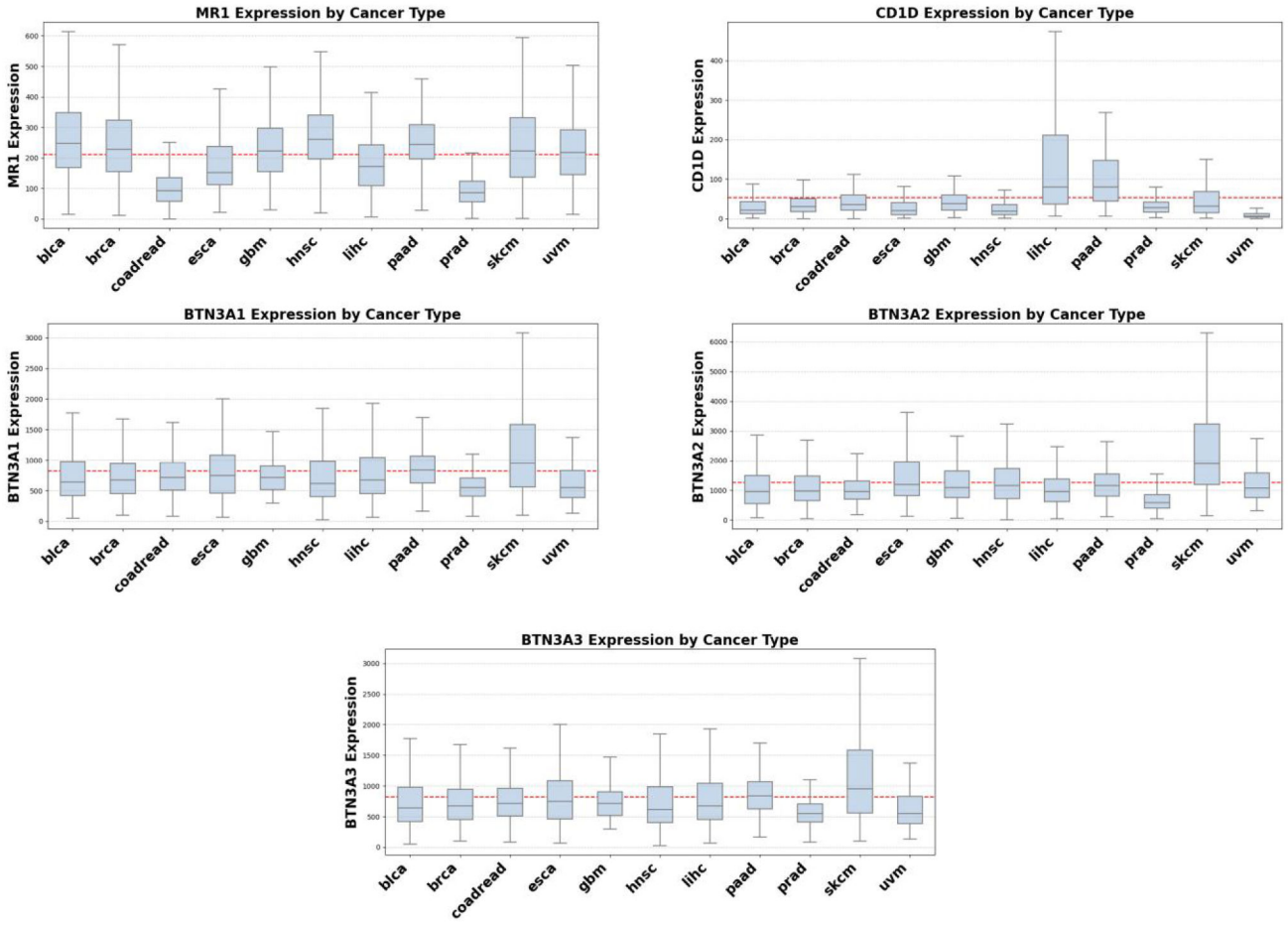

Fig. 2.

TCGA data showing the expression of the MHC-associated genes BTN3A1–3, MR1, and CD1d in multiple cancer types: bladder urothelial carcinoma (blca), breast invasive carcinoma (brca), colon adenocarcinoma and rectum adenocarcinoma (coadread), esophageal carcinoma (esca), glioblastoma multiforme (gbm), head and neck squamous cell carcinoma (hnsc), liver hepatocellular carcinoma (lihc), pancreatic ductal adenocarcinoma (paad), cutaneous melanoma (skcm), and uveal melanoma (uvm). The dotted line indicates the mean expression of the indicated gene across all 11 cancer types.

Table 1.

Summary of common markers associated with exhaustive differentiation and dysfunction of conventional $CD8^+$ T cells, MAIT cells, iNKT cells, and γδ T cells in human cancer and viral infections. The information listed here corresponds with the information in Fig. 1.

Conventional CD8+ T cell			
Disease	**Markers of exhaustive differentiation**	**Dysfunction**	**Citation**
Cancer	PD-1^+CTLA4^+Tim3^+LAG3^+BTLA$^+$TIGIT$^+$	↓ IFNγ, IL-2, TNFα, granzyme B ↑ Tbet/Eomes, FoxP1, Tox, NR4A1, BATF	1,6,8
HIV	PD-1^+2B4^+LAG3^+TIGIT$^+$ CD39^+CD160^+	↑ TOX ↓ IL-2, proliferation	7
Lymphocytic choriomeningitis virus	PD-1^+LAG3^+Tim3^+CD39^+CD160^+	↓ IFNγ, IL-2, TNFα, granzyme B	6,8
MAIT cell			
Colon cancer	PD-1^{High}Tim-3^+CD39^+	↓ IFNγ and granzyme B ↑ IL-17A	12,18
Epithelial ovarian cancer	LAG3^+VISTA$^+$	↓ IFNγ and IL-17A	22
Esophageal adenocarcinoma	PD-1^{High}	↓ IFNγ and TNFα	23
Hepatitis B	PD-1^{High}	↓ Granzyme B and IFNγ	21
Hepatocellular carcinoma	PD-1^{High}CTLA4^+CD25^+Tim3^+	↓ IL-17A, granzyme B, IFNγ, and perforin ↑ IL-8	24
HIV	PD-1^{High}CD39^+	↓ Decreased frequency in peripheral blood ↑ IRF4	19,20
Non-small cell lung carcinoma	PD-1^{High}Tim-3^+CD39^+	↓ IFNγ ↑ IL-8 and IL-17A	25
Prostate	PD-1^{High}	↓ IFNγ	26
Other (colon resident MAIT cells)	PD-1^+Tim3^+TIGIT$^+$CTLA4^+ LAG3^+4–1BB$^+$	Not defined	27
iNKT cell			
Sarcoidosis	PD-1^+CD57^+	↓ IFNγ, TNFα	28
HIV	PD-1^+CD57^+2B4^+	↓ Decreased frequency in peripheral blood	29,30
γδ T cell			
AML	PD-1^+	↑ Terminally differentiated effector memory (TEMRA) Vδ1T cells in bone marrow ↓ IFNγ and TNFα	31,32
Ovarian cancer	PD-1^+Tim3^+CD39^+TIGIT$^+$	↑ TEMRA Vδ1T cells	31
HIV	PD-1^+CD95^+	↓ IFNγ Vδ1 expansion Vδ2 depletion	31

Conventional CD8+ T cell			
Disease	**Markers of exhaustive differentiation**	**Dysfunction**	**Citation**
Multiple myeloma	PD-1^{+}Tim3^{+}CD39^{+}TIGIT^{+}	↑ TEMRA V61 T cells in bone marrow ↓ Proliferation capacity	32
Renal cell carcinoma	PD-1^{+}TIGIT^{+}Tim3^{+}4–1BB^{+}	Retention of effector molecules	33