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Review Article on Lung Cancer and The Immune System

Holding our breath: the promise of tissue-resident memory T cells in lung cancer

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Abstract: T cell memory is critical in controlling infection and plays an important role in anti-tumor responses in solid cancers. While effector memory and central memory T cells circulate and patrol non-lymphoid and lymphoid organs respectively, tissue resident memory T cells (T_{RM}) permanently reside in tissues and provide local protective immune responses. In a number of solid tumors, tumor-specific T cell memory responses likely play an important role in keeping tumors in check, limiting cancer cell dissemination and reducing risk of relapse. In non-small cell lung cancer (NSCLC), a subset of tumor infiltrating lymphocytes (TILs) display phenotypic and functional characteristics associated with lung T_{RM} (T_{RM}-like TILs), including the expression of tissue-specific homing molecules and immune exhaustion markers. High infiltration of T_{RM}-like TILs correlates with better survival outcomes for lung cancer patients, indicating that T_{RM}-like TILs may contribute to anti-tumor responses. However, a number of T_{RM}-like TILs do not display tumor specificity and the exact role of T_{RM}-like TILs in mediating anti-tumor response in lung cancer is unclear. Here we review the characteristics of T_{RM}-like TILs in lung cancer, the role these cells play in mediating anti-tumor immunity and the therapeutic implications of T_{RM}-like TILs in the use and development of immunotherapy for lung cancer.

Keywords: Tissue-resident memory T cells; immunotherapy; lung cancer; immunosurveillance

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Introduction

The lungs are constantly exposed to pathogens throughout life and continually need to develop and maintain immunity against infection. T cell immunity is provided by central memory T cells (T_{CM}), effector memory T cells (T_{EM}) and resident memory T cells (T_{RM}) to enable rapid responses against re-infection. Each memory cell type has a distinct location: T_{CM} are mostly present in secondary lymphoid organs and the circulation, T_{EM} patrol the blood, transiently entering peripheral organs, and T_{RM} permanently reside in most tissues such as the lung, gut, skin, brain and liver (1-4). T_{RM} form a defensive barrier against viral and bacterial infections and have also emerged as an important population in regulating anti-tumor immunity (5,6). High numbers of tumor infiltrating lymphocytes (TILs) that display a $CD103^+$ T_{RM}-like phenotype in carcinomas have been associated with improved survival in multiple solid cancers (7-9). These T_{RM}-like cells likely play

Table 1 Summary table depicting distinct characteristics of healthy lung T_{RM} and T_{RM}-like tumor-infiltrating lymphocytes

Healthy lung T_{RM}	T_{RM}-like tumor infiltrating lymphocytes
CD39 is not expressed by healthy lung T_{RM}	CD39 marks tumor neoantigen-specific T_{RM}-like TILs
Rapid response to repeated infection	High number of T_{RM}-like TILs associated with better cancer survival
Relatively quiescent	Proliferative
Poised for cytotoxicity: RNA encoding pro-inflammatory cytokine (IFN-γ, IL-17, IL-2, IL-10 and TNF-α) and cytotoxic mediators (granzyme B, perforin)	Strong cytotoxic activity expressing high levels of granzyme B, perforin, IFN-γ, TNF-α
Express co-inhibitory molecules PD1, LAG3, TIM3	Express higher levels of co-inhibitory molecules PD1, LAG3, TIM3
Broad TCR repertoire	Clonal expansion of T_{RM}-like TILs with a narrow TCR repertoire against tumor-specific neoantigens

an important role in cancer immune surveillance and maintenance of a cancer-immune equilibrium in latent tumors before immune-escape mechanisms are developed by tumor cells and invasive tumors form (10). In lung cancer, vast diversity exists in both the abundance and composition of tumor-infiltrating cells, even between patients with the same tumor types (11). For the last few years, lung cancer research has focused on understanding the diverse immune populations infiltrating lung tumors with the overall aim of identifying the populations that play critical roles in anti-tumor immunity. There is now evidence that non-small cell lung cancer (NSCLC) TILs comprise not only tumor-specific T cells but also bystander T cells that do not have a T-cell receptor (TCR) specificity for tumor neoantigens, yet may still play a role in the overall cancer immune response (12). T_{RM}-like TILs appear to participate in anti-tumor immunity, either through direct cytotoxic activity (5) or by promoting the recruitment of functional activated cytotoxic T cells to the tumor site (13), raising the possibility of targeting these cells for novel immunotherapies.

Despite the potential role for T_{RM}-like TILs in controlling lung cancer, several fundamental questions remain about the identification of T_{RM} in cancerous lesions, how T_{RM} function in healthy lung, and how they may protect against lung cancer spread. This knowledge is important for the design of strategies that manipulate anti-tumor T_{RM} in lung cancer for potential immunotherapies. Here, we provide an overview of lung T_{RM} in health and lung cancer (*Table 1*) and discuss how this crucial T cell subset may be harnessed in the development of lung cancer immunotherapies.

Identification and phenotype of lung T_{RM} in health and cancer

Human T_{RM} were first identified following HLA-mismatched transplantation (14) and depletion of circulating memory T cells with anti-CD52 antibody studies (Alemtuzumab) (15), which demonstrated a population of tissue-resident cells in disequilibrium with the blood. These initial studies established CD69 and the integrin CD103 to be canonical markers associated with T_{RM} (14,15). Further studies have elucidated markers specific to T_{RM} and factors responsible for their maintenance [reviewed in (16,17)]. Like T_{EM}, T_{RM} express the memory marker CD45RO, and do not express CD45RA, a marker of naïve T cells and terminally differentiated effector memory T cells (T_{EMRA}), nor the lymph node homing marker CCR7 (18). T_{RM} can be distinguished based on their expression of CD69, CD103$^{+/-}$ and CD49a$^{+/-}$, although T_{RM} that do not express these markers are likely to exist, as is found in mice (19). CD69 is a transmembrane C-Type lectin protein that prevents egress of T cell from tissues by interfering with the activity of the receptor for the bioactive lipid sphingosine-1 phosphate (20). CD103, or integrin alpha E (*ITGAE*) is induced by TCR engagement in the presence of transforming growth factor beta (TGF-β) (21) and forms a complex with integrin beta 7 (*ITGB7*). This complex binds to e-cadherin present on epithelial cells to promote the intra-epithelial retention of CD103$^+$ T_{RM} (22). The majority of CD8$^+$ mouse and human lung T_{RM} express CD103, but CD103 is only expressed in less than a quarter of human CD4$^+$ T_{RM} and absent on mouse CD4$^+$ T_{RM} (19,23). CD49a [integrin alpha 1 (*ITGA1*)] and CD11a [integrin alpha L (*ITGAL*)] are expressed by CD8$^+$ and CD4$^+$ T_{RM} although the expression of CD11a appears restricted to alveolar T_{RM} (23-28). These integrins

are involved in cellular adhesion and costimulatory signaling, and CD11a is also required for $CD8^+$ T cell entry in the lung parenchyma (29).

While CD103 and CD69 are commonly used markers of T_{RM} in healthy tissues, relying on these markers to identify T_{RM} in TILs may be problematic. The expression of CD103 can be induced on circulating $CD8^+$ T cells in the presence of TGF-β (30) which is secreted in the tumor microenvironment. CD69 is transiently expressed in response to antigen or inflammation, such that CD69-expressing T cells in tissues may be activated and transiting through the tissue, but not resident (31). Nevertheless, multiple studies have now shown that TILs in NSCLC and other solid tumors display a phenotype reminiscent of T_{RM}, describing them as T_{RM}-like cells (5,6,8,32). In lung tumors, not all $CD69^+CD8^+$ or $CD4^+$ TILs express CD103 (6). While the number of $CD69^+CD103^+CD8^+$ T cells increases in lung tumors compared with normal lung, $CD69^+CD103^-CD4^+$ are more prevalent in the tumor than in the normal lung, and are more abundant than $CD69^+CD103^+CD4^+$ TILs (6). The T_{RM} pool in TILs therefore consists of diverse subsets of cells, with varying levels of expression of CD69 and CD103. CXCR6 and CD49a have also been shown to define T_{RM}-like TILs being expressed in both $CD69^+CD103^{+/-}CD4^+$ and $CD69^+CD103^{+/-}CD8^+$ TILs (6).

Insights into markers of tumor-specific T_{RM}-like cells has also come from the analysis of markers of T_{RM} in chronic infectious diseases. CD39, a marker of exhausted T cells in patients with chronic viral infection was found to be expressed in tumor infiltrating $CD103^+CD8^+$ TILs in head and neck squamous cell carcinoma, lung, melanoma, ovarian, and colorectal cancer (32). CD39 is highly expressed in $CD103^+CD8^+$ TILs compared with $CD103^-CD8^+$ TILs in early stage NSCLC tumors (5). $CD39^+CD103^+CD8^+$ T cells were also detected in metastatic lymph nodes, but not in non-tumor involved lymph node or the circulating blood, suggesting that CD39 is a marker of tumor-specific T_{RM}-like cells (32). CD39 is a cell surface ectonucleotidase that dephosphorylates ATP to AMP. Excess ATP can be toxic for cells, suggesting that CD39 expression by $CD103^+CD8^+$ cells may be a way to protect T_{RM}-like TILs from ATP-induced cell death. CD39 may prove to be a critical marker to distinguish tumor neoantigen-specific T_{RM}-like TILs from other antigen-specific T_{RM}. Simoni *et al.* observed that in lung tumor and colorectal cancer, only $CD39^+CD8^+$ TILs and not $CD39^-CD8^+$ TILs had undergone neoantigen-driven clonal expansion (12). Analysis of 40 human lung cancer samples by mass cytometry revealed that a large portion of TILs were bystander $CD8^+$ TILs that express CD103 and CD69 but did not express cancer-related epitopes (12). These bystander $CD8^+$ T_{RM}-like TILs express coinhibitory molecules such as PD1, but do not express CD39. CD38, another ectonucleotidase that also regulates adenosine signaling, was found highly expressed in NSCLC $CD103^+$ TILs further suggesting that regulation of the adenosine pathway may be an important mechanism for tumor-specific T_{RM} (5).

Further insight in the heterogeneity and distinguishing features of cancer T_{RM}-like TILs compared to healthy tissue T_{RM} will come from the use of novel single cell technologies including single cell RNA sequencing (scRNAseq), cytometry-based assays including mass cytometry (CyTOF), CITE-seq, and multi-parametric immunostaining technics. Single cell RNAseq analysis of lung $CD103^+$ TILs compared with non-malignant tissue $CD103^+$ T cells has revealed greater activation of these T_{RM}-like cells in NSCLCs, where the protein expression of additional cell surface markers could further clarify the heterogeneity and tumor-specificity of T_{RM}-like TILs (33). High parameter mass cytometry experiments (34), or CITE-seq that takes advantage of DNA-barcoded antibodies combined with scRNA sequencing (35) constitutes novel technologies to explore distinct features of T_{RM} in cancer and healthy tissue from the same organ. Multi-parameter immunostaining methodologies in tissue would also complement these approaches to provide in depth information on the spatial organization of these cell types (36).

Localization and regulation of lung T_{RM}

The location and turnover of lung T_{RM} is tightly controlled to ensure rapid defense against infection while preserving tissue integrity. Lung T_{RM} are localized in two different compartments in the human and mouse lung: the lung parenchyma or alveolar region, and the airways (bronchi and bronchioles) (24,28,37-40). Studies analyzing the precise localization of T_{RM} have been conducted in the mouse lung following influenza infection (41,42). A month after flu infection, $CD4^+T_{RM}$ were found clustered in a niche surrounding the mouse airways, in proximity to the primary site of reinfection, consistent with their helper T cell role (28). In contrast, $CD8^+$ T_{RM} did not form clusters and were detected within the epithelial repair region in the parenchyma and peribronchial area (25) (*Figure 1A*). In the human lung, $CD4^+$ and $CD8^+$ T_{RM} are detected in both the

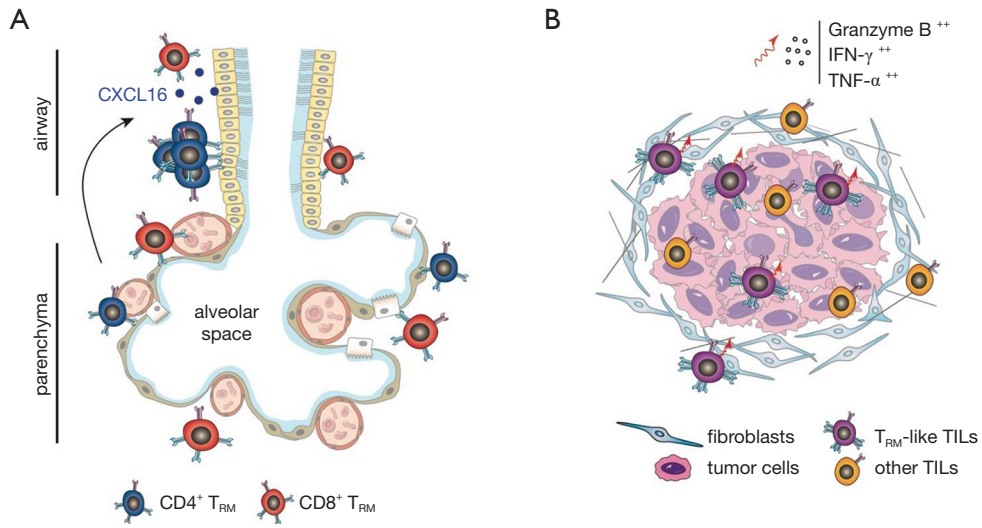

Figure 1 T_{RM} in lung health and cancer. (A) In the healthy lung $CD4^+$ and $CD8^+$ T_{RM} are located near the airways and in the alveolar space. $CD4^+$ T_{RM} form clusters closer to the airways while $CD8^+$ T_{RM} are more dispersed. Alveolar T_{RM} have a low turn-over and replenish airway T_{RM} that have a shorter half-life. (B) Tumor T_{RM}-like TILs are present in the cancer stroma and within the carcinoma, express higher levels of inhibitory molecules and secrete more inflammatory cytokines compared to healthy lung T_{RM} and non T_{RM}-like TILs.

parenchyma and the airways (38). The 3D localization of T_{RM} within bronchiolar and alveolar regions is still unclear, and how these locations may facilitate ready exposure to inhaled memorized antigens remains to be elucidated.

To maintain cellular homeostasis and prevent unnecessary immunopathology, cell death and proliferation of lung T_{RM} are tightly regulated (37,43). Particularly, mouse airways T_{RM} have a relatively short half-life of 14 days (39,40,43). Mouse studies have shown that alveolar $CD8^+$ T_{RM} will only form after exposure to an antigen within the lung and are maintained through a slow regenerative turn-over in the alveoli (24,44). Alveolar $CD8^+$ T_{RM} constantly replenish the pool of short-lived airways $CD8^+$ T_{RM} by migrating to the front line to respond to re-infection events (24) (*Figure 1A*). CXCL16 expressed by the airway epithelium appears to be the most prevalent chemokine responsible for the migration of $CD11a^+CXCR6^+CD8^+$ T_{RM} from the parenchyma to the airways where the expression of CD11a and CXCR6 is then downregulated (23,24,40,45-47). Interestingly, there are some differences in the level of expression of CD103 and CD69 in T_{RM} present in human lung airways or the parenchyma where the airways have more than a two-fold increase in the proportion of $CD69^+CD103^+$ expressing $CD8^+$ and $CD4^+$ T cells compared with the alveolar parenchyma that has a higher proportion of $CD69^+CD103^-$ cells (38). These results are reminiscent to what is observed in the human skin where the epidermis contains $CD69^+CD103^+$ CD4 and CD8 T cells, whereas the $CD69^+CD103^-$ subset is more prevalent in the dermis for both CD4 and CD8 T_{RM} (48). In the skin $CD69^+CD103^+$ T_{RM} express more effector cytokines such as IFN-γ and TNF-α than $CD69^+CD103^-$ T_{RM} (48). It remains to be evaluated whether the same is true in the different subpopulations of lung T_{RM}.

Molecular mechanisms controlling tissue residency of lung T_{RM} appear to be distinct from other tissue-specific T_{RM}. Mouse studies have shown that Hobit, Blimp1 and Runx3 are important mediators of tissue residency in the small intestine, liver, kidney and skin, by directly down-regulating the expression of tissue egress receptor CCR7 and S1PR1 (49-51). However, detailed investigation of transcriptional programs regulating mouse lung $CD8^+$ T_{RM} showed that Blimp1, but not Hobit, was required for their formation following influenza virus infection (52). In human lung, RUNX3 and HOBIT may be involved in the generation and/or maintenance of $CD8^+$ T_{RM} (38,50), although the expression of HOBIT appears lower in human lung T_{RM} than mouse T_{RM} (23), suggesting RUNX3 may be a common regulator of lung T_{RM} in both species. Indeed, human and mouse T_{RM} share a core transcriptional signature associated with RUNX3 expression (50). Runx3 also plays an important role in regulating TILs in a mouse

model of melanoma, where Runx3-deficient T-cells fail to accumulate in the tumor, resulting in increased tumor growth. Conversely, overexpression of Runx3 increased the recruitment of $CD8^+$ T cells to the tumor and these TILs expressed a transcriptomic signature of tissue-residency (50). Further investigation will be necessary to specifically delineate the role of these distinct transcription factors in regulating T_{RM} in healthy lung and lung tumors.

In early stage NSCLC, infiltration of $CD103^+CD8^+$ T_{RM}-like TILs in the tumors correlated with better patient survival (8). Interestingly, while a large proportion of these T_{RM}-like cells resided in the tumor stroma, high infiltration of T_{RM}-like TILs within the tumor was associated with an increase in the number of all TILs, irrelevant of the histological subtypes of NSCLC (*Figure 1B*). Those tumor-penetrating T_{RM}-like cells were more frequently observed in patients with a history of cigarette-smoking and were associated with better outcome (8). This observation in lung cancer was similar to endometrial adenocarcinoma where $CD103^+CD8^+$ T cells were found in the carcinoma region but not in the stromal region, consistent with the role of CD103 in homing to epithelial cells (53). These studies highlight the importance of the spatial organization of the tumor microenvironment and suggest that *in situ* analysis will be critical to understand the role played by T_{RM}-like TILs in anti-tumor immunity.

In cancer progression, tumor cells utilize high levels of oxygen and nutrients leading to an aberrant metabolic state within the tumor-microenvironment (54). This nutrient deprivation may limit the lifespan and effector functions of lung T_{RM} (37) suggesting that the function of T_{RM}-like TILs may also be impacted. Whether this nutrient-deprived environment limits the survival and function of T_{RM}-like TILs or drives a reprograming of their metabolic state promoting their survival and activity remains to be investigated.

Lung T_{RM} function in health and cancer

In the healthy lung, T_{RM} express a gene signature significantly different to peripheral blood circulating T cells, expressing genes encoding for effector molecules but also inhibitory regulators, indicating that these cells are poised for prompt response to infection, while maintaining immune tolerance (55). To rapidly recognize and respond to a large spectrum of invading pathogens, lung T_{RM} express a large repertoire of TCR (26), with a higher TCR clonal diversity for $CD4^+$ T_{RM} compared with $CD8^+$ T_{RM} (49). T_{RM} act rapidly against pathogens due to their high expression of mRNAs encoding pro-inflammatory cytokine (IFN-γ, IL-17, IL-2, IL-10 and TNF-α) and cytotoxic mediators (granzyme B, perforin) which would presumably prevent delays required by transcription (23,26,37,38,49).

Similarly, T_{RM}-like TILs are primed for cytotoxic activity. The interaction of CD103 with tumor cells through e-cadherin triggers lytic granule polarization and exocytosis, promoting anti-tumor cytotoxicity (30,56). Classification of early stage NSCLC tumors based on TIL expression of CD103 showed that in TIL-$CD103^{hi}$ tumors, TILs expressed higher levels of genes associated with proliferation (Ki67, cell cycle genes) and cytotoxicity (granzyme B, perforin, IFN-γ) compared with TIL-$CD103^{lo}$ tumors (5) (*Figure 1B*). Contrary to normal lung T_{RM} that have a relatively quiescent phenotype (57,58), $CD103^+$ TILs were shown to be more proliferative than $CD103^-$ TILs (32,33). When $CD103^+CD8^+$ cells were cultured *in vitro* in the presence of recombinant IL-2, cytotoxic degranulation was much more prominent in $CD103^+$ cells compared with $CD103^-$ cells isolated from the same tumor, as measured by granzyme B and CD107a expression (8). Further subdividing $CD103^+$ cells with $CD39^+$ showed that $CD8^+CD39^+CD103^+$ could kill autologous tumor cells in an *in vitro* co-culture assay three-times more efficiently than $CD8^+CD103^+CD39^-$ cells in an MHC Class I-dependent manner, indicating that CD39 is an important marker to select for cytotoxic tumor-specific T_{RM} like TILs (32).

IFN-γ produced by T_{RM} has been shown to increase the recruitment of circulating T cells to potentiate robust immune response to pathogens in infected tissue. IFN-γ stimulates chemokine production by epithelial cells and increases the expression of adhesion molecules by the vasculature resulting in higher T cell infiltration (59,60). Similarly, high production of IFN-γ by tumor-specific T_{RM}-like cells may play a role in the recruitment of non-exhausted circulating T cells to the tumor. $CD103^+CD4^+$ TILs were found to express the highest levels of TNF-α and IFN-γ upon CD3/CD28 stimulation compared with $CD103^+CD8^+$ TILs or their lung T_{RM} counterparts (6). These cytokines may contribute to the recruitment of functional T cells to the tumor site (*Figure 2A*). Indeed, Wu *et al.* recently showed that recruitment of peripheral T cells may be an important factor in response to immune checkpoint blockade (ICB) (13). The authors combined scTCR-sequencing and scRNA sequencing of T cells in tumors, unaffected adjacent tissue and blood samples and showed an expansion of $CD8^+$ T cell clones in the blood

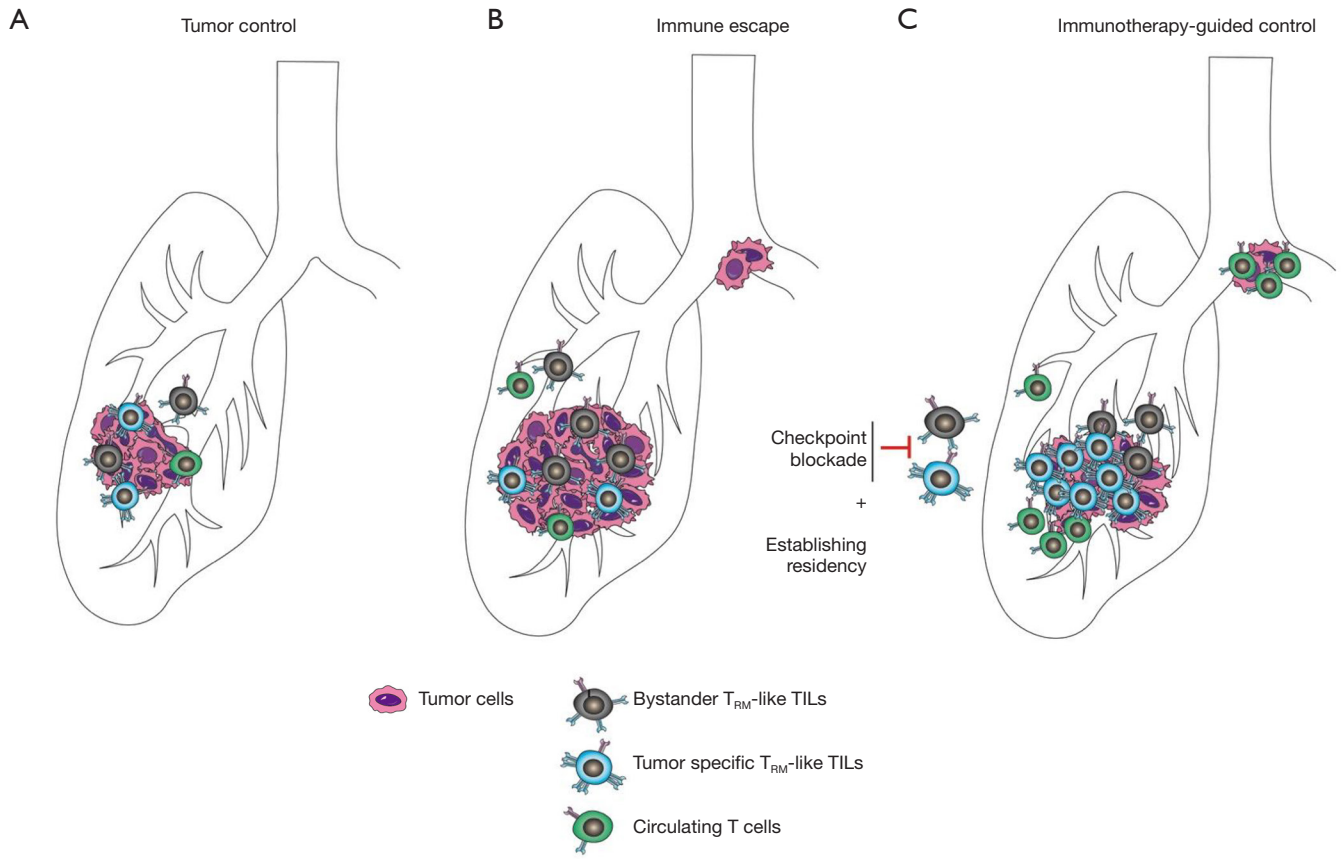

Figure 2 Harnessing T_{RM} for cancer immunosurveillance at steady state and for long term tumor control. (A) Tumor neoantigen-specific T_{RM}-like TILs participate in maintaining the tumor-immune equilibrium to keep the tumor in check. (B) During immune escape, T_{RM} guided surveillance is evaded through checkpoint molecules to allow for uncontrolled tumor cell growth and metastasis to pulmonary lymph nodes. (C) Checkpoint inhibitors, such as anti-PD-1, anti-CTLA4, anti-TIM3 and anti-LAG3, activate T_{RM}-like TILs (tumor-specific and bystander) and increase the recruitment of functional circulating T-cells to the tumor site. Combined with an understanding of factors driving long-term residency of tumor-specific T_{RM} cells, longer-term local and systemic control of tumor growth could be achieved with appropriate personalized therapies.

that were also detected in the unaffected and tumor tissue. These data offer a novel paradigm in our understanding of anti-tumor immunity and T_{RM} biology, where $CD103^+CD4^+$ tumor T_{RM}-like cells may mediate recruitment of non-exhausted peripheral $CD8^+$ T cells to the tumor site. It remains to be seen how ICB act upon these $CD4^+$ T_{RM}-like TILs, in parallel to reverting to a T cell exhaustion phenotype in $CD8^+$ TILs.

Implications for therapy

Therapies that activate the anti-tumor response of cytotoxic T cells have shown great promises in the management of lung cancer patients. However, there is still a wide disparity in responses to these checkpoint immunotherapies, where only 20% of lung cancer patients show response with different degrees of duration (61,62). The mechanisms behind a lack of response are still unclear, but recognition of a tumor-specific neoantigen by TILs is necessary for T cell mediated tumor cell destruction (63,64). Lung cancer-specific T_{RM} are ideally placed as targets for immunotherapy which aims to enhance tumor immunosurveillance. Their rapid response upon re-exposure to antigen compared to circulating memory cells, their residency within the lung and their close contact with epithelial cells at risk of malignant transformation ensures their molecular features

and spatial environment are primed for tumor control. Although neoantigen-reactive T cells can be detected in the circulation (65), it is still unknown if subsets of neoantigen-specific T cells become resident in the lung upon recognition of tumor neoantigens, likely due to the unsuitability of surface markers in tumors and the great heterogeneity of patients possessing *bona fide* tumor-reactive T cells (66). Approaches to target cancer-specific T_{RM} for lung cancer treatment center around three major strategies: targeting existing cancer-specific T_{RM} that are being prevented from enacting anti-tumor immunity, harnessing bystander lung T_{RM} for tumor immunity and activating cancer-specific T_{RM} in tumors with inherently low immunogenicity.

Current FDA-approved ICB for NSCLC treatment include those blocking CTLA4, PD1 or its ligand PDL1 (67). PD-1, CTLA4 as well as TIM3 and LAG3 are co-inhibitory molecules expressed by lung T_{RM} where they counteract the expression of activation molecules to limit inflammation-induced tissue damage and ensure immune tolerance (23,55). Compared with their normal lung counterparts, tumor-infiltrating T_{RM}-like cells express higher levels of these co-inhibitory molecules. $CD8^+CD103^+$ tumor T_{RM}-like TILs express higher levels of TIM3, LAG3 and PD1 than $CD8^+CD103^-$ TILs indicating that T_{RM}-like TILs are the likely targets of immune checkpoint inhibitors (5,8,32,33) (*Figure 2B*). Although patients with TIL-$CD103^{hi}$ lung tumors have a better overall survival outcome (5), this has not been linked to response to specific therapeutic strategies, including ICB. Exploring the exact balance of expression of co-inhibitory molecules on tumor-specific T_{RM}-like cells and bystander T_{RM}-like TILs will be necessary to evaluate these correlations. Nonetheless, *in vitro* studies showed that anti-PD1 or anti-PDL1 treatment was necessary to induce autologous tumor cell lysis by TILs and this effect was blocked in the presence of anti-CD103 antibody, indicating the critical role of $CD103^+$ cells in restoring anti-tumor immunity upon ICB treatment (8). It is likely that current ICB therapies are effective both upon cancer-specific T_{RM} and also by inducing the recruitment of T cells from the periphery (5,8,13,32,68). Other immune checkpoints highly expressed by T_{RM}-like TILs are under investigation in clinical trials, including TIM3 and LAG3 (69), which may be effective under similar mechanisms to current ICBs. Consistently, $CD103^+$ TILs in patients responding to anti-PD1 therapy expressed higher level of TIM3 than non-responders, indicating that inhibiting TIM3 may provide an additional therapeutic approach for tumors with primary or acquired resistance to anti-PD1 (33). Other sophisticated strategies include adoptive T cell therapy, where neoantigen-specific T cells are isolated and expanded from circulating T cells before re-infusion into the patient (70). A remaining question in the field is how long-term residency might be induced in cancer-specific T_{RM} after successful immunotherapy for long term cancer immune-surveillance and control.

Recent studies have revealed a preponderance of bystander T_{RM} within lung TILs that are reactive to unrelated epitopes (12). These cells are characterized by their lower expression of immune checkpoints compared to cancer-specific T_{RM}-like TILs, yet it is tempting to speculate they may still contribute to current ICB sensitivity by producing effector cytokines to support cancer-specific T_{RM}-like TILs or peripheral recruitment of effector T cells (*Figure 2C*). In contrast, bystander lung and other organ T_{RM} could also contribute to immune-related adverse events (irAEs), common in patients who are sensitive to ICB (71). IrAEs include pneumonitis, dermatologic, gastrointestinal, endocrine and hepatic inflammatory events, indicating that ICBs induce augmentation of systemic immunity. Whether these effects are mediated differentially through circulating immune cells and/or tissue-specific T_{RM} remains to be explored. Use of genetically engineered mouse models will help understand the role of bystander and tumor-specific T_{RM}-like TILs in mediating anti-tumor immunity, and to determine how this response is accentuated by ICB. Tracing of circulating T-cells and their recruitment to the tumor site may also provide insights into the effect of ICB and T_{RM}-like TILs in the recruitment of T cells from the circulation. Analysis of the phenotype and TCR repertoire of T-cell in bronchio-alveolar lavage fluid of patients who have developed pneumonitis as irAEs in response to ICB will also provide some clues on the mechanisms participating in the toxicity associated with ICB.

Tumor immunogenicity is necessary to induce an immune response. Immune escape mechanisms developed by tumor cells include loss of heterozygosity in major histocompatibility molecules I/II responsible for antigen presentation or reduced expression of neoantigens that can be recognized by T cell clones (72,73). The vast heterogeneity in the TCR repertoire detected in early stage tumors correlates with the genetic heterogeneity of tumor cells and diversity in predicted neoantigens (74). However, best responses to ICB appear to come from T cells responding to neoantigens that are universally present in every tumor clone (75). It is tempting to speculate that

the TILs recognizing a common neoantigen may have a T_{RM}-like phenotype, due to their tumor-retention and persistence in the tissue. Indeed, TCR sequencing of $CD103^+$ and $CD103^-$ lung cancer TILs demonstrated a much narrower TCR repertoire of $CD103^+$ TILs compared with $CD103^-$ TILs, indicating a clonal expansion of T_{RM}-like TILs against a restricted number of tumor-specific neoantigens (33). Validating this observation could have significant therapeutic implications, notably to permit the use of compounds activating co-stimulatory molecules on T cells. Such strategies include antibodies targeting 41BB, OX40, CD27 and ICOS, for which progress in the clinic has been hampered by both immune side-effects and complex overlapping roles of these molecules in other tumor-infiltrating immune cells (76-78). However, activating these co-stimulatory molecules specifically within T_{RM}-like TILs may circumvent such issues. Other interventions to increase immunogenicity of tumors include oncolytic viruses in small cell lung cancer (79), dendritic cell vaccines, targeting myeloid-derived suppressor cells, chemotherapy and radiotherapy [reviewed in (80)]. The ability to induce residency of tumor-reactive T cells in these techniques should also be explored.

Conclusions

Tumor-specific T_{RM}-like cells could play an important role in early stage and advanced disease. T cell recognition of neoantigens and subsequent residency of T_{RM}-like TILs may help to prevent tumor relapse after successful treatment, and in late tumor evolution, T_{RM}-like TILs recognition of clonal neoantigens could prevent further metastatic dissemination (75). An emerging challenge in these strategies is immune evasion developed by tumors, including loss of MHC Class I expression and ability to present antigens. Further research into the mechanisms inducing tumor residency and immune evasion by tumors will enable personalized medicine for the immunotherapy era.

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Footnote

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