Skin resident T cells

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Skin-resident memory T cells as a potential new therapeutic target in vitiligo and melanoma

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The presence of a pathogen stimulates naïve T cells to differentiate into memory and effector T cells in order to eliminate pathogen-infected cells. Memory T cells can be subgrouped into central memory T (T<sup>CM</sup>) cells, effector memory T (T<sup>EM</sup>) cells and migratory memory T (T<sup>MM</sup>) cells.

The T<sup>CM</sup> cell pool predominates in secondary lymphoid organs and expresses markers, such as chemokine receptor CCR7 and the vascular addressin L-selectin (CD62L). In contrast, T<sup>EM</sup> cells migrate into the non-lymphoid tissues to clear the infection with their high cytokine production capacity and perforin expression. T<sup>EM</sup> cells express low CCR7 and CD62L levels, but can express high levels of the tissue-homing addressin and E-selectin ligand Cutaneous Lymphocyte Antigen (CLA), which enables them to enter into the skin.

Expression of CCR7 and absence of CD62L characterizes migratory memory T (T<sup>MM</sup>) cells, which recirculate between blood and tissues and are excluded from the lymph nodes.

Memory T cells were initially considered to be circulatory and to enter the tissues only when needed, to clear an infection. Work over the past years has defined another pool of memory T cells, called resident memory T (T<sup>RM</sup>) cells.

T<sup>RM</sup> cells do not recirculate, but reside permanently in tissues such as skin, intestine, lung, brain and female reproductive tract, where they provide rapid protective immunity against re-infecting pathogens.

Upon viral or bacterial infection, antigen-specific primary and memory CD8<sup>+</sup> T cells become present throughout the body. Resident memory CD8<sup>+</sup> T cells isolated from non-lymphoid tissues showed higher antigen-specific response than circulatory memory cells isolated from lymphoid tissues. T<sup>RM</sup> can even respond more rapidly to tissue infection than circulatory memory cells.

The T<sup>RM</sup> cell population within each tissue is capable of recognizing the specific pathogens that most commonly affect those tissues, and T<sup>RM</sup> cells remain in place long after pathogen elimination.

Besides eliminating pathogens, T<sup>RM</sup> cells may also contribute to various disorders when aberrantly activated. These cells can develop not only after pathogen infection, but also after sensitization to otherwise harmless environmental or self-antigens.

The involvement of T<sup>RM</sup> cells has been demonstrated in various skin diseases, such as psoriasis, fixed drug eruptions, allergic contact dermatitis, cutaneous T cell lymphoma—a malignancy of T<sup>RM</sup> cells and vitiligo.

Interestingly, autoreactivity and tumor immunity are often linked, as exemplified by the association between vitiligo and melanoma. Overwijk et al. (2003) showed that the same specific lymphocytic response could promote tumor destruction and vitiligo, in the exact same mouse.

Adaptive transfer of gp100-specific CD8<sup>+</sup> T cells in mice bearing B16
INTRODUCTION

The presence of a pathogen stimulates naïve T cells to differentiate into memory and effector T cells in order to eliminate pathogen-infected cells. Memory T cells can be subgrouped into central memory T (T_C) cells, effector memory T (T_E) cells and migratory memory T (T_M) cells. The T_C cell pool predominates in secondary lymphoid organs and express markers, such as chemokine receptor CCR7 and the vascular addressin L-selectin (CD62L). In contrast, T_E cells migrate into the non-lymphoid tissues to clear the infection with their high cytokine production capacity and perforin expression. T_E cells express low CCR7 and CD62L levels, but can express high levels of the tissue-homing addressin and E-selectin ligand Cutaneous Lymphocyte Antigen (CLA), which enables them to enter into the skin. Expression of CCR7 and absence of CD62L characterizes migratory memory T (T_M) cells, which recirculate between blood and tissues and are excluded from the lymph nodes.

Memory T cells were initially considered to be circulatory and to enter the tissues only when needed, to clear an infection. Work over the past years has defined another pool of memory T cells, called resident memory T (T_R) cells. T_R cells do not recirculate, but reside permanently in tissues such as skin, intestine, lung, brain and female reproductive tract, where they provide rapid protective immunity against re-infecting pathogens. Upon viral or bacterial infection, antigen-specific primary and memory CD8+ T cells become present throughout the body. Resident memory CD8+ T cells isolated from non-lymphoid tissues showed higher antigen-specific response than circulatory memory cells isolated from lymphoid tissues. T_R cells can even respond more rapidly to tissue infection than circulatory memory cells. The T_R cell population within each tissue is capable of recognizing the specific pathogens that most commonly affect those tissues, and T_R cells remain in place long after pathogen elimination.

Besides eliminating pathogens, T_R cells may also contribute to various disorders when aberrantly activated. These cells can develop not only after pathogen infection, but also after sensitization to otherwise harmless environmental or self-antigens. The involvement of T_R cells has been demonstrated in various skin diseases, such as psoriasis, fixed drug eruptions, allergic contact dermatitis, cutaneous T cell lymphoma - a malignancy of T_R cells and vitiligo.

Interestingly, auto-immunity and tumor immunity are often linked, as exemplified by the association between vitiligo and melanoma. Overwijk et al. (2003) showed that the same specific lymphocytic response could promote tumor destruction and vitiligo, in the exact same mouse. Adaptive transfer of gp100-specific CD8+ T cells in mice bearing B16
melanoma cured the mice of the tumor, but also caused vitiligo. The vitiligo started at the former tumor site, and even one year after therapy these mice remained tumor-free with progressive vitiligo. Gp100 is a member of a family of “self” (i.e., unmutated), melanoma/melanocyte differentiation antigens that are widely expressed by melanoma cells. Hence, vitiligo was caused by activated anti-melanoma immunity that not only targeted malignant cells, but also healthy melanocytes. A subsequent study reported that tumor-bearing mice with vitiligo generated 10-fold larger CD8+ memory T cell populations that are specific for shared melanoma/melanocyte antigens than mice without vitiligo. These responses were not observed in melanocyte-deficient mice. CD8+ T cells in mice with vitiligo acquired phenotypic and functional characteristics of T_{EM} cells, suggesting that they were supported by ongoing antigen stimulation. Conversely, melanocyte-deficient mice did not generate such protective responses, indicating a requirement for melanocyte destruction as antigen source in maintaining CD8+ T cell immunity to melanoma.

In humans, it has been observed that vitiligo can occur in melanoma patients spontaneously or during immunotherapy treatment and correlates with prolonged survival. Conversely, vitiligo patients have 3-fold lower probability of developing melanoma during their lifespan than non-vitiligo patients. Recent work has indicated the pathogenic involvement of T_{RM} cells in human vitiligo and data on this is still emerging. Other studies have demonstrated a protective role for T_{RM} cells in melanoma. These responses were not observed in melanocyte-deficient mice. CD8+ T cells in mice with vitiligo acquired phenotypic and functional characteristics of T_{EM} cells, suggesting that they were supported by ongoing antigen stimulation. Conversely, melanocyte-deficient mice did not generate such protective responses, indicating a requirement for melanocyte destruction as antigen source in maintaining CD8+ T cell immunity to melanoma.

FEATURE OF SKIN-RESIDENT MEMORY T CELLS

I. Phenotypic characteristics of skin-resident T_{RM} cells

The human skin contains approximately one million T cells per cm², which amounts to almost 20 billion T cells in total. This is nearly twice as many T cells as those circulating in the blood. T_{RM} cells, like all memory T cells, can be distinguished from naive T cells by expression of CD44, a marker of antigen experience. Furthermore, T_{RM} cells lack expression of CD62L and CCR7; which differentiates them from recirculating T_{CM} and T_{MM} cells (Figure 1). The chemokine receptor CCR7 interacts with CCL19 and CCL21, thereby helping T cells to migrate towards lymph nodes. As CCR7 expression is needed for T cell egress from peripheral tissues, CCR7+ T cells in tissue can be considered tissue-resident. Another study showed that in normal skin under resting conditions, more than 90% of CCR7+ CD62L+ T cells co-expressing the skin homing molecule CLA are skin-resident.

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To discriminate T_{RM} cells from T_{EM} cells, more phenotypic markers are needed. In human skin, 50-70% of T cells express CD69 and CD103. Although CD69 has been characterized as a T cell activation marker, it has been shown to be constitutively expressed by a subset of T cells within peripheral tissues under steady-state conditions (Figure 1).^{48}

A subset of T_{RM} cells also expresses CD103, which is the α-subunit of the α3β7 integrin receptor (Figure 1). In healthy human skin, its expression is most prominent on epidermal CD4^{+} and CD8^{+} T_{RM} cells, where it enables T_{RM} cell tethering within the epidermal compartment by binding to E-cadherin, which is widely expressed by epithelial cells.^{15} Nevertheless, binding to E-cadherin is not required for skin residency.^{49} Although CD4^{+} and CD8^{+} CD103^{+} T_{RM} cells are less proliferative than CD103^{-} T_{RM} cells, CD103^{+} T_{RM} cells have a larger effector cytokine-production capacity. Relative proportions of resident and recirculating memory T (T_{CIRC}) cells have been measured in highly immunocompromised NOD SCID IL-2Rγ-deficient (NSG) human-engrafted mice and in lymphoma patients upon alemtuzumab treatment, which is an antibody specific to CD52 (expressed by T cells). Alemtuzumab depletes T cells by antibody dependent cellular cytotoxicity. This requires neutrophils and/or natural killer cells, which are relatively abundant in the circulation, but are rare in peripheral tissues. Alemtuzumab, therefore, only depletes T_{CIRC} cells, but not T_{RM} cells, which makes it possible to determine the relative proportions of both subsets. In healthy adult human skin, most T_{RM} cells are CD103^{-} CD4^{+} and reside in the dermis. While CD103^{+} T_{RM}, both CD4^{+} and CD8^{+}, are more frequent in the epidermis, recirculating T cells are the minority among both CD4^{+} and CD8^{+} T cell populations in skin.^{2} The resident T cell populations in human skin thus differ in their migration compartments and functional capacities.

The α-subunit of the α1β1 integrin receptor, CD49a (also known as very-late antigen (VLA)-1) was identified to delineate a subset of CD8^{+} T_{RM} cells in human skin epithelia that preferentially localize to the epidermis. These cells are poised towards IFN-γ production and acquire high cytotoxic capacity upon IL-15 stimulation.^{26} In the same study, CD49a^{+} CD8^{+} T_{RM} cells excelled at IL-17 production, and expression of CD49a was restricted solely to CD8^{+} T cells. Moreover, CD49a binds to collagen IV, a major component of the basement membrane between epidermis and dermis.
II. Tissue retention and transcriptional signatures shared by T<sub>RM</sub> cells

Various molecular factors have been implicated in tissue retention. CD69 transcriptionally downregulates the sphingosine-1-phosphate receptor (S1P1), a G protein-coupled receptor for sphingosine 1-phosphate (S1P).<sup>50</sup> This limits egress of these memory cells out of tissues, showing that S1P1 downregulation is needed for long-term residency of T<sub>RM</sub>.<sup>51</sup> Conversely, S1P1, through detection of its ligand S1P in the blood and lymph, is essential for naive lymphocytes to access the circulatory system from the thymus and lymph nodes.<sup>52</sup> Effector T cells also use S1P1 to sense S1P gradients among tissues, lymph and blood, thereby guiding entry in efferent lymphatics from lymphoid tissues.<sup>53</sup>
Expression of S1P1 can also be regulated by the transcription factor Kruppel-like factor 2 (KLF2).\textsuperscript{51} KLF2 was not expressed by CD69\textsuperscript{+} mouse memory CD8\textsuperscript{+} T cells isolated from non-lymphoid tissues. Hence, T\textsubscript{RM} did not express its target gene S1pr1 (encoding S1P1), while forced S1P1 expression prevented establishment of T\textsubscript{RM} cells. Furthermore, cytokines capable of inducing the CD69\textsuperscript{+} CD103\textsuperscript{+} resident phenotype (including TGF-\(\beta\), IL-33 and TNF), provoked KLF2 downregulation and thus downregulation of S1P1.

Expression of CD103 (or its ligand, E-cadherin) by T\textsubscript{RM} cells contributes to their maintenance in some non-lymphoid tissues\textsuperscript{54}, but is not a universal mechanism for residency retention in all tissues. For example, Casey \textit{et al.} (2012) showed that while CD103 was required for maintenance of T\textsubscript{RM} cells in the small intestinal intraepithelial lymphocyte population, it was found to be dispensable for memory cell establishment in the lamina propria lymphocyte population of the same organ.\textsuperscript{55}

Other factors involved in tissue retention include inflammatory cytokines such as transforming growth factor (TGF)-\(\beta\), interleukin (IL)-33 and tumor-necrosis factor (TNF)-\(\alpha\). TGF-\(\beta\) was shown to induce CD103 expression on mouse memory CD8\textsuperscript{+} T cells, and IL-33 and TNF-\(\alpha\) were found to synergize with TGF-\(\beta\).\textsuperscript{55} This resulted in memory cells that adopted a resident phenotype (CD69\textsuperscript{+} CD103\textsuperscript{+}) and indicates that tissues can intrinsically support differentiation of T\textsubscript{RM} cells by the cytokine milieu. Stromal cells control tissue residency of memory T cells by expression of integrins, thereby regulating activation of TGF-\(\beta\).\textsuperscript{56} Moreover, TGF-\(\beta\) and IL-15 signaling were shown to be needed for development of T\textsubscript{RM} cells in skin.\textsuperscript{57} IL-15 promoted formation and survival of T\textsubscript{RM} cells in mice. IL-15 deficient mice had reduced T\textsubscript{RM} cell formation, and this correlated with reduced Bcl-2 expression, a prosurvival molecule, in CD103\textsuperscript{+} T\textsubscript{RM} cells. Similarly, CD69 is rapidly induced in response to type 1 interferon (IFN) and suppresses S1P1 expression.\textsuperscript{48}

It has been shown that T\textsubscript{RM} have a transcriptional profile that is distinct from their memory T cell counterparts and includes transcription factors Hobit, Blimp1, and Runx3. In mice, the transcription factor Hobit is specifically upregulated in T\textsubscript{RM} cells and, together with Blimp1, instructs tissue retention in different epithelial barrier tissues.\textsuperscript{58} While Hobit was found to be essential for T\textsubscript{RM} cell development, Blimp1 by itself was not, but synergized with Hobit. Also, Blimp1 was shown to initiate cytotoxic effector function while Hobit was essential in the long-term maintenance of granzyme B-driven cytotoxicity.\textsuperscript{59} The expression of Hobit is regulated by IL-15 and the transcription factor T-bet.\textsuperscript{60} In the absence of IL-15, T\textsubscript{RM} cells had decreased Hobit levels, and upon IL-15 stimulation, activated CD8\textsuperscript{+} T cells upregulated Hobit expression in a T-bet-dependent manner.\textsuperscript{58} Blimp1 expression, however, is not induced by IL-15 or T-bet. Its expression is regulated by
the transcription factor Runx3\(^{61}\), which also promotes the expression of the \(T_{RM}\) retention markers CD69 and CD103.\(^{62}\)

Data on human \(T_{RM}\) cell transcriptional profiles are now emerging. Compared to their circulating counterparts, CD8\(^+\) \(T_{RM}\) cells isolated from human lungs expressed high levels of \(GZMB\), \(IFNG\), \(TNF\), and \(NOTCH1\) transcripts.\(^{63}\) Additionally, CD69\(^+\) memory cells from lung, spleen, and blood exhibited a transcriptional signature including CD103 and CD49a, chemokine receptors CXCR6 and CX3CR1, and immune checkpoint PD-1.\(^{64}\) Despite similar core signatures with mouse \(T_{RM}\) cells, human \(T_{RM}\) cells lacked expression of Hobit.

**IMMUNOSURVEILLANCE AND PROTECTION BY \(T_{RM}\) CELLS**

Although \(T_{RM}\) cells do not recirculate throughout the body, they can migrate slowly within their environment. Antigen-specific CD8\(^+\) T cells have been shown to crawl slowly between keratinocytes.\(^{65}\) This enables \(T_{RM}\) cells to identify antigen-expressing target cells at different tissue locations within minutes to hours.\(^{65,66}\) Their ability to scan the environment in which they persist after a primary infection is associated with enhanced pathogen detection upon reinfection by pathogens.\(^{65}\) \(T_{RM}\) cells are located in frontline sites of infection, such as the skin, lungs and intestines and, therefore tend to respond rapidly to pathogen re-challenge.

Additionally, upon antigen resensitization \(T_{RM}\) cells trigger rapid innate and adaptive immune responses by secreting cytokines. Initially, \(T_{RM}\) cells can attract circulating memory T cells within hours by producing IFN-\(\gamma\).\(^{67}\) Moreover, \(T_{RM}\) cell-derived IFN-\(\gamma\) initiates an anti-pathogen state at the local tissue site.\(^{65}\) At the same time, activated \(T_{RM}\) cells express TNF-\(\alpha\), which is essential for dendritic cell maturation.\(^{14}\) Also, CD4\(^+\) and CD8\(^+\) CD69\(^+\) T cells are able to produce IL-22, IL-17 and anti-inflammatory IL-10. \(T_{RM}\) cells can thus trigger inflammation by pro-inflammatory cytokines, but prevent excessive inflammation through IL-10.\(^{64}\) Within 12 hours of local reactivation \(T_{RM}\) cells express IL-2, which leads to elevated levels of granzyme B secreted by both \(T_{RM}\) and natural killer cells.\(^{14}\) After local pathogen challenge, \(T_{RM}\) cells proliferate in situ and recruit memory T cells from the circulation, which subsequently undergo \(T_{RM}\) cell differentiation.\(^{68}\) As a result, secondary \(T_{RM}\) cells are generated from pre-existing \(T_{RM}\) cells, as well as from recirculating precursors. However, the pre-existing \(T_{RM}\) cell populations are not displaced and remain in place in the tissue.
Despite their role in conferring protective immunity, T_{RM} cells can become pathologically activated and can cause tissue-specific autoimmunity and inflammatory disease.\textsuperscript{16} The clinical characteristics of inflammatory lesions caused by T_{RM} cells manifest as fixed, delineated zones of lesions, with an abrupt cut-off from nonlesional tissues. The pathogenic role of T_{RM} cells has been shown in many diseases, including psoriasis\textsuperscript{17-20} and cutaneous T cell lymphoma\textsuperscript{24}. Commonly used treatments for psoriasis cannot fully deplete the pathogenic T_{RM} cells from skin lesions.\textsuperscript{17,19} This appears to explain why psoriatic lesions often reoccur at exactly the same anatomical location after therapy cessation.

THE ROLE OF T\textsubscript{RM} CELLS IN AUTOIMMUNE VITILIGO

I. T_{RM} cells in the pathogenesis of vitiligo

Vitiligo is a common autoimmune disease, affecting approximately 1% of the general population. It results from the loss of epidermal melanocytes.\textsuperscript{69} Genetic predisposition, environmental factors, and metabolic and immune alterations have been implicated in melanocyte destruction.\textsuperscript{70-72} Previous studies have clarified the autoimmune etiology in human vitiligo. For example, vitiligo patients have melanocyte-specific CD8\textsuperscript{+} T cells that are capable of killing melanocytes\textsuperscript{73-75} and initiating antibody responses against melanocyte antigens, such as tyrosinase and TRP-2.\textsuperscript{76}

Vitiligo lesions often recur at the same locations as those previously affected, suggesting that T_{RM} cells could be involved. A mouse model of melanoma-associated vitiligo showed that both lesional and nonlesional skin contained resident memory T cells, although they were preferentially localized in hair follicles containing white hairs.\textsuperscript{41} To induce melanoma-associated vitiligo, mice were inoculated with B16 melanoma cells and depleted from regulatory T cells, after which the tumor was surgically removed. Vitiligo-affected skin was shown to have CD8\textsuperscript{+} T cells recognizing tumor/self-antigens and to exhibit a T_{RM} cell phenotype (CD44\textsuperscript{hi} CD62L\textsuperscript{lo} CD69\textsuperscript{+} CD103\textsuperscript{+}). In line with this, autoreactive CD8\textsuperscript{+} T cells with a CD69\textsuperscript{+} CD103\textsuperscript{+/-} T_{RM} phenotype have been found in the skin of vitiligo patients (Figure 2).\textsuperscript{25-28} Compared to healthy unaffected donor or psoriasis skin, lesional skin from vitiligo patients was shown to be enriched with CD49a\textsuperscript{+} CD103\textsuperscript{+} CD8\textsuperscript{+} and CD69\textsuperscript{+} CD103\textsuperscript{+/-} CD8\textsuperscript{+} T_{RM} cells, independent of disease activity.\textsuperscript{25,26} In the same study it was suggested that the remaining CD8\textsuperscript{+} T_{RM} cells could possibly mediate disease flares or, alternatively, block repigmentation. CD8\textsuperscript{+} T_{RM} cells may prevent repigmentation by blocking either renewal of epidermal melanocytes or entry from the follicular reservoir of melanocyte precursors.
The chemokine receptor CXCR3 (the receptor for the chemokines CXCL9 and CXCL10) was shown to be important for epidermal localization of effector T cells and T<sub>Rm</sub> cell development<sup>57</sup>, which led to studies on CXCR3 expression in vitiligo patients. Perilesional skin from patients with progressive disease showed high CXCR3 expression.<sup>77</sup> Expression of CXCR3 was found on the majority of CD8<sup>+</sup> T<sub>Rm</sub> cells in human vitiligo, including melanocyte-specific cells, and these T<sub>Rm</sub> cells were poised for the secretion of IFN-γ and TNF-α.<sup>25</sup> These results indicated that targeting the CXCL9/10-CXCR3 pathway could be an attractive strategy for the treatment of vitiligo. In line with this, another study reported an enrichment of IFN-γ-producing CD49a<sup>+</sup> CD103<sup>+</sup> CD8<sup>+</sup> T<sub>Rm</sub> cells in vitiligo lesions, with a rapid granzyme B and perforin production response upon IL-15 stimulation.<sup>26</sup> Two studies showed that IFN-γ and granzyme B are key cytokines in the pathogenesis of vitiligo because they induce melanocyte apoptosis.<sup>78,79</sup> These results add to improved understanding of the autoimmune response in human vitiligo and suggest a profound role for CD8<sup>+</sup> T<sub>Rm</sub> cells in human vitiligo, which explains the interest in targeting this cell subset in the treatment of vitiligo patients.

**Figure 2. Resident memory T cells in vitiligo**

The role of resident memory T cells in human vitiligo is shown. Firstly, Cheuk et al. (2017) reported an increase in CD49a<sup>+</sup> T<sub>Rm</sub> cells in vitiligo skin, which produce IFN-γ, granzyme B and perforin upon IL-15 stimulation.<sup>26</sup> Furthermore, a substantial proportion of CD49a<sup>+</sup> T<sub>Rm</sub> cells recognised melanocyte-antigens, indicating a pathogenic role. Secondly, Boniface et al. (2018) showed vitiligo perilesional skin to be enriched with melanocyte-specific CXCR3<sup>+</sup>CD8<sup>+</sup> T<sub>Rm</sub> cells and CD8<sup>+</sup> T<sub>Rm</sub> cells were poised for secretion of IFN-γ and TNF-α with moderate cytotoxic activity.<sup>25</sup>
II. Therapeutic intervention in vitiligo

Based on the fundamental pathogenic role of $T_{RM}$ cells in human vitiligo, novel strategies specifically targeting $T_{RM}$ cells may improve the treatment outcome of vitiligo. $T_{RM}$ cell formation has been shown to be highly dependent on IL-15, and IL-15 promoted $T_{RM}$ cell function ex vivo. A subsequent study therefore looked at IL-15 signaling as a therapeutic target for vitiligo. Treatment with anti-CD122 antibody, a subunit of the IL-15 receptor on human and mouse $T_{RM}$ cells, was shown to reverse disease in mice with established vitiligo (Figure 3). A 2-week short-term treatment decreased IFN-$\gamma$ production, while an 8-week long-term treatment depleted autoreactive CD8$^+$ $T_{RM}$ cells. These findings indicate that targeting IL-15 signaling via CD122 may be an effective strategy to treat vitiligo and possibly other $T_{RM}$ cell-mediated diseases. $T_{RM}$ cell survival and function also depends on the uptake of exogenous lipids and on their oxidative metabolism. Future studies targeting this pathway might reveal if it can affect or even deplete $T_{RM}$ cells from peripheral tissues.

Besides melanocyte-specific $T_{RM}$ cells, autoreactive $T_{CM}$ cells have been found in the blood of vitiligo patients, and these cells have the potential to home to the skin. However, the functional capacity of $T_{CM}$ has remained unknown. A study examining the functional relationship between $T_{CM}$ and $T_{RM}$ in a vitiligo mouse model reported that $T_{CM}$ cells cooperate with $T_{RM}$ cells to maintain disease (Figure 3). Both subsets recognized self-antigen and secreted IFN-$\gamma$ and chemokines. Gp100-specific CD69$^+$ CD103$^+$ CD8$^+$ $T_{RM}$ cells produced CXCL9 and CXCL10, which are chemokines recognized by CXCR3, potentially to recruit $T_{CM}$ cells towards melanocytes in the skin. Treatment of mice with FTY720, as a means of blocking T cell access to the skin, or low-dose Thy1.1 antibody, to deplete $T_{CM}$, resulted in reversal of disease. This study suggests that circulating and resident T cells cooperate in vitiligo pathogenesis. However, the extent of such a relationship between circulating and resident memory T cell subsets in human vitiligo remains unclear. Better understanding of this relationship may give clues on how pathogenic T cells can most effectively be targeted in vitiligo.
Figure 3. **Therapeutic intervention in vitiligo**
Potential therapeutic approaches for vitiligo are illustrated. Two murine studies showed that targeting TRM cells can reverse disease in mice with established vitiligo. The left model shows TRM cells express the IL-15 receptor subunit CD122 and treatment with anti-CD122 antibody led to repigmentation. Short-term treatment led to less IFN-γ production by TRM cells and long-term treatment depleted autoreactive TRM cells and other memory T cell pools. The right model shows that TRM and TCM cells cooperate to maintain vitiligo. Treatment of mice with FTY720, which limits T cell access to the skin, or low-dose Thy1.1 antibody, which depletes TCM, resulted in repigmentation.
SKIN-RESIDENT T CELL RESPONSES IN MELANOMA

I. Prognostic significance of resident memory-like tumor-infiltrating lymphocytes (TILs)

While T<sub>RM</sub> cells have been widely characterized in viral infections, their role in mediating tumor immunity is not yet fully known. Studies analyzing the infiltration of T<sub>RM</sub> cells in human tumors have shed some light on their relevance in anti-tumor immunity. Tumor infiltration of CD8<sup>+</sup> T cells exhibiting a resident phenotype (CD69<sup>+</sup> CD103<sup>+</sup> and/or CD103<sup>+</sup>) correlates with a more favorable prognosis for various human cancers. Similar correlations were shown for human melanoma (Figure 4).<sup>38,42</sup> CD103<sup>+</sup> CD8<sup>+</sup> T cells, residing in the tumor microenvironment, were strongly correlated with increased melanoma-specific survival in immunotherapy naïve stage III melanoma patients.<sup>38</sup> High CD103<sup>+</sup> CD8<sup>+</sup> T<sub>RM</sub> cell counts led to a 5-year survival rate of 50% compared to 20% in those with lower counts. Also, expression of CD49a by vaccine-induced CD8<sup>+</sup> T cells was shown to predict a prolonged overall and disease-free survival in stage III/IV melanoma patients (Figure 4).<sup>42</sup> CD49a<sup>+</sup> CD8<sup>+</sup> TILs were found to be enriched in human melanoma metastases in various peripheral tissues. Most interestingly, CD49a was frequently co-expressed with CD69 and CD103, and in vivo blockade of CD49a or CD103 in a C57BL/6 melanoma mouse model significantly impaired control of subcutaneous B16-OVA tumors, supporting the notion of T<sub>RM</sub> cell-mediated anti-tumor immunity. CD49a<sup>+</sup> B16-OVA derived T<sub>RM</sub> cells produced higher levels of IFN-γ and granzyme B and exhibited a high activation status, which was even more prominent in the CD103<sup>+</sup> subset. Moreover, in human melanoma, local IL-15 levels strongly correlated with tumor-resident CD8<sup>+</sup> T cell numbers, and high IL-15 levels were associated with a more favorable prognosis.<sup>38</sup> IL-15 seems to be essential in retaining T cells within the tumor microenvironment, indicating that IL-15 is worthy of further investigation, as supported by the murine vitiligo data discussed previously.<sup>28</sup>

II. Expression of immune checkpoints by resident memory-expressing TILs

Upregulation of immune checkpoints on TILs has emerged as a major barrier to effective anti-tumor immunity. Interestingly, not all TILs express immune checkpoints. Identifying which subpopulations among tumor-infiltrating immune cells – defined both phenotypically and functionally – express immune checkpoints is important in evaluating anti-tumor immunity. Counterintuitively, in human melanoma metastases, tumor-associated CD8<sup>+</sup> T cells with a T<sub>RM</sub> phenotype were shown to express the highest levels of immune checkpoints, such as PD-1 and TIM-3, independent of CD103 expression (Figure 4).<sup>37</sup> In the same study, TILs simultaneously produced less cytokines, which is consistent with an exhausted phenotype.<sup>87</sup> Another study reported that mainly the CD103<sup>+</sup> CD8<sup>+</sup> subset within TILs expressed high levels of PD-1, LAG-3, 2B4 and TIM-3 (Figure 4).<sup>38</sup> Upon
anti-PD-1 therapy, CD103-expressing CD8+ T\textsubscript{RM} cells significantly expanded, suggesting that these cells have been released from the negative effect of PD-1 checkpoint signaling by anti-PD-1 therapy. Tumor-resident TILs may thus represent a major target for immune checkpoint blockade. However, these findings were based on markers that are redundant with other T cell states. For example, CD69 and PD-1 can be co-expressed as a result of recent antigen stimulation. Similarly, TGF-β has been shown to induce expression of CD103 on CD8+ T cells\(^{38}\) and TGF-β is often produced within the tumor environment\(^{89}\). It therefore remains unclear whether inhibitory checkpoint molecules are particularly enriched on resident memory-expressing TIL or whether resident cell markers are expressed as result of environmental factors or antigen stimulation.

Another consideration is that less immune checkpoint expression is found on T\textsubscript{RM} cells in normal skin or during autoimmune response or infection. In the B16 mouse model of melanoma-associated vitiligo, it was shown that cutaneous tumor/self-antigen-specific CD8+ T\textsubscript{RM} cells located within depigmented hair follicles, lacked PD-1 and LAG-3 expression.\(^{41}\) Likewise, skin CD8+ T\textsubscript{RM} cells lacked PD-1 expression in a murine model of viral infection.\(^{8}\) In a vitiligo mouse model, however, autoreactive CD8+ T\textsubscript{RM} cells did express PD-1.\(^{27}\) This indicates that expression of immune checkpoints has not been fully elucidated and requires more attention in future research.

### III. Clinical implications of resident memory-like TILs

Current data suggest an important role for TILs that express T\textsubscript{RM}-associated markers in providing anti-tumor immunity and their potential as biomarkers. Immune checkpoints seem to be enriched particularly on T\textsubscript{RM} cells, suggesting that the T\textsubscript{RM} subset of TILs may be the major target for immune checkpoint blockade. Hence, cancer immunotherapy vaccination strategies should aim at priming tumor-reactive T\textsubscript{RM} cells subsets, which could synergize with immune checkpoint blockade.

Human metastatic lesions are enriched with CD8+ T\textsubscript{RM}-like cells\(^{37}\), and adoptive transfer of resident memory-like TILs might be a promising therapeutic option to melanoma patients. However, individual metastasis in the same patient may contain a distinct repertoire of T\textsubscript{RM}-like cells. Sequencing of the T cell receptor (TCR) revealed interlesional heterogeneity of TILs, which was also found in the resident T cell population.\(^{37}\) This heterogeneity was not due to variance in mutations or neoepitopes in tumor cells. Consequently, patients may experience mixed responses, with some tumor lesions regressing and others progressing, as occasionally observed following immunotherapy. It is therefore logical to explore adoptive transfer of T\textsubscript{RM}-like TIL isolated from multiple lesions, as this may provide a more diverse repertoire. However, considering the limited sample number in the study of Boddupalli et al. (2016), interlesional heterogeneity should be confirmed in larger studies.\(^{37}\)
IV. Anti-tumor immunity by TRM cells

Despite the great interest in immunotherapy, its clinical success still requires substantial optimization. To improve the efficacy of cancer immunotherapy, it is important to induce a potent effector response together with a stable, functional memory response, thus protecting the patients from cancer recurrence or relapse. Mouse models of melanoma have shown that tumor-specific TRM cells can protect against highly aggressive melanoma. CD8+ TRM cells driven by a model of autoimmune vitiligo were shown to inhibit melanoma growth in a CD103-dependent manner. Also, infecting the skin with recombinant vaccinia virus expressing full-length ovalbumin (OVA) protein generated CD8+ T CIRC and TRM cells that delayed the growth of OVA-expressing melanoma (Figure 4).

Intraperitoneal vaccination, which generates T CIRC only, or FTY720 treatment, which blocks T cell access to the skin, revealed that either T CIRC or TRM cells were sufficient for protection against B16-OVA re-challenge in the skin, but that the presence of TRM cells improved anti-tumor efficacy. Gálvez-Cancino et al. (2018) showed that intradermal administration of vaccines, which are known to induce strong CD8+ T cell responses, efficiently induced TRM cell responses against tumor antigens and self-antigens (Figure 4). Moreover, growth of cutaneous melanoma tumors was strongly suppressed, independently of circulating CD8+ T cells and other adaptive immune cells. Similarly, CD8+ TRM cells promoted a melanoma-immune equilibrium in the epidermal layer of the skin (Figure 4). In the B16/B16 mouse melanoma model, approximately 40% of mice that received epicutaneous inoculation of B16 melanoma cells remained free of macroscopic tumor growth. Tumor cells were dynamically surveyed by CD69+ CD103+ CD8+ TRM cells, and TRM cell responses were observed more often and at higher densities in peritumoral skin than in the skin of tumor-bearing mice. In line with the findings of Enamorado et al. (2017), melanoma development was also suppressed in the majority of mice, irrespective of depletion of T CIRC cells, but protection was most pronounced in mice harboring both T CIRC and TRM cells. These studies clearly affirm the potential of intradermal vaccine-induced TRM cells to achieve potent protection against skin cancer. To effectively protect against malignancies, cancer vaccines should therefore evoke potent TRM cell responses within the tissue.
Emerging evidence has shown that non-recirculating T$_{RM}$ cells constitute a large fraction of the memory T cell pool and are involved in controlling various infectious diseases, cancer or in mediating autoimmunity. The growing appreciation that T$_{RM}$ cells are central players in immunity to vitiligo and melanoma has led to increased interest in T$_{RM}$ cells as promising targets for future vaccines and immunotherapies. T$_{RM}$ cells are likely to have a prominent role in disease development and flare-up in human vitiligo. Therefore, targeting T$_{RM}$ cells appears to be an attractive treatment strategy. Blocking the generation, maintenance and coordination of T$_{RM}$ cells efficiently inhibits melanocyte killing in mice models. Future trials in patients will provide important insights into targeting T$_{RM}$ cells for the treatment of human vitiligo. Although not fully confirmed by all studies so far, inhibitory immune checkpoints appear to be particularly enriched on cells with T$_{RM}$ cell properties in human melanoma. At the same time, however, their expression on T$_{RM}$ cells in the context of autoimmune vitiligo remains unstudied. In vitiligo, the autoimmune reaction is not downregulated, but remains present; future studies might therefore clarify whether immune checkpoint expression is possibly dispensable on vitiligo-associated T$_{RM}$ cells. The evidence on the contribution of T$_{RM}$ cells in cancer suppression also shows how the manipulation of T$_{RM}$ cells can be beneficial in optimizing the anti-tumor immunity. Vaccination strategies have successfully generated T$_{RM}$ cell populations that have effectively suppressed tumor growth in mouse models of melanoma. However, developing these therapies will require additional experimental studies to obtain more insight into the exact phenotype and function of T$_{RM}$ cells in mice and humans. Furthermore, to validate data from mouse experiments in human clinical trials, it is crucial to study the potential of targeting T$_{RM}$ cells in human disease. The research highlighted in this review has focused on CD8$^+$ T$_{RM}$ cells as key mediators of anti-tumor immunity. However, the role of CD4$^+$ T$_{RM}$ cells in immunity to cancer remains undefined. Future studies should clarify whether tumor immunity benefits from local helper T$_{RM}$ cells, and whether regulatory T$_{RM}$ cells are detrimental to this immunity. Moreover, studies on vitiligo have not reported data on CD4$^+$ helper or regulatory T$_{RM}$ cell subsets either. With more knowledge becoming available on the involvement of T$_{RM}$ cells in autoimmunity and cancer, future research will hopefully overcome barriers to effectively block or to promote effective responses of T$_{RM}$ cells to vitiligo and melanoma.

**Figure 4. Resident memory T cells in melanoma**

Skin-resident memory T cells can have various roles in melanoma; (1) T$_{RM}$ cells can mediate anti-tumor immunity, upon vitiligo induction strategies or intradermal vaccine administration (murine data), (2) CD8$^+$ T$_{RM}$ cells promote a melanoma-immune equilibrium in the epidermis (murine transplanted melanoma model), (3) tumor infiltration of T cells expressing either CD103 or CD49a, frequently co-expressed with CD69 and CD103, is correlated with improved survival of melanoma patients and (4) metastatic melanoma patients show expression of the immune checkpoints PD-1, LAG-3, 2B4 and TIM-3 on intratumoral T$_{RM}$ cells, with or without CD103 co-expression.
CONCLUSIONS

Emerging evidence has shown that non-recirculating T_{RM} cells constitute a large fraction of the memory T cell pool and are involved in controlling various infectious diseases, cancer or in mediating autoimmunity. The growing appreciation that T_{RM} cells are central players in immunity to vitiligo and melanoma has led to increased interest in T_{RM} cells as promising targets for future vaccines and immunotherapies.

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