vaccines

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Review

Resident Memory T Cells and Their Effect on Cancer

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Abstract: Resident memory T (T_{RM}) cells are a unique subset of $CD8^+$ T cells that are present within certain tissues and do not recirculate through the blood. Long term memory establishment and maintenance are dependent on tissue population of memory T cells. They are characterized by dual CD69/CD103 positivity, and play a role in both response to viral infection and local cancer immunosurveillance. Human T_{RM} cells demonstrate the increased expression of adhesion molecules to facilitate tissue retention, have reduced proliferation and produce both regulatory and immune responsive cytokines. T_{RM} cell phenotype is often characterized by a distinct expression profile driven by Runx3, Blimp1, and Hobit transcription factors. The accumulation of T_{RM} cells in tumors is associated with increased survival and response to immunotherapies, including anti-PD-1 and anti-CTLA-4. In this review, we explore potential mechanisms of T_{RM} cell transformation and maintenance, as well as potential applications for the use of T_{RM} cells in both the development of supportive therapies and establishing more accurate prognoses.

Keywords: memory T cells; immunotherapy; cancer vaccine

1. Introduction

$CD8^+$ T cells are a critical component of the adaptive immune system that target intracellular pathogens, such as virus-infected host cells, as well as host cells with oncogenic mutations. Based on the heterogeneity of cell-surface receptors isolated from peripheral blood samples, $CD8^+$ T cells are classically stratified into two groups, effector memory T (T_{EM}) cells or central memory T (T_{CM}) cells [1]. Antigen presenting cells, such as dendritic cells, utilize the major histocompatibility complex I (MHC-I) system to present intracellular antigens to both recirculating and non-recirculating $CD8^+$ T cells resulting in cell death [2]. More recent examination of nonlymphoid tissue specimens have revealed a unique population of non-recirculating T cells, called resident memory T (T_{RM}) cells. T_{RM} cells do not re-circulate, and it is for this reason that these cells were not originally observed in samples obtained from peripheral blood [3]. Instead, T_{RM} cells reside in peripheral tissues long after an infection has cleared. After exposure to an antigen, T_{RM} cells persist, poised to respond quickly in the event of re-exposure by rapidly secreting cytotoxic granules as well as cytokines that recruit both innate and adaptive immune cells. This allows patients to respond to re-exposure much faster than T_{EM} or T_{CM} cells allow [4–6]. In addition to viral infections, T_{RM} cells are thought to take part in local cancer immunosurveillance because they often accumulate in a variety of human solid tumors, and are associated with positive patient outcomes [7–11]. This aligns with previous observations

concerning the role that MHC-I antigen presentation systems play in detecting intracellular insults, such as viral infections and malignant transformation of host cells [12]. The T_{RM} cell phenotype is induced when effector-like $CD8^+$ T cells—which enter a tissue early on in an infection—are activated by TGF-β, IL-33, and IL-15. Activation results in the expression of the transcription factors Hobit and Blimp1 [13]. This, in turn, initiates a signaling cascade that upregulates genes associated with tissue retention and downregulates genes associated with recirculation. The functional phenotype of $CD8^+$ T_{RM} cells is largely due to their dual presentation of CD69 and CD103 surface markers, as well as an absence of the lymph node homing receptors CD62L and CCR7 (Figure 1) [7,14,15]. CD69 is a C-type lectin that is upregulated prior to CD103 and antagonizes sphingosine-1-phosphate-receptor-1 (S1P1), rendering T_{RM} cells unresponsive to tissue egress signals and resulting in tissue retention [15,16]. CD103 is an αEβ7 integrin that binds to the epithelial marker E-cadherin, which allows T_{RM} cells to maintain close contact with both peripheral lymphoid and non-lymphoid tissues, such as the lung, skin, gastrointestinal, and genitourinary tracts [17]. CD103-mediated binding to E-cadherin also allows T_{RM} cells to maintain close contact with malignant cells residing within these tissues [18,19]. This allows T_{RM} cells to act as an immunosurveillance population within the tissue microenvironment (TME). T_{RM} cells are primed with both cytotoxic granules and effector molecules, such as IFN-γ and TNF-α, which can further activate additional immune cells [4,20]. The differentiation and maintenance of T_{RM} cells also requires Runx3 expression, which serves as a master regulator of T_{RM} cell phenotype by regulating expression of CD103 and CD69, and increasing cytotoxic activity [21,22]. This is evident in preclinical murine melanoma models, where *Runx3*-shRNAmir knockdown resulted in diminished T_{RM} cell accumulation within tumors, uncontrolled tumor growth, and low survival. Conversely, T_{RM} cells that overexpress Runx3 resulted in growth inhibition and improved survival [21].

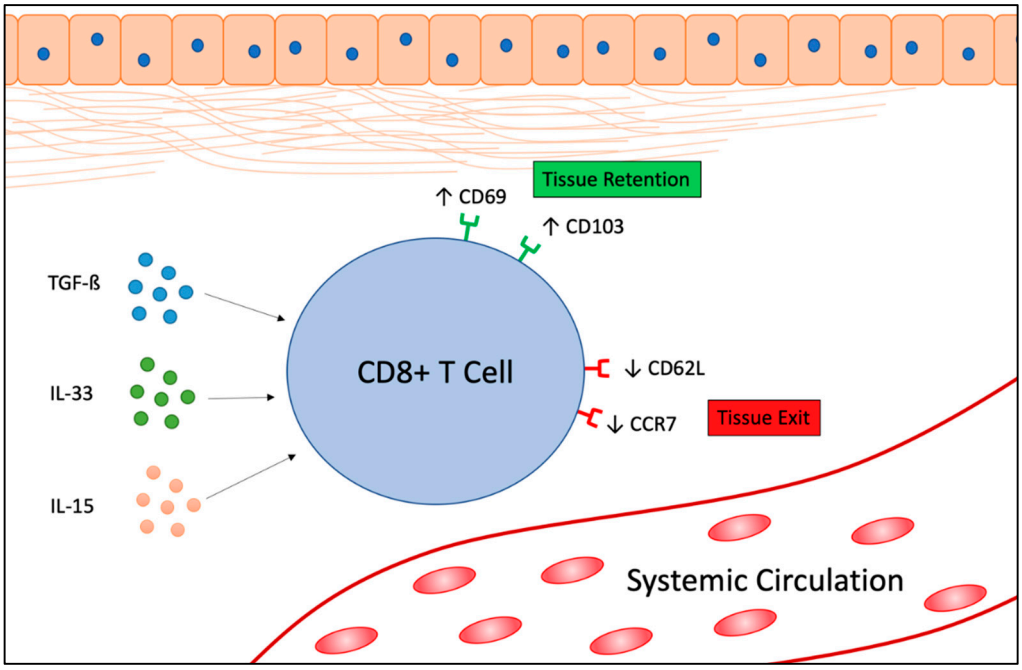

Figure 1. $CD8^+$ T cells enter tissues and are stimulated by TGF-ß, IL-33, and IL-15 to upregulate the tissue-retention surface markers, CD69 and CD103, while simultaneously down-regulating the tissue-egress markers, CD62L and CCR7. This creates a resident memory T (T_{RM}) cell phenotype, enabling T_{RM} cells to maintain close contact with malignant cells residing within tissues.

2. T_{RM} Cells in Cancer

2.1. Function of T_{RM} Cells in Cancer

The presence of T_{RM} cells in clinical tumor samples is associated with an improved outcome in a variety of cancers, including melanoma [23], non-small cell lung cancer (NSCLC) [9], breast cancer [24–26], cervical cancer [11], and ovarian cancer [27]. In addition, T_{RM} cells confer a degree of antitumor immunity in murine melanoma models. Specifically, knockout mice lacking CD69 or CD103 were more susceptible to transplantable melanoma challenge relative to wild-type mice [28,29]. Furthermore, T_{RM} cells pre-generated using vaccines containing tumor neoantigen DNA are protective against transplantable melanoma or head and neck tumors even when circulating T cells are depleted, using targeting antibodies [30,31]. Mackay et al. demonstrated that local signaling by IL-15 and TGF-β were required for T_{RM} cell transformation and the maintenance of CD69 and CD103 status in skin epithelium [15]. T_{RM} cells utilize a variety of tumor-killing methods depending on the cancer type. For example, samples from NSCLC patients demonstrate marked antigen-independent increases in inflammatory cytokine mRNA expression of granzyme B, IFN-γ, and TNF-α [32], providing a potential explanation for the ability of T_{RM} cells to act quickly following antigen exposure. Tumor samples from urothelial urinary bladder cancer (UBC) patients were obtained during diagnostic transurethral resections, and T_{RM} cells were isolated using flow cytometry. The pyrosequencing of genes associated with cytotoxicity reveal that the perforin gene (PRF1) is hypomethylated in T_{RM} cells present in UBC samples. This corresponds to the increased perforin expression previously reported in T_{RM} cells from other cancers [32,33]. In addition, T_{RM} cells derived from a variety of primary ovarian tumors, including endometrioid, mucinous, clear cell, and high-grade serous carcinoma, were obtained and sorted using flow cytometry and immunohistochemistry. The authors found that the T_{RM} cells universally expressed high amounts of TIA-1, a marker of cytotoxic potential, relative to non-resident $CD103^{-}$ T cells [34]. The number and frequency of T_{RM} cells vary from cancer to cancer, patient to patient, and even from lesion to lesion within the same patient [23,35,36]. This suggests that environmental cues from the tumor microenvironment are essential for the recruitment and maintenance of T_{RM} cells. Metabolically active tumor cells are heavily dependent on glucose metabolism, commonly referred to as the "Warburg effect," resulting in elevated lactate levels in the tumor microenvironment [37]. This setting of nutrient deprivation and local acidosis favors metastasis, angiogenesis, and immunosuppression. T_{RM} cells have a unique ability to adapt to this by metabolizing free fatty acids. This suggests that these cells are better suited to survive in the TME [38].

In addition to predicting survival, the induction or presence of T_{RM} cells enhance response to certain therapeutics. T_{RM} cells are often present in normal tissues and tumors which express higher amounts of immune inhibitory and costimulatory receptors, such as PD-1, CTLA-4, and Tim3. This prevents autoimmune reactions [15,32,39]. Interestingly, the combination of immune inhibitory and costimulatory receptors varies depending on tumor type [27]. This opens the door for immunotherapies that seek to target these receptors and enhance T cell response. Enamorado et al. demonstrate this in a preclinical murine model in which anti-PD-1 antibody administration and adoptive T_{RM} cell transfer inhibit the growth of subcutaneously injected MC38-OVA tumors, as well as intradermal B16-OVA tumors, when compared to adoptive T_{RM} cell therapy alone [40]. Edwards et al. isolated T_{RM} cells from both immunotherapy-naïve melanoma samples and those derived from patients undergoing anti-PD-1 therapy using multiparameter flow cytometry. T_{RM} cells were quantified using quantitative multiplex immunofluorescence staining to show that the presence of T_{RM} cells in immunotherapy-naïve melanoma samples was associated with significantly increased melanoma-specific survival, and this cell population was further expanded using anti-PD-1 therapy [23]. Additionally, Blanc et al. showed that the T_{RM} cell population increases within tumors during the early stages of anti-PD-1 treatment [41]. In addition, T_{RM} cells isolated from lung carcinomas co-cultured with autologous tumor cells demonstrate enhanced cytotoxic activity in the presence of PD-1 targeting antibodies compared to those not treated with PD-1 antibodies [9]. Also, the combination of PD-1 targeting antibodies with PPAR-α agonists or

the administration of free fatty acids increased functionality of T_{RM} cells in a melanoma model [42]. These studies provide evidence for the superior prognostic value of the number of T_{RM} cells present in multiple tumor types compared to circulating $CD8^+$ cells. This evidence demonstrates that T_{RM} cells are not only associated with protective immunity, but they may also be effective in increasing the response to anti-PD-1 therapy [23,43].

2.2. Identification of T_{RM} Cells in Patient Samples

T_{RM} cells have been identified in both human and murine tissues, including liver, lungs, pancreas, lymphoid tissues, genital mucosa, stomach, jejunum, ileum, colon, bone marrow, and in brain obtained from autopsies [44–51]. However, since T_{RM} cells do not recirculate in blood, point-of-care collection and analysis of T_{RM} cells is limited by the need for tissue biopsy or surgical resection [44]. Once appropriate samples are obtained, there are a variety of methods for characterizing T_{RM} cells. For example, the cell surface epitopes CD103 and CD69 can be used to separate T_{RM} cells from other $CD8^+$ T cells using flow cytometry. T_{RM} cells can also be identified through the use of unique transcriptional signatures that include the increased expression of IL-2, IFN-γ, IL-17, and IL-10, as well as multiplex immunohistochemistry [34,39]. In addition, T_{RM} cells can be differentiated from circulating T cells via functional characterization by assessing T_{RM} disequilibrium in a parabiosis model [52–54]. Another method of identifying T_{RM} cells is by their migration patterns using methods such as photoreaction, as seen in transgenic murine and lymphatic cannulation models [53,55–57]. The accurate and quantitative measurement of T_{RM} will facilitate potential use as a biomarker involved in clinical testing of immunotherapy.

2.3. Improving Vaccine Efficacy

The human papilloma virus (HPV) cancer vaccine provides long-term protection against certain cancers associated with HPV, including cervical cancer, as well as head and neck cancer. The success of these vaccines is likely associated with the presence of T_{RM} cells which are knowledgeable of the HPV-induced cancer antigen profile [58]. Despite the fact that HPV-induced cancers primarily develop at mucosal sites such as the oral and vaginal cavities, the majority of preclinical vaccines are administered subcutaneously or intramuscularly [59]. In murine models, intravaginal boosters, following systemic (intramuscular) vaccination, resulted in the local accumulation of T_{RM} cells and was associated with increased survival compared to intramuscular vaccination alone, which did not induce local T_{RM} cell accumulation [60]. In addition, intranasal administration of the HPV vaccine in an orthotopic head and neck cancer model provided long-lasting protection by recruiting and maintaining T_{RM} cells in the local tissue. Intranasal vaccination for respiratory syncytial virus (RSV) also generated robust and durable T_{RM} cell populations that were not detectable after subcutaneous vaccination [61].

Vaccine trials involving intramuscular injections for herpes simplex virus 2 (HSV-2) have largely been ineffective. This is likely due to the minimal recruitment of T_{RM} cells to the site of infection. To address this limitation, Cuburu et al. generated a replication-defective HPV pseudovirus that expressed HSV-2 glycoproteins B (gB) and D (gD), for intravaginal vaccination using a murine model. Mice vaccinated intravaginally demonstrated a significantly reduced viral load and reduced severity of HSV lesions compared to intramuscular vaccination. Importantly, intravaginal vaccination resulted in the accumulation of T_{RM} cells that were able to secrete IFN-γ, TNF-α, and moderate levels of neutralizing antibodies [62].

This phenomenon is further supported by preclinical glioblastoma models, whereby the injection of tumor cells by different routes (intraperitoneal, intracranial, and subcutaneous) resulted in T cells displaying different patterns of integrins depending on the sentinel lymph nodes they were obtained from. This suggests that the route of immunization plays an important role in vaccine efficacy [63,64]. Importantly, the use of a potent circulating memory T cell inhibitor (FTY720) provided evidence that the T_{RM} cells alone could partially control tumor growth, although the presence of circulating memory T cells improved vaccine efficacy [30,65]. However, while the interplay between both T_{RM} cells and

circulating memory T cells is critical to vaccine efficacy, the presence of local T_{RM} cells appears to be paramount [40].

Reports describing the Vigil vaccine in various solid tumors provide additional evidence supporting the efficacy of immunization. Vigil is an autologous vaccine produced from harvested tumor tissue and transfected ex vivo, using a plasmid containing the GM-CSF gene and short hairpin RNA that knocks down furin expression [66]. Furin is a convertase which is responsible for the cleavage and activation of TGFβ1 and TGFβ2. Phase I clinical trials in Ewings sarcoma, melanoma, and solid tumor malignancies demonstrate both safety and efficacy [67–70]. A phase I trial investigating the Vigil vaccine in solid tumor patients reports a significant correlation between γ-IFN-ELISPOT positive response and improved overall survival [69,70]. Subsequently, a Phase IIa trial of Vigil in ovarian cancer patients demonstrated safety and improved relapse-free survival compared to control [71]. A Phase IIb trial has recently completed and significant survival advantage in relapse free survival (RFS) was demonstrated in patients with *BRCA*-wt tumors [72]. Based on the durability of clinical response observed in Phase I testing and long term follow up, it was suggested that, Vigil induces persistent circulating "self" mononuclear cell function activity against "self" tumor following treatment and persists after discontinuation. Evidence supports enhanced memory T cell function which maybe relevant to clonal neoantigens [70,71,73]. Further study is indicated.

2.4. Improving Adoptive T Cell Therapy

In addition to cancer vaccines, direct infusion of cancer neoantigen experienced T_{RM} cells through adoptive T cell therapy (ACT) is a promising strategy. Traditional ACT involves harvesting tumor-specific circulating T cells and expanding them exponentially in vitro. These T cells are then reinfused into the patient, where they mediate tumor destruction [74]. Milner et al. provided evidence that ACT with T_{RM} cells inhibited tumor growth and increased overall survival in mice [21]. Using a murine model for adoptive T cell therapy in melanoma, they demonstrated that transfer of Runx3-deficient $CD8^+$ T cells (recirculating phenotype) resulted in increased mortality due to their inability to accumulate in tumors. Conversely, the transfer of $CD8^+$ T cells overexpressing Runx3 (T_{RM} cell phenotype) resulted in an accumulation of T_{RM} cells in tumors and prolonged survival [21]. In addition, reprogramming tumor infiltrating dendritic cells using β-glucan curdlan resulted in increased dendritic cell TGF-β production and the differentiation of $CD103^+$ T cells in a humanized murine model of breast cancer. Importantly, this resulted in tumor rejection, highlighting how indirect adoptive cell therapy may lead to local T_{RM} cell transformation and maintenance [75].

A more recent branch of adoptive cell transfer has been developed, in which a patient's T cells are collected and modified to produce and present chimeric antigen receptors (CARs) on their surface. These receptors enable the new CAR T-cells to latch onto tumor-specific antigens on the cell's surface. Currently, CAR-T therapy is used to treat chemotherapy-resistant acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL) [76–80]. Unfortunately, attempts to use CAR-T therapy to treat solid tumors have not been nearly as successful [81]. At least part of the difficulty in treating solid tumors is ensuring that the CAR-T cells reach and infiltrate the tumor sites [82]. Although the precise mechanism of differentiation and maintenance of T_{RM} cells are still being elucidated, researchers may be able to use CAR-T therapy to increase and maintain functional T_{RM} cells in cancer patients with solid tumors by inducing the T_{RM} cell phenotype with TGF-β and IL-15, or via other relevant molecular profile induction [83].

2.5. T_{RM} Cell Clinical Trials

Few clinical trials have explored how the presence of T_{RM} cells in various cancers are related to response rates with immune checkpoint inhibitors (Table 1). However, Savas et al. demonstrated the importance of both the $CD8^+/CD103^+$ and $CD8^+/CD103^-$ T cell subtypes in prolonging overall survival in triple-negative and HER2-positive breast cancer using single-cell profiling ($p = 0.03$) [26]. The authors found a 37-gene T_{RM} signature associated with better response to cancer treatments, using

both multi-cell RNA-Seq of $CD8^+/CD103^+$ T cells (T_{RM} phenotype) compared to $CD8^+/CD103^-$ T cells, as well as single-cell RNA-Seq [26]. Among the 37 genes included in the gene signature, there was a significant decrease in the tissue-egress genes, S1PR1 and KLF2, and a significant increase in expression of immune checkpoint genes, PD1 and CTLA-4, in $CD8^+/CD103^+$ T cells, compared to $CD8^+/CD103^-$ T cells, suggesting a functionally and phenotypically distinct subset of $CD8^+$ T cells.

Table 1. Studies in solid tumors evaluating T_{RM} cell populations reporting clinical benefit.

Tumor Type	T_{RM} Markers	No. of Samples/Patients	Reference
Ovarian Cancer	CD103	489	[27]
	CD103	497	[34]
	CD103, CD3, TCRαβ, CD8αβ, CD4	186	[84]
Cervical Cancer	CD103	460	[11]
Melanoma	CD69, CD103, TNFRSF18, CD8	44	[23]
	CD8, CD103, CD69	18	[85]
Lung Cancers	CD8, CD103, CD3	101	[9]
	CD8, CD103	77	[10]
	CD8, CD103	510	[86]
Pancreatic Cancer	CD8, CD103	136	[87]
Breast Cancer	CD8, CD103	424	[8]
	CD8, T cell gene signatures	989	[88]

The presence of CD103+ TILs was also evaluated in a cohort of breast cancer cases from the Manitoba Breast Tumor Bank. CD103 TILs in the intraepithelial compartment correlated with increased relapse free and overall survival (OS) in basal-like tumors (HR 0.28; CI 0.17–0.72, $p = 0.0047$ and HR 0.25; CI 0.17–0.66 $p = 0.0017$ respectively). Increased levels of $CD8^+$ /$CD103^+$ TILs were also strongly associated with increased clinical benefit in both RFS and OS (HR 0.10 CI 0.07–0.62, $p = 0.006$ and HR 0.09 CI 0.07–0.57, $p = 0.003$ respectively) [8]. These results further confirm the importance of $CD8^+/CD103^+$ TILs in breast cancer.

The I-SPY 2 trial (NCT01042379) is an ongoing neoadjuvant platform trial evaluating the efficacy of a variety of experimental agents/combinations when added to standard chemotherapy to treat multiple breast cancer types. The goal of this study is to identify molecular signatures that serve as early indicators of treatment success. The trial is open to all women 18 and over with radiologically diagnosed stage II-III breast malignancies who have not received any prior cytotoxic treatment. Patients are enrolled and assessed for HER2, HR, and MammaPrint status, using IHC, FISH, and HER2 expression, generating eight defined subgroups based on biomarker status. Yau et al. evaluated a subset of this data to compare the prognostic value of published T/B cell signatures including subsets exhibiting a $CD8^+$ T resident memory (T_{RM}) phenotype or $CD8^+$ T effector memory (T_{EM}) phenotype. Using gene expression data from pretreatment biopsies of 989 patients enrolled in I-SPY 2 and logistic modeling to predict pathologic complete response (pCR), they found that the T_{RM} cell phenotype was most predictive of survival in the $HR^-/HER2^-$ subtype [88].

The Keynote-086 trial (NCT02447003) is a recently published international, open-label, multicohort, phase II clinical trial exploring the efficacy of the anti-PD-1 antibody pembrolizumab (MK-3475) in the treatment of metastatic TNBC. This study enrolled patients in two cohorts. Cohort A consisted of 170 patients with recurrent disease irrespective of PD-L1 expression status, who received at least 1 line of prior therapy that did not include: an anti-PD1, anti-PD-L1, anti-PD-L2, or another co-inhibitory T-cell receptor therapy at any time; an antineoplastic monoclonal antibody within four weeks; chemotherapy, targeted small molecule therapy, or radiation therapy within two weeks. Patients submitted a tumor

biopsy sample that was evaluated for TNBC status and determination of PD-L1 status using gene expression measurement. Patients received 200 mg intravenous pembrolizumab every three weeks for up to two years until evidence of disease progression through radiologic confirmation, adverse effects or patient withdrawal. The overall response rate was 5.3% (95% CI 2.7–9.9), with 5.7% (95% CI 2.4–12.2) in the PD-L1-positive population, and 4.7% (95% CI 1.1–13.4) in the PD-L1 negative population. Median progression free survival was 2.0 months (95% CI 1.9–2.0) and median overall survival was 9.0 months (95% CI 7.6–11.2) [89]. Cohort B enrolled 84 patients with no prior lines of systemic treatment who were PD-L1 positive using gene expression. Patients received 200 mg intravenous pembrolizumab every three weeks for up to two years, until evidence of disease progression through radiologic confirmation, adverse effects or patient withdrawal. The overall response rate was 21.4% (95% CI 13.9–31.4), with 4 patients receiving a complete response and 14 exhibiting a partial response. Median progression free survival was 2.1 months (95% CI 2.0–2.2) and median overall survival was 18.0 months (95% CI, 12.9–23.0) [90]. Loi et al. examined Phase II of the study, which expanded the investigation into the efficacy of pembrolizumab to subgroups of patients from Phase I, including patients who demonstrated a T_{RM} gene signature based on RNA-seq analysis from their biopsy specimens. The authors found that expression of the T_{RM} signature was associated with increased PFS ($p < 0.001$) and OS ($p < 0.001$) in patients with advanced-stage TNBC treated with pembrolizumab monotherapy [91]. Several ongoing studies are evaluating the use of gene expression profiles to predict improved clinical outcomes (NCT02841748 and NCT03516981).

3. Future Directions

Checkpoint inhibitors have demonstrated remarkable clinical efficacy to achieve durable responses, however, a subset of patients do not respond to checkpoint inhibition, despite having an immunologically "hot" tumor, including PD-L1 expression, high TMB or other prognostic factors [92]. While clinical trial data is limited, generation of T_{RM} cells has shown prognostic value to determine which patients will have the most clinical benefit to checkpoint inhibitors. Moreover, the generation of T_{RM} cells through vaccination may provide a mechanism to sensitize tumors to check point inhibition. Ovarian cancer in particular has not responded to checkpoint inhibition, despite early trials indicating a favorable immune profile [93–95]. There have been clinical trials involving combination vaccination and checkpoint inhibitors, with most administering the combination concurrently. In melanoma, gp100, a glycoprotein peptide vaccine, was administered concurrently with ipilimumab, and no clinical benefit was seen with the combination compared to ipilimumab alone [96]. However, in a murine preclinical model of prostate cancer, mice who received GVAX prior to anti-CTLA-4 demonstrated increased $CD8^+$ and $CD4^+$ T cells compared to those who received anti-CTLA-4 treatment first [97]. These findings may indicate that the timing of combination vaccination and checkpoint inhibitor is critical to achieve response. This may be due to the ability of vaccination to prime T cells and promote the generation of T_{RM} prior to checkpoint inhibition which would rapidly expand this population. Further research is needed to determine if this approach can be used to sensitize patients to checkpoint inhibition.

TILs could also be used to identify a sensitive patient population prior to checkpoint therapy. The GeparNuevo phase II double-blind study in TNBC investigated the safety and efficacy of durvalumab stratified patients, based on stromal tumor infiltrating (sTILs) lymphocytes. Pathological complete response (pCR) was increased in patients with higher sTILs compared to low sTILs, indicating that the presence of sTILs prior to therapy start may serve as a prognostic marker [98]. Further analysis is ongoing to characterize the sTILs to determine the subpopulation molecular characteristics. It would be beneficial to determine the population of T_{RM} and how they relate to response and clinical outcome. More research is needed to determine if sTILs are a prognostic factor in other cancer types and with other checkpoint inhibitors.

The drawback to both of these methods would be the necessity of the tissue. These approaches would be limited to solid tumors with easily assessable surgical or biopsy sites, to obtain adequate

tissue specimens in addition to those used for standard of care practices. In the case of vaccination, autologous vaccines require sufficient tissue to construct the vaccine, which then must be manufactured.

4. Conclusions

While much has been uncovered regarding the mechanism of T_{RM} cell transformation and maintenance, there are still missing pieces to the puzzle. Further evaluation of the molecular signal patterns may provide direction to additional therapies. In addition to developing supportive regimens, the further characterization of T_{RM} cell status in clinically derived patient samples may also be used as a biomarker to predict response to therapy and prognosis, particularly immunotherapies involving checkpoint inhibitors, CAR-T approaches and/or vaccines, such as those with multiple immune stimulatory functions (i.e., Vigil).

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