



# Decoding the Interplay: Exploring Immunotherapy Resistance in the Tumor Microenvironment

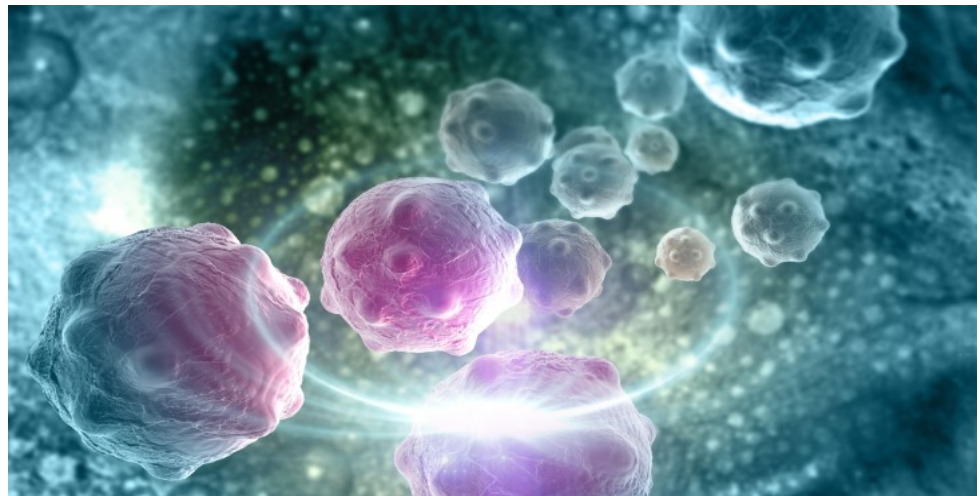
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## Abstract

*The tumor microenvironment (TME) surrounds the tumor and includes blood vessels, immune cells, fibroblasts, signaling molecules, and the extracellular matrix. This review examines the cellular components and pathways within the TME, highlighting their potential as targets for immunotherapy. It also covers recent advances from the past several years in TME, immunotherapy, and combination therapy. The review emphasizes the role of CD8+ T cells in the TME and their relevance to immunotherapy. It discusses various T cell-targeted treatments, including PD-1/PD-L1, CTLA-4, VEGF, Interferon- $\gamma$ , LAG-3, and ER stress-XBP1. Recognizing the complexity and uniqueness of each tumor's immunotherapy network, the review aims to understand and compare the TMEs of different cancers and the respective immunotherapies. Cancers covered include non-small cell lung Cancer (NSCLC), pancreatic ductal adenocarcinoma (PDAC), cervical cancer, chronic lymphocytic leukemia, and gastric cancers. The review also addresses future research directions and applications of immunotherapies, aspiring to advance TME understanding and research.*

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## 1. Introduction

The tumor microenvironment (TME) constitutes the immediate surroundings of a tumor, forming a complex ecosystem inclusive of nearby blood vessels, immune cells, fibroblasts, signaling molecules, and the extracellular matrix (ECM).<sup>1</sup> Within the TME, there exist hypoxic and acidic conditions, alongside altered expression patterns of ECM proteins. These conditions foster the presence of resident and infiltrating immunosuppressive cells, trigger the expression of immune checkpoint proteins, and promote the exclusion and exhaustion of cytotoxic T lymphocytes (CTLs).<sup>2</sup> Such circumstances serve as the foundation for immunotherapy targeting the TME. While contemporary interest in TME immunotherapy appears recent, investigations into utilizing immune cells for combating tumors date back to the 1900s.<sup>3</sup>

New studies are directed towards pinpointing cellular pathways within the TME with the use of immunotherapy components to hinder growth. Among the primary pathways are lactic acid accumulation, metabolic processes, and diverse signaling routes within the TME.<sup>5,6,7</sup> All these pathways represent potential targets for immunotherapy interventions. Moreover, treatments are geared towards enhancing the immune system's ability to eradicate cancer cells and impede their proliferation. To achieve this goal, two primary strategies are employed: immune checkpoint inhibition and adoptive cellular therapy (ACT).<sup>4</sup> Immune checkpoint inhibition involves obstructing certain proteins, known as checkpoints, to enhance the responsiveness of immune cells.<sup>4</sup> Adoptive cell therapy (ACT) entails administering T cells to patients to bolster their ability to combat tumor cells.<sup>4</sup>

In this discussion, our focus lies on T cell-based immunotherapies, including anti-PD-1/PD-L1, anti-CTLA-4, VEGF, Interferon- $\gamma$  (IFN- $\gamma$ ), LAG-3, and ER stress-XBP1. However, the complexity of immunotherapy arises from the variable responses of individual cancers to different immunotherapeutic approaches. This review delves into the distinctive attributes of cancers such as non-small cell lung cancer (NSCLC),

melanoma, pancreatic cancer, cervical cancer, and chronic lymphocytic leukemia.

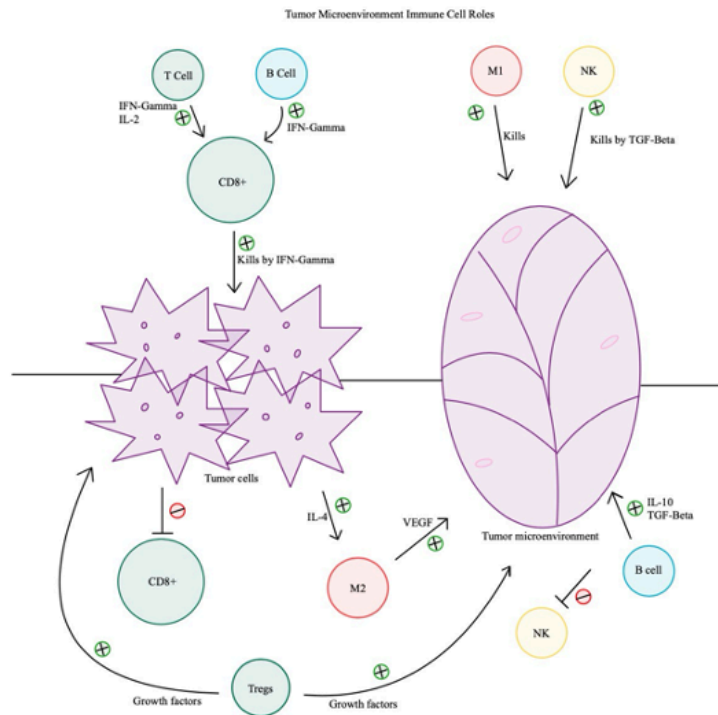
## 2. Immune Cell Overview

The extracellular matrix (ECM) is comprised of collagen, fibronectin, elastin, and laminin, serving as the structural framework within the tumor microenvironment (TME). Within this environment, various immune cells including T cells, B cells, Natural Killer cells (NK), macrophages (M1 and M2), stromal cells, endothelial cells, and cancer-associated fibroblasts (CAFs) play pivotal roles in either promoting or suppressing tumor growth.<sup>8</sup>

T cells, equipped with T-cell receptors, target specific antigens on tumor cells, halting angiogenesis—the formation of new blood vessels—and leading to tumor cell destruction. However, T cells can become infiltrated and dysfunctional within the immune system.<sup>8,9</sup> B cells contribute to antibody production, antigen presentation, and cytokine secretion.<sup>8,9</sup> In the context of lymphocytes, both T and B cells, particularly tumor-infiltrating lymphocytes (TILs), are crucial in research. While TILs effectively eliminate tumor cells, they can be recruited by the TME, compromising immune response efficacy.<sup>4</sup>

Regulatory T cells (Tregs) maintain immune response balance, but when recruited to the TME, they hinder T cell formation, posing a danger to immune function. Consequently, immune mechanisms originally aimed at protecting the body now facilitate TME growth.<sup>10</sup>

NK cells surveil the bloodstream for tumor cells, inhibiting metastasis.<sup>8,9</sup> Macrophages regulate immune responses by phagocytosing pathogens and presenting antigens. Notably, increased macrophage infiltration in tumors, particularly the M2 phenotype, and cytokine secretion, support tumor growth.<sup>8,9</sup>



**Figure 1:** This figure illustrates the distinct contributions of various immune cells to the Tumor Microenvironment (TME). It delineates the overall immune response attributed to each mentioned immune cell type: T cells, B cells, Macrophages (M1 and M2), Natural Killer cells (NK), Tregs, and CD8+ cells. The upper side represents the positive immune response, while the lower side depicts the autoimmune suppressive response.

Stromal cells, including endothelial cells, fibroblasts, adipocytes, and stellate cells, play crucial roles in tumor development. Tumor cells recruit these supporting cells from nearby tissue to aid in tumor formation. Stromal cells secrete various factors that influence processes like angiogenesis, proliferation, invasion, and metastasis.<sup>8,9</sup>

Endothelial cells, for instance, are pivotal in orchestrating blood vessel formation and significantly contribute to cancer progression by promoting cancer cell migration, invasion, angiogenesis, and metastasis. They transition into cancer-associated fibroblasts (CAFs), facilitating communication between tumor cells and the tumor microenvironment (TME).<sup>1</sup> This interaction often leads to the disruption of cell connections and detachment. CAFs can derive from various immune cells, but within the TME, they become

significant producers of extracellular matrix (ECM), tumor growth factors, cytokines, and other essential components. Each stromal cell type contributes uniquely to the TME, and targeting their specific functions presents an opportunity for novel immunotherapeutic approaches.<sup>1</sup>

The immune response involves numerous intricate processes, among which angiogenesis stands out—a phenomenon where endothelial cells proliferate and migrate, forming new blood vessels.<sup>11</sup> This process is pivotal as it grants tumors the capability to metastasize, spreading to other locations within the body. Conversely, inhibiting angiogenesis can effectively curtail tumor growth and metastasis.<sup>11</sup> For instance, research conducted by Chen et al. demonstrates that the administration of anti-malarial drugs like dihydroartemisinin and artesunate resulted in a reduction in the size of cell lines. These drugs were found to hinder the growth factors crucial for cancer cell proliferation, thereby impeding angiogenesis.<sup>11</sup>

Anti-angiogenic (AA) therapy plays a significant role in remodeling the extracellular matrix (ECM), altering the distribution of cell types and populations.<sup>12</sup> This therapy redistributes pericyte proteins along blood vessels, increasing coverage and eliciting a pro-aggressive tumor response. Notably, glycosylation of these proteins is speculated to contribute to malignant resistance to AA therapy.<sup>12</sup> However, a complication arises as resistance can develop post-AA treatment, fostering tumor cell migration and invasion. Tumor cell receptors possess mechanisms to detect AA-induced alterations in their environment, prompting a remodeling of the entire tumor microenvironment (TME) to facilitate tumor growth once more.<sup>12</sup>

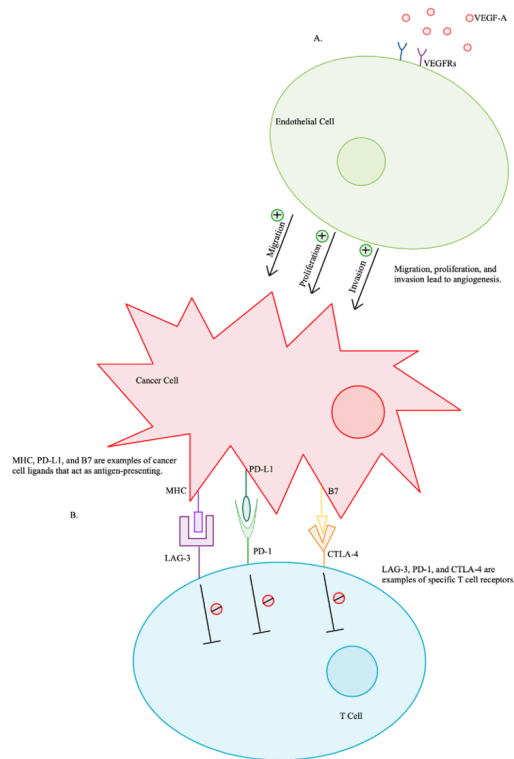
### **3. T Cell Based Immunotherapy**

This section of the review will delve into several key surface cell receptors found on endothelial cells, tumor cells, and T-cells. It will explore their roles in promoting tumor growth, strategies to overcome resistance, and the

underlying mechanisms of resistance. Emphasis will also be placed on the critical functions of infiltrating tumor lymphocytes and cytotoxic T cells.

Within the tumor microenvironment (TME), various surface cell receptors recognize specific antigens, initiating signaling cascades that ultimately impact downstream events. These cascades often lead to the inactivation or impairment of effective T-cells, which are typically responsible for targeting and eliminating tumor cells. The TME, being highly adaptable, fosters conditions conducive to tumor cell growth, proliferation, and survival.<sup>13</sup>

Several immune response cells contribute to immune resistance, including myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and endothelial cells. The secretion of negative regulatory factors by these cells can induce T-cell exhaustion, dysfunction, and apoptosis.<sup>13</sup>



**Figure 2:** A. Vascular Endothelial Growth Factor-A (VEGF-A) binds to Vascular Endothelial Growth Factor Receptors (VEGFRs) situated on endothelial cell surfaces, initiating a series of reactions that stimulate angiogenesis. B. The interplay between proteins located on T cell surfaces and their corresponding ligand surface cell receptors on tumor cells contributes to T cell exhaustion.

One effective therapeutic approach, immune checkpoint inhibition, has demonstrated success in rejuvenating the cytotoxic function of CTLs and T-cells. This therapy works by alleviating the suppression of antitumor immunity, freeing CTLs from exhaustion.<sup>13</sup> The FDA has approved antibodies targeting Programmed Cell Death 1 (PD-1/PD-L1) and Cytotoxic T Lymphocyte-Associated Protein 4 (CTLA-4) for treating various cancers. Additionally, ongoing clinical research is targeting other immune checkpoints like LAG3, CD39, CD73, and CD47, all showing promising potential in advancing cancer immunotherapy.<sup>14</sup>

### **3.1 PD-1/PD-L1**

Immune checkpoint inhibition stands out as an effective therapeutic approach in reviving the cytotoxic capabilities of CTLs and T-cells. By alleviating the exhaustion-induced suppression of antitumor immunity, this therapy unleashes CTLs to combat tumors.<sup>13</sup> The FDA has approved the use of antibodies targeting Programmed cell death 1 (PD-1/PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) as treatments across various cancer types.<sup>13</sup>

Numerous other immune checkpoints are currently under investigation in clinical research, including LAG3, CD39, CD73, and CD47, all of which show promising potential in advancing the landscape of cancer immunotherapy.<sup>14</sup> Among these, PD-1 is a pivotal protein implicated in T cell apoptosis, or programmed cell death. It is predominantly expressed on the surface of activated immune cells such as macrophages, dendritic cells, B cells, and T cells.<sup>14</sup> Notably, PD-1 expression is particularly elevated on exhausted T cells, where it plays a role in inhibiting their normal immune function. When PD-1 interacts with its ligand receptor, PD-L1, on tumor cells, it initiates a series of signaling events that directly impede the response of activated cells by enhancing local evasion mechanisms.<sup>14,15</sup> These immune checkpoint inhibitors (ICIs) interfere with T cell functionality, leading to

an increase in exhausted T cell populations, both of which contribute to adaptive resistance against immune checkpoint blockade therapy.<sup>16</sup>

Researchers conducted clinical trials investigating the interaction between PD-1 and PD-L1 on cytotoxic T cells in mice. They observed accelerated tumor growth, which was mitigated when treated with an anti-PD-L1 antibody or through PD-1 knockout, resulting in reduced tumor growth. Similar findings were noted in human trials.<sup>17</sup> For instance, Qian et al. demonstrated in a study focusing on glioma cancer that tumor-infiltrating T cells become activated and can increase PD-1 expression, leading to T cell dysfunction mediated by Immune Checkpoint Inhibitors (ICIs). Consequently, the researchers suggested that anti-PD therapy could impede glioma cancer progression.<sup>17</sup>

Another instance illustrating the effectiveness of immune checkpoint inhibition therapy in microsatellite instability/deficient mismatch repair (MSI/dMMR) tumors comes from the ongoing trial conducted by IMHOTEP, which showcases a group of 120 patients benefiting from anti-PD therapy.<sup>18</sup> While anti-PD therapy has shown success in certain patients, others have exhibited poor responses due to both primary and acquired resistance mechanisms working against the treatment. Effective anti-PD therapy hinges on the precise blockade of the PD-1 and PD-L1 pathways. However, if either protein lacks expression, the treatment proves ineffective against the tumor cells—a direct primary resistance mechanism observed in some cases.<sup>15</sup> Furthermore, certain cancers lack tumor-infiltrating T cells or PD-L1, rendering them unresponsive to anti-PD therapy.<sup>15</sup> To combat this adaptive resistance, combination therapy involving both immuno- and chemo-therapies has been proposed as a more effective approach to treating cancer compared to immunotherapy alone.

### **3.2 CTLA-4**

CTLA-4, a protein associated with cytotoxic T lymphocytes, plays a role in dampening the anti-tumor immune response by regulating the activation of CTLs, thus hindering an efficient immune reaction.<sup>14</sup> Unlike PD-1, which manages programmed cell death, CTLA-4 dictates whether a T cell



undergoes activation or remains in a state of rest termed exhaustion. It is present on the surface of stimulated functional T cells, and activation occurs upon stimulation from a T cell receptor.<sup>14</sup>

CTLA-4 competes with CD28 for binding to the T cell ligand, blocking the crucial costimulation signals required for activation.<sup>14</sup> Due to its higher affinity for the ligand, CTLA-4 binds more effectively, leading to T cell exhaustion and inhibiting activation. Using an antibody that selectively targets and binds to CTLA-4 can restore T cell activation by eliminating the competition for T cell ligand binding with costimulation signals.<sup>14</sup> Additionally, CTLA-4 is prominently expressed on the surface of Tregs, which play a role in immune response suppression.<sup>14</sup>

Research indicates that CTLA-4 plays a pivotal role in regulating the function and production of Tregs,<sup>14</sup> which in turn directly suppress the activation of target immune cells by increasing CTLA-4 expression.<sup>19</sup> Utilizing CTLA-4 blockade therapy has emerged as an effective strategy for enhancing anti-tumor immune responses.<sup>14</sup> The FDA-approved monoclonal anti-CTLA-4 antibody, ipilimumab, has significantly enhanced overall survival rates among patients with malignant melanoma.<sup>4</sup> It's worth noting that while anti-CTLA-4 antibodies show efficacy in late-stage melanoma, their effectiveness in other tumor types is limited. Consequently, clinical trials are exploring combination therapies to address this limitation.<sup>4</sup>

### **3.3 LAG-3**

Lymphocyte Activation Gene 3 (LAG-3) serves as an immune checkpoint receptor, capable of dampening responses orchestrated by T and NK cells, thereby fostering a hyporesponsive condition, which aids tumors in escaping immune surveillance. Typically found on activated and exhausted T and NK cells, B cells, dendritic cells, and Tregs, LAG-3 signaling inhibits T cell proliferation, cytokine generation, and cytolytic activity.<sup>20</sup> Additionally, its presence on Tregs contributes to immunosuppression.<sup>20</sup>

An illustrative instance of this phenomenon is evident in individuals afflicted with chronic lymphocytic leukemia (CLL), where there is significant dysregulation of LAG-3. Both NK cells and T cells exhibit

heightened LAG-3 expression, and elevated levels of LAG-3 or soluble LAG-3 (sLAG-3) are associated with adverse cytogenetics and unfavorable outcomes among CLL patients. Consequently, treatment involving the application of an anti-LAG-3 blocking antibody called relatlimab to peripheral blood mononuclear cells (PBMCs) has demonstrated efficacy in reducing leukemic cell counts and restoring NK cell and T cell mediated responses.<sup>20</sup> As a result, interventions aimed at reinstating T and NK cell mediated responses through LAG-3 blockade have emerged as promising therapies for hematological malignancies such as CLL, follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), and acute myeloid leukemia (AML).<sup>20</sup>

### **3.4 ER Stress/IRE1 $\alpha$ -XBP1**

A fresh angle in cancer therapy involves focusing on the ER stress pathway, with a particular emphasis on the XBP1 gene.<sup>21</sup> In a 2022 study conducted by Zundell et al., investigating ovarian clear cell carcinomas (OCCC), it was discovered that the ARID1A gene, responsible for epigenetically regulating gene expression via the SWI/SNF chromatin remodeling complex, is mutated in over 50% of OCCCs. This mutation results in inadequate expression of ARID1A protein, leading to advanced-stage disease and early recurrence.<sup>21</sup>

This element plays a vital role by transcriptionally inhibiting the IRE1 $\alpha$ -XBP1 pathway of the ER stress response. Their research using mouse models indicates that disabling XBP1 and inhibiting the IRE1 $\alpha$ /XBP1 pathways significantly enhances the survival rate of mice with OCCC, as it suppresses the growth of ARID1A mutant OCCC cells.<sup>21</sup> In the face of ER stress, the IRE1- $\alpha$  component, a type of unfolded protein response (UPR), undergoes a structural change, splicing the mRNA encoding the XBP1 transcription factor. This splicing facilitates the translation of XBP1, which in turn aids cancer cell survival by resolving ER stress.<sup>21</sup>

ER stress itself fosters the survival of cancer cells by activating adaptive programs through the unfolded protein response (UPR) once detected.

Consequently, inhibiting the UPR is emerging as a therapeutic strategy for cancers characterized by heightened ER stress response.<sup>21</sup> In this study, the efficacy of a therapeutic intervention targeting the IRE1 $\alpha$  RNase, B-I09, was examined. By inhibiting IRE1 $\alpha$  RNase activity, B-I09 effectively suppressed the growth of ARID1A-activated cells in ovarian clear cell carcinomas (OCCCs), indicating the potential of targeting the IRE1 $\alpha$ -XBP1 axis of the ER stress response as a promising approach for treating ARID1A mutant OCCCs.<sup>21</sup>

A study by Ma et al. illustrated the role of cholesterol, the primary sterol distributed throughout the human body, in the ER stress-XBP1 pathway.<sup>22</sup> Through staining techniques to observe cholesterol levels in tumor-infiltrating T cells in mice, researchers found that elevated cholesterol content triggers CD8+ T cell exhaustion via the ER stress-XBP1 pathway.<sup>22</sup> Inhibiting the XBP1 pathway or reducing cholesterol levels in CD8+ T cells restored normal antitumor function, while increased cholesterol was consistently linked to elevated PD-1 expression on tumor-infiltrating T cells.<sup>22</sup> Similar patterns were observed in human colon cancer and myeloma samples, underscoring the significance of cholesterol content in T cell exhaustion.<sup>22</sup>

### **3.5 VEGF/VEGF-A:**

Vascular endothelial growth factors (VEGFs) are signaling proteins crucially involved in the microvasculature of tumors, the suppression of immune cells, and the promotion of immunosuppression, both locally and systemically, in cancer.<sup>19</sup> These proteins are predominantly secreted by the endothelial cells lining blood vessels, with additional production occurring in immune cells within the tumor microenvironment (TME).<sup>19</sup> VEGFs play a pivotal role in fostering tumor development and progression by interacting with receptors on tumor cells through autocrine and paracrine signaling pathways. Among them, VEGF-A primarily binds to VEGFR-2, triggering the proliferation and migration of endothelial cells, thereby facilitating angiogenesis.<sup>19</sup>

VEGF-A boosts the expression of both PD-1 and CTLA-4, dampening the body's ability to fight tumors and reducing the activity of CD8+ T cells by transitioning them from an active state to an exhausted one, impairing their normal function. Blocking VEGF with anti-angiogenic agents can reverse the immune checkpoints' inhibitory effects.<sup>19</sup> In a phase I trial led by Daniel Chiu et al., a combination of anti-PD-1 therapy and antiangiogenic agents was used to treat glioblastoma, a challenging form of brain cancer known for its resistance to ICI therapy. The findings revealed that this combination treatment was well tolerated, safe, and did not lead to any previously unreported adverse events. Furthermore, the results showed decreased levels of VEGF-A and other angiogenic factors in the tumor microenvironment, underscoring the relationship between VEGF and PD-1 expression.<sup>16</sup>

#### **4. Overcoming Resistance to Immune Checkpoint Blockade**

Although the immune checkpoint blockade strategy has shown efficacy in reviving the antitumor response within tumor-infiltrating lymphocytes (TILs) and cytotoxic T cells, the tumor microenvironment (TME) employs adaptive resistance mechanisms against this therapy. Specifically, the upregulation of immune checkpoints such as PD-1 and CTLA-4 in response to targeted antibodies can foster this adaptive resistance.<sup>14</sup> Moreover, the TME utilizes evasion tactics, such as enhancing PD-1 expression in response to T cell attacks, leading to the functional impairment of TILs.<sup>13,17</sup> Additionally, the differentiation of CD8+ T cells poses a significant challenge, as TILs develop resistance to antitumor treatments, resulting in a diverse CD8+ T cell subset.<sup>17</sup> Researchers are exploring combination therapies, pairing immunotherapy with chemotherapy or radiation therapy, to address malignant tumors. In a study by Feng Y. et al., a dual gene therapy approach was investigated to counter adaptive resistance to immune checkpoint blockade therapy in both CD4+ and CD8+ T cells. The scientists developed a dual gene delivery system aimed at eliminating tumor adaptive resistance and restoring T cell function by modulating the expression of VEGF-A and PD-L1 proteins.<sup>14</sup> Gene-silencing techniques were employed to decrease VEGF-A expression in the TME, reducing angiogenic activity and blocking PD-L1 immune

checkpoint. The findings demonstrated an increase in the number of CD8+ T cells expressing granzymes B, leading to inhibited tumor growth.<sup>14</sup>

Furthermore, increasing VEGF-A gene silencing effectively alleviated tumor hypoxia. However, it's crucial to regulate the dosage of each treatment, as excessive pShVEGF-A antibody treatment can exacerbate tumor hypoxia by overly depleting tumor vessels. Upon treating tumor-infected mice with the dual gene therapy, scientists observed substantial inhibition of tumor growth and prolonged overall survival rates compared to mice treated with monotherapy.<sup>14</sup> This dual gene therapy not only countered immune checkpoint blockade-induced adaptive resistance but also reversed the immunosuppressive tumor microenvironment simultaneously. This dual gene delivery system represents a promising avenue for immunotherapy across various tumor types, though further research is warranted

#### **4.1 Lactic Acid in the Tumor Microenvironment**

One of the key areas of exploration for scientists has been the manipulation of lactic acid within the tumor microenvironment (TME). Lactic acid, a byproduct of altered metabolism, plays a significant role in shaping the TME. Its acidic nature fosters various processes including angiogenesis, metastasis, and drug resistance. A 2020 study highlighted that heightened lactic acid production can detrimentally affect anticancer immunity by suppressing immune responses due to the low pH environment it creates.<sup>7</sup> Lactic acid impedes the differentiation of monocytes into dendritic cells, leading to a reduction in antigen-presenting functions. Additionally, it hampers the anti-tumoral activities of immune cells like natural killer cells and cytotoxic T cells.<sup>7</sup> Consequently, the targeting of lactate and lactic acid has emerged as a compelling area of focus within contemporary immunotherapy research.

Scientists have been investigating the neutralization of lactic acid as a potential method. By buffering the tumor microenvironment using oral bicarbonate and coupling it with anti-PD-1 immunotherapy and adoptive T-cell transfer, there's potential for enhancing survival rates.<sup>5</sup> Furthermore, research has shown that V-domain Ig suppressor of T cell activation (VISTA) can inhibit T cells in an acidic pH environment. Consequently,

blocking VISTA in conjunction with PD-1 blockade, and the development of pH-specific antibodies against VISTA, have demonstrated efficacy in tumor rejection.<sup>5</sup>

Another significant focus lies in targeting the lactate-producing enzyme LDH, which shows promising anti-cancer effects. However, its impact on immune cells remains largely unexplored, as LDH inhibition can decrease T cell levels<sup>5</sup>. An alternative approach involves inhibiting lactate transporters. Notably, researchers identified SLC4A4 as the predominant bicarbonate transporter in pancreatic ductal adenocarcinoma (PDAC). Inhibiting SLC4A4 in PDAC cells reduces TME acidification by accumulating bicarbonate in the extracellular space and lowering lactate production. Combining SLC4A4 targeting with immune checkpoint blockades has been found to overcome immunotherapy resistance.<sup>6</sup> Targeting lactic acid holds significant promise for advancing immunotherapy in the tumor microenvironment, as it plays a pivotal role in TME acidification and subsequent immune response suppression.

#### **4.2 Targeting Metabolism in the Tumor Microenvironment**

Focusing on metabolism to enhance the tumor microenvironment for cancer immunotherapy holds significant promise. Cancer cells exhibit heightened metabolic activity, fueling their rapid proliferation. By zeroing in on metabolism, scientists can slow down cancer cell spread and impede their function. The realm of immune metabolism offers potential metabolic targets to bolster anti-cancer immunity. The dynamic metabolism of immune cells significantly influences their functions as well.<sup>25</sup>

A primary approach involves targeting amino acid metabolism, where specific amino acids such as glutamine, tryptophan, and arginine have emerged as key targets for inhibiting tumor progression and enhancing immunity. Glutamine, in particular, plays a vital role as a nutrient for cancer cells. Enzymes like glutaminase (GLS) facilitate the conversion of glutamine to glutamate, making GLS a prime target to curb cancer cell metabolism and glucose utilization. However, targeting GLS poses challenges as cancer cells employ alternative methods to boost glucose metabolism. Additionally, directing GLS inhibition towards immune cells, particularly T cells, affects

their differentiation, survival, proliferation, and effector functions.<sup>5</sup> This presents a significant hurdle, as it necessitates reducing glutamate levels in cancer cells while ensuring adequate glutamine for proper T cell function. Consequently, a strategy has been devised to broadly block glutamine metabolism alongside anti-PD-1 immunotherapy. This combined approach enhances anti-tumor effects by dampening tumor metabolism while maintaining robust glucose metabolism in T cells.<sup>5</sup>

Furthermore, researchers have shown keen interest in targeting arginine and nitric oxide, delving into amino acid metabolism. Arginine plays a pivotal role in cancer and immune cell functions, particularly in proliferation, survival, and protein synthesis. The metabolic pathways of arginine heavily rely on enzymes like arginase (ARG) and nitric oxide synthase (NOS). However, given arginine's significance to both cancer and immune cells, its potential as a therapeutic target presents complexities. While arginine depletion proves effective in certain scenarios, it can hinder anti-tumor T cell responses and bolster the population of myeloid-derived suppressor cells (MDSCs), thus exacerbating arginine depletion.<sup>5</sup> Yet, inhibiting ARG offers promise by restoring arginine levels, leading to tumor regression and enhancing T cell functionalities.<sup>5</sup>

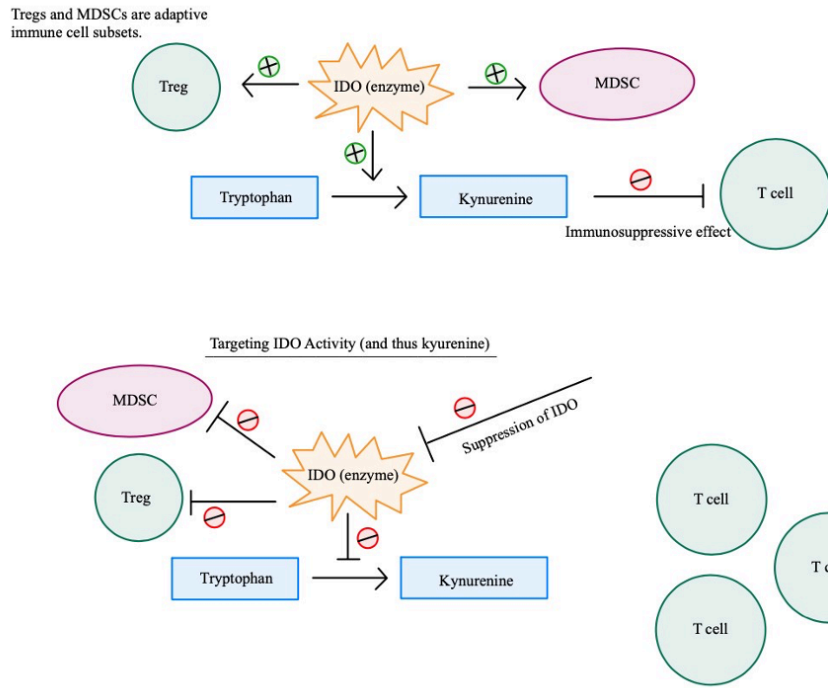
Targeting arginine and nitric oxide in myeloid cells could potentially enhance immunotherapy, but further research is needed to understand its impact on other immune cells. Tryptophan is another significant amino acid being investigated for immunotherapy due to its role in cell growth and maintenance. Adaptive immune cell subsets such as Tregs, tolerogenic dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs) exhibit high levels of indoleamine 2,3-dioxygenase (IDO), the enzyme that converts tryptophan to kynurenine.<sup>5</sup> Kynurenine has immunosuppressive effects on T cells, so targeting IDO activity could not only mitigate these immunosuppressive effects on T cells but also inhibit these adaptive immune cell subsets.<sup>5</sup>

Lipid metabolism has emerged as a recent target in immunotherapy. Cancer cells exploit lipid metabolism to support their rapid proliferation, survival, migration, invasion, and metastasis.<sup>26</sup> Reprogramming lipid metabolism can

prevent effector T cell senescence, suggesting that lipid metabolism plays a role in regulating senescence development in T cells.<sup>27</sup> Fatty acid metabolism is crucial for the differentiation of immune cells, and the upregulation of fatty acid oxidation (FAO) can enhance the functions of CD8+ T cells. Reprogramming fatty acid metabolism in immune cells could promote an inhibitory immune microenvironment.<sup>28</sup> It is often used in combination therapies with other immunotherapeutic treatments, such as immune checkpoint blockades (ICBs).<sup>28</sup> Recent studies have shown that targeting fatty acid metabolism in T cells can improve the efficacy of ICBs. For instance, a study found that combining bezafibrate with PD-L1 antibody treatment yielded better results in LLC xenograft mouse models. Bezafibrate treatments increased the expression of FAO-related genes in cytotoxic T lymphocytes (CTLs), such as PGC-1 $\alpha$ , CPT1a, and LCAD, and maintained the survival and function of CTLs.<sup>29</sup>

Scientists have explored manipulating iron metabolism as a strategy for treating tumors, given iron's crucial role in tumor proliferation. Iron metabolic dysfunction directly impacts cancer pathophysiology, with the availability of iron regulating the aggressive phenotypes of tumors.<sup>30</sup> Many tumor microenvironment (TME) cells rely on iron to thrive and function properly. For instance, activated M2 tumor-associated macrophages (TAMs) can disrupt iron homeostasis within tumor cells by exporting high amounts of iron and increasing the production of other iron-related proteins.<sup>30</sup> It has been observed that iron chelation, the bonding of ionic molecules to metal ions, can reverse the iron-exporting phenotype of M2 TAMs.<sup>30</sup> Blocking transferrin receptor 1 (TFR1), a receptor protein that recognizes transferrin-bound iron, has proven effective in suppressing tumor growth, as TFR1 is often upregulated in cancer cells to enhance their iron supply.<sup>30</sup> Thus, cutting off a tumor's iron supply appears to be an effective method to reduce tumor growth.





*Figure 3: The interactions between tryptophan and kynurenine. The enzyme IDO converts tryptophan into kynurenine, which exerts an immunosuppressive effect on T cells. Blocking IDO would not only eliminate the immunosuppressive effect on T cells but also suppress adaptive immune cell subsets, including MDSCs, Tregs, and DCs.*

### 4.3 Targeting Signaling Pathways

Different signaling pathways, such as oxygen and nutrient sensing pathways, have significant potential for reducing the glucose metabolism of cancer cells. In low-glucose environments, AMP-activated protein kinase (AMPK) becomes activated, and in high-glucose environments, AMPK is promoted, which can lead to the persistence of immunosuppressive cells.<sup>5</sup> High levels of AMPK can inhibit T cells, but disabling AMPK may result in increased glycolysis in cancer cells. Metformin is one drug that targets AMPK by activating its signaling. Studies have shown that metformin reduces cancer risk in both mice models and humans. AMPK activation is believed to reduce tumor burden by slowing tumor growth, supporting the growth of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment (TME), and blocking certain glycolytic enzymes necessary for cancer cell

proliferation. However, AMPK activation can also increase the number of immunosuppressive cells while promoting tumor regression.<sup>5</sup> It is crucial to examine the effects on both cancer cells and the phenotypic ratios of immune cells.

Another crucial nutrient pathway is the mTOR pathway, which is activated by high levels of glucose and amino acids. In T cells, mTOR supports glycolysis, differentiation, and effector functions. However, because mTOR enhances memory formation in CD8+ T cells while low mTOR levels boost Treg activity, it is vital to balance mTOR levels to enhance memory formation without promoting Treg activity.<sup>5</sup> Like AMPK, the mTOR signaling pathway has a complex nature, necessitating further research to develop nuanced methods that induce beneficial immune responses without creating a tumor-protecting immune environment.<sup>5</sup>

Two critical targets of immunotherapy are the immune checkpoint pathways PD-1/PD-L1 and CTLA-4. Anti-CTLA-4 antibodies can deplete Treg cells from the tumor microenvironment (TME), leading to successful tumor rejection in animal studies.<sup>31</sup> PD-1/PD-L1 are involved in signaling mediated by antigen recognition through T cell receptors.<sup>31</sup> Antibodies that block the PD-1/PD-L1 checkpoint have shown significant therapeutic efficiency.<sup>31</sup> These immune checkpoints mainly function by suppressing the metabolic reprogramming of immune cells, inhibiting glycolysis, and increasing lipolysis. Blocking these pathways promotes anabolic metabolic pathways and glycolysis, thereby restoring the effector function of tumor-infiltrating lymphocytes (TILs). Antibodies against CTLA-4, PD-1, and PD-L1 reverse the glycolysis restrictions on T cells, essentially restoring T-cell glycolysis and interferon production.<sup>6</sup>

Another signaling pathway that has been extensively studied is the CSF-1/R pathway, which is expressed in myeloid cells. This pathway regulates the infiltration, phenotypic and functional differentiation, and survival of myeloid cells, including tumor-associated macrophages (TAMs). Blocking the CSF-1/R pathway promotes the TME by facilitating the infiltration and reactivation of cytotoxic T lymphocytes (CTLs).<sup>32</sup> Consequently, studying

various signaling pathways can pave the way for developing nuanced cancer immunotherapy treatments.

As discussed, the TME consists of various components, and understanding these differences in TME is crucial for developing effective treatments and immunotherapy approaches for different cancers. Identifying and targeting the most relevant aspects of the TME for each specific disease is key to creating successful therapies.

## 5. Non-small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death and has become a primary focus for the development of TME-based immunotherapy. Similar to other cancers, NSCLC features an immunosuppressive tumor microenvironment (TME) and CTL exhaustion. However, it also has distinct characteristics such as variable blood flow, areas of acidosis and hypoxia, and increased expression of hypoxia markers. These features can lead to metastasis and elevated resistance to treatment.<sup>32,33</sup> Consequently, past therapies targeting the TME have focused significantly on addressing hypoxia and angiogenesis.<sup>33</sup>

A recent study conducted by Zhao et al. adopts a similar strategy focusing on the Tumor Microenvironment (TME). The study delves into a Phase III trial combining bevacizumab, atezolizumab, and chemotherapy for metastatic non-squamous Non-Small Cell Lung Cancer (NSCLC). Bevacizumab, an IgG antibody that obstructs the Vascular Endothelial Growth Factor (VEGF) pathway by binding to VEGF-A, has demonstrated efficacy in treating NSCLC. The VEGF pathway is a prime target due to its overexpression in NSCLC, correlating with tumor recurrence, low survival rates, metastasis, and mortality.

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Bevacizumab, an IgG antibody that obstructs the Vascular Endothelial Growth Factor (VEGF) pathway by binding to VEGF-A, has demonstrated efficacy in treating NSCLC.<sup>19</sup> The VEGF pathway is a prime target due to its overexpression in NSCLC, correlating with tumor recurrence, low survival rates, metastasis, and mortality.<sup>19</sup>

Apart from bevacizumab, other antibodies like ramucirumab and nintedanib have demonstrated enhancements in overall survival (OS), along with tyrosine kinase inhibitors (TKIs) capable of inhibiting the Vascular Endothelial Growth Factor (VEGF) pathway.<sup>19</sup> However, the VEGF pathway primarily addresses the angiogenic aspect of tumors, prompting its utilization in combination therapies with chemotherapy and immunotherapy, which have proven effective. In a similar vein, this Phase III trial combines bevacizumab with atezolizumab, an immune checkpoint inhibitor targeting the PD-L1 ligand (anti-PD-1), alongside chemotherapy. This multifaceted therapeutic approach to Non-Small Cell Lung Cancer (NSCLC) aims to enhance progression-free survival and OS.<sup>32</sup>

### **5.1 NSCLC Combination Therapy**

The primary treatment modality for Non-Small Cell Lung Cancer (NSCLC) typically involves Immune Checkpoint Inhibitors (ICIs) like pembrolizumab, an anti-PD-1 antibody. Pembrolizumab has emerged as the first-line treatment for metastatic NSCLC due to its notable response rates and sustained tumor regression.<sup>14</sup> However, despite its efficacy, many patients encounter resistance to these treatments or suffer from immune-related adverse events.<sup>14</sup> Consequently, combination therapy has emerged as a pivotal focus for advancing cancer treatments. A recent study conducted in 2022 by Reckamp et al. explored combination therapy involving ICIs and Vascular Endothelial Growth Factor (VEGF) receptor inhibition in NSCLC patients. Patients were administered a combination of either ramucirumab (VEGF inhibitor) and pembrolizumab (ICI), or the investigator's standard of care, such as docetaxel, gemcitabine, or pemetrexed.<sup>34</sup> Among the 136 eligible patients, those who received ramucirumab and pembrolizumab exhibited significantly higher overall

survival (OS), suggesting another promising combination therapy avenue for treating NSCLC.<sup>34</sup>

In September 2022, Casarrubios et al. conducted a study focusing on a specific form of combination therapy called neoadjuvant chemoimmunotherapy. This approach utilizes both chemotherapy and immunotherapy to reduce tumor size before more targeted treatment aimed at tumor removal. Through bulk RNA sequencing, the researchers analyzed samples from 41 patients with stage III Non-Small Cell Lung Cancer (NSCLC) who underwent three cycles of nivolumab (an anti-PD-1 drug) and chemotherapy. Their findings revealed downregulation of tumor and proliferation markers, along with genes associated with IFN $\gamma$  signaling.<sup>35</sup> Additionally, they observed the regulation of numerous genes such as IFNG and NKG7, distinguishing between complete pathological response (CPR) and non-CPR tumors before and after treatment. This study lays the groundwork for future personalized immunotherapy approaches based on predictive biomarkers of CPR, in conjunction with neoadjuvant immunotherapy.<sup>35</sup>

## 5.2 Melanoma

Anti-PD-1 therapy represents a standard immunotherapy regimen for melanoma, a type of skin cancer. However, this treatment modality is only effective in providing long-term clinical benefits to approximately 40% of patients with advanced melanoma.<sup>36</sup> To address this challenge, Davar et al. conducted a study involving the administration of pembrolizumab, an anti-PD-1 drug, alongside a fecal microbiota transplant (FMT) in 16 patients with advanced melanoma who did not respond to anti-PD-1 therapy alone.<sup>36</sup> This combined approach of FMT and PD-1 blockade resulted in the reprogramming of the Tumor Microenvironment (TME), overcoming resistance to anti-PD-1 therapy. Notably, the treatment led to the upregulation of CD8+ T cells, a decrease in the percentage of naive CD8+ T cells, and an increase in activated and differentiated CD8+ T cells. Responders also exhibited downregulation of cytokines and chemokines associated with anti-PD-1 resistance, along with upregulation of biomarkers linked to improved outcomes.<sup>36</sup>

A recent approach in melanoma treatment involves categorizing the Tumor Microenvironment (TME) to anticipate the cancer's responsiveness to immunotherapy. While combining different Immune Checkpoint Inhibitors (ICIs) like anti-PD-1 and anti-CTLA-4 may appear promising, trials on advanced melanoma patients showed severe immune-related adverse events (irAEs) such as hypophysitis, pneumonitis, and thyroiditis, likely due to the distinct mechanisms of T cell inhibition—CTLA-4 acting on T cell activation, while PD-1 intervenes later.<sup>14</sup> A recent study identified 29 functional gene expression signatures (Fges) delineating major functional and cellular components of melanoma tumors, classifying them into four subtypes: immune-enriched/fibrotic (elevated angiogenesis and Cancer-Associated Fibroblast activation), immune-enriched/non-fibrotic (more immune-active TME), fibrotic (low lymphocyte infiltration, high fibroblast activity), and immune-depleted (immune-desert).<sup>37</sup> This classification aids in determining treatment effectiveness and alternative approaches. For instance, responses to anti-CTLA-4 treatment varied across subtypes, with 82% of immune-enriched subtypes responding compared to only 10% of fibrotic subtype, suggesting appropriate utilization of ICI immunotherapy.<sup>37</sup>

### **5.3 Other Cancers (Novel Treatments/Combination Therapies)**

Pancreatic cancer (PDAC) poses a significant challenge for immunotherapy due to its low T cell infiltration, low tumor mutational burden, and highly suppressive Tumor Microenvironment (TME), making it the fourth leading cause of cancer-related deaths.<sup>44</sup> Combination therapy emerges as a promising approach, as demonstrated in a recent study by Padron et al. This study investigated the administration of sotigalimab and/or nivolumab (both anti-PD-1 drugs) alongside chemotherapy.<sup>38</sup> The results revealed that combining nivolumab with chemotherapy was associated with a less suppressive TME, increased numbers of activated and circulating T cells, and achieved the primary endpoint of one-year Overall Survival (OS). Although sotigalimab combined with chemotherapy did not meet the OS endpoint, it led to greater infiltration and differentiation of CD4+ cells. Interestingly, administering all three components together did not yield additional improvements.<sup>38</sup>

Cervical cancer (CC) ranks as the fourth most prevalent cancer, with concurrent chemoradiotherapy (CCRT) serving as the standard treatment.<sup>39</sup> However, individuals with advanced-stage disease often experience diminished long-term outcomes, prompting investigation into combination therapies. A recent study by Huang et al. in August 2022 explored the utilization of adoptive cell therapy (ACT), a form of immunotherapy involving the introduction of Tumor-Infiltrating Lymphocytes (TILs) to evoke an immune response, following CCRT and its impact on survival.<sup>39</sup> Among the 27 patients receiving ACT alone, 20 saw successful expansion of TILs. Subsequently, 12 patients received TILs post-CCRT, with 75% experiencing complete response and a disease control duration ranging from 9 to 22 months. Treatment-related adverse events were minimal, with only one patient encountering severe toxicity, underscoring the potential of post-CCRT ACT in managing CC.<sup>39</sup> Effective combination therapy entails strategic timing of treatments. Another study focused on determining the optimal timing for immune therapy concerning CCRT revealed the benefit of administering Immune Checkpoint Inhibitors (ICIs) before CCRT. This strategy capitalizes on maintaining tumor-specific immune response, as it was observed to diminish following CCRT. Post-CCRT, reduced numbers of Cytotoxic T Lymphocytes (CTLs), decreased T Cell Receptor (TCR) diversity, and increased Regulatory T cells (Tregs) signified a weakened antitumor immune response.<sup>40</sup>

Researchers are investigating new treatment avenues for chronic lymphocytic leukemia (CLL) that focus on targeting LAG-3, a protein involved in inhibiting the function of natural killer (NK) cells and CD8+ T cells. In CLL, LAG-3 expression on leukemic cells is associated with poorer outcomes and decreased response to treatment.<sup>20</sup> Relatlimab, an IgG4 antibody that blocks LAG-3, has shown promise in preclinical studies. Administering relatlimab increased the proliferation of NK cells and CD8+ T cells without affecting the growth of leukemic cells. This suggests that LAG-3 blockade can reverse the inhibition of NK and T cells, potentially restoring their anti-leukemic activity.<sup>20</sup>

Moreover, combining relatlimab with lenalidomide, an angiogenesis inhibitor, led to increased T cell numbers and enhanced anti-leukemic activity. This combination therapy approach may offer a synergistic effect in treating CLL by targeting both LAG-3-mediated immune suppression and angiogenesis. Overall, these findings provide a promising direction for the development of novel immunotherapies for CLL, particularly for patients who have not responded well to PD-1 or CTLA-4 blockade.<sup>20</sup>

Cancer often spreads to the liver, complicating treatment with immunotherapy. Liver metastases attract activated CD8+ T cells, which then undergo apoptosis, resulting in a significant reduction in effective T cells.<sup>41</sup> A study comparing patients with metastatic melanoma and NSCLC who received immunotherapy and targeted therapy, or immunotherapy and chemotherapy respectively, revealed that liver metastases were linked to reduced response to immunotherapy but not targeted therapy.<sup>41</sup> Similarly, in NSCLC cases, immunotherapy had limited efficacy compared to chemotherapy, while radiotherapy showed promising results. These findings suggest that liver metastasis may be more effectively managed through non-immunotherapeutic methods, informing future treatment strategies.<sup>41</sup>

## 6. Practical Applications/Discussion

Numerous innovative combination therapies extend beyond traditional chemotherapy and immunotherapy, delving into the modulation of the tumor microenvironment (TME) to bolster immune responses. For instance, one approach involves delivering a fecal microbiota transplant (FMT) via colonoscopy alongside immunotherapeutic anti-PD-1 drugs.<sup>36</sup> This strategy effectively reshapes the TME, overcoming primary resistance to anti-PD-1 in advanced melanoma patients. Following treatment, there's an increase in the proportion of CD56+CD8+T cells, indicating heightened circulation of active and differentiated CD8+ T cells.<sup>36</sup> Responders exhibit decreased levels of certain cytokines and chemokines linked to anti-PD-1 resistance, while biomarkers associated with improved survival are upregulated.<sup>36</sup> Notably, variations in patient response may occur due to factors like tumor immunogenicity or unsuccessful colonization by



FMT, necessitating further investigation for its establishment as a standard treatment.<sup>36</sup>

Another method, as explored by Ribas et al., involves combining anti-PD-1 therapy (specifically pembrolizumab) with intratumoral administration of an oncolytic virus, such as talimogene laherparepvec, in advanced melanoma patients. Initially, tumor size may transiently increase post-injection; however, this approach boosts CD8+ T cell infiltration and peripheral CD4+ T cell presence.<sup>48</sup> Pembrolizumab promotes the proliferation of CD8+ T cells, leading to a comprehensive shift in the TME and an augmented immune response compared to anti-PD-1 therapy alone. Importantly, T cells expressing PD-1 encounter tumor cells expressing PD-L1, a dynamic that, alongside PD-1 blockade, moderates the antitumor efficacy of the administered virus.<sup>48</sup>

As previously discussed, extensive research has already been conducted on PD-1, PD-L1, CTLA-4, and VEGF/VEGF-A as targets for treating various cancers. Scientists are now focusing on identifying new potential biomarkers to better predict patient responses to specific treatments, given that the existing biomarkers are not perfect indicators of a patient's response to immune checkpoint inhibitor (ICI) therapy.<sup>49</sup> Vanhersecke et al. observed that the tertiary lymphoid structure (TLS) within tumors is associated with the population of CD8+ T cells. Higher TLS densities correspond to increased densities of CD8+ T cells. Moreover, the presence of more mature TLS was linked to improved survival rates, higher objective response rates, and longer progression-free survival. Notably, mature TLS emerged as the most significant predictive factor for an objective response, independent of PD-L1 expression and CD8+ T-cell infiltration. Patients with tumors containing mature TLS had better outcomes compared to those without mature TLS.<sup>49</sup> Studying the TLS in tumors opens a new avenue for identifying biomarkers to predict patient responses to specific ICI treatments.

Two ongoing clinical trials are exploring recent advances in immunotherapy and tumor microenvironment (TME) mediated treatments, focusing on combination therapies involving LAG-3, anti-PD-1, and VEGF signaling.

The first trial is investigating the effects of anti-LAG-3 (urelumab) alone and in combination with nivolumab (an anti-PD-1 drug) in patients with recurrent glioblastoma. The study aims to determine which treatment regimen is more effective at killing tumor cells. Currently, 63 patients are enrolled, and the trial is expected to conclude by November 2023.<sup>50</sup>

The second trial, entering phase II, is evaluating the efficacy and safety of a combination of anti-PD-L1/VEGF drugs with traditional chemotherapy in treating non-small cell lung cancer (NSCLC). The primary objectives are to measure the objective response rate to the treatment and progression-free survival, which involves monitoring the time from the start of treatment until the cancer progresses or the patient dies from any cause.<sup>51</sup>

Other monitored outcomes include overall survival rate, disease control, response duration, treatment-related adverse events, and the correlation between PD-L1 expression and antitumor effect. The clinical trial aims to enroll 374 patients and is expected to be completed by December 2025.<sup>51</sup>

## **7. Conclusion**

The tumor microenvironment (TME) is a promising target in immunotherapy. Due to its variability and adaptability, further research is needed to effectively target different TME components. Current techniques include immune checkpoint blockades like PD-1 and CTLA-4, and targeting lactic acid and metabolism. Future prospects lie in combination therapies with chemotherapy or neoadjuvant therapy, microbiota fecal transplants to enhance TME for therapy, classifying CPR versus non-CPR tumors, and optimizing the timing of immunotherapy in combination treatments.<sup>36</sup>

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