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REVIEW ARTICLE

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Understanding the tumor microenvironment for effective immunotherapy

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Abstract

Advances in immunotherapy have led to durable and long-term benefits in a subset of patients across a number of solid tumor types. Understanding of the subsets of patients that respond to immune checkpoint inhibitors at the cellular level, and in the context of their tumor microenvironment (TME) is becoming increasingly important. The TME is composed of a heterogeneous milieu of tumor and immune cells. The immune landscape of the TME can inhibit or promote tumor initiation and progression; thus, a deeper understanding of tumor immunity is necessary to develop immunotherapeutic strategies. Recent developments have focused on

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characterizing the TME immune contexture (type, density, and function) to discover mechanisms and biomarkers that may predict treatment outcomes. This has, in part, been powered by advancements in spatial characterization technologies. In this review article, we address the role of specific immune cells within the TME at various stages of tumor progression and how the immune contexture determinants affecting tumor growth are used therapeutically.

KEYWORDS

biomarkers, immune checkpoint inhibitors, immune contexture, immunotherapy, tumor microenvironment

1 | INTRODUCTION

Over the last decade, immunotherapies have come to the fore in the treatment of a number of malignancies.[1] Immunotherapy acts to take the "brakes off" the immune system; so that it can hone in on the cancer cells and destroy them.[2] The first generation of antibody-based immunotherapy, known as immune checkpoint inhibitors (ICIs), exert their role by blocking the receptor/ligand interactions between different molecules involved in the regulation of T cell activation or function, such as programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4).[3] ICI therapies have been successful in providing significant benefit to a subset of patients who demonstrate long term, durable responses.[1] However, there is a need to develop biomarkers that better describe the tumor microenvironment (TME) and may be predictive of patient outcomes to identify responders/nonresponders.[4]

The TME is considered to be an essential part of cancer initiation and dissemination.[4] Several studies have been carried out to shed light on how the TME plays a role in tumor progression.[4] However, knowledge of mechanisms involved in the development of the TME and disease progression is in its infancy.[5] Emerging data showed that an intricate understanding of the TME is needed to identify predictive biomarkers of response that can routinely be used in the clinic.[5] It has been thought that the immune contexture, that is, the type, density, and location of cells in the TME may associate with disease outcome.[4] To this end, studies characterizing the TME have provided the foundation for insights into the composition, location, and function of different cells within the TME.[5] In this review, we will provide a broad overview of the development of TME during cancer progression, the role of systemic immune and nonimmune factors that influence the characteristics of the TME, biomarkers within the TME so far used in clinical settings, and patient stratification based on recent findings in the TME.

2 | TUMOR MICROENVIRONMENT

2.1 | Establishment of the TME during cancer progression

The complexity of the TME is thought to be due to the unregulated cancer cell proliferation and defective blood vessel development.[6] Acidic pH conditions, hypoxia, endogenous H_2O_2, and the alteration in the expression of the extracellular matrix (ECM) proteins are the hallmarks of the TME, which play a key role in tumor progression and cancer metabolism.[7] The acidic pH is derived from anaerobic glycolytic excretion of protons (H^+) and lactate by

membrane proteins, such as ATPase, monocarboxylate transporter 1 (MCT1), and MCT4.[7] The acidic pH is one of the major players in cancer cell migration and invasion by increasing the expression of angiogenic factors, including vascular endothelial growth factor A (VEGFA) and interleukin (IL)-8.[7] Hypoxia (partial pressure of oxygen <10 mmHg) has been found to occur in several solid tumors.[8,9] Regarding this, with uncontrolled growth of tumor cells, the cells in the center of the mass gradually become hypoxic due to the distance from existing blood vessels, resulting in the deprivation of available oxygen and nutrient supplies.[8] Therefore, tumor cells need to upregulate the hypoxia-inducible angiogenic factors, such as VEGF, to overcome the proliferation limitations.[8,10] However, the blood vessels formed during neovascularization are different from normal vessels in the context of phenotype and function, which means that the tumor-associated vessels have a blunt-ended and a weak perfusion.[8] Also, compared to endothelial cells in normal vessels which have a laminar flow, endothelial cells of tumor-associated vessels have multiple gaps that contribute to vascular leakiness and nonlaminar flow that, in turn, cause blood clotting and local tissue edema.[8] In addition, overexpression of hypoxia-inducible factor-alpha (HIF-α) and hypoxia-inducible factor-1 beta (HIF1β), the major mediators of the hypoxic response pathway, has been shown to be related to tumor growth as they are capable of binding to hypoxia response elements of genes involved in tumor survival and angiogenesis.[8]

Moreover, reactive oxide species (ROS), in particular hydrogen peroxide (H_2O_2), have been found to have critical effects on a variety of physiological processes.[11] To wit, the intracellular concentration of H_2O_2, a two-electron oxidant, has been reported to be maintained in a low nanomolar range (approximately 1–100 nM).[11] This low state of H_2O_2 maintenance and its associated redox physiological signaling is referred to as "oxidative stress."[12] This steady-state is necessary for the orchestration of different processes in cells and organs, including cell proliferation, differentiation, migration, and angiogenesis.[11] However, in some pathophysiological conditions, known as "oxidative distress," the levels of intracellular H_2O_2 were found to be above 100 nM, leading to non-specific oxidation of proteins and reversible or irreversible damage to various types of other intracellular macromolecules, thereby impairing their function.[11] However, tumor cells take advantage of ROS production to modulate signaling pathways and transcription factors, increase the proliferation rate, mediate cell metabolism, and adapt to hypoxic stresses.[11,13–15] Nevertheless, evidence suggests that cancer cells balance the production of oxidants and antioxidants to become resistant to chemotherapeutic agents that elicit cancer cell cytotoxicity through the generation of oxidants.[11,14,16] Therefore, for anticancer drugs to be effective, the extent of oxidant- or antioxidant production should be taken into account in concert with the oxidant levels in the tumor niche and the endogenous oxidant capacity of the respective tumor.[11]

Furthermore, a number of studies have indicated that cancer cells express different levels of ECM proteins, often called the matrisome, compared with normal cells.[17–19] During tumor development, the expression levels of ECM proteins that are involved in cell–cell attachment decrease, including laminin subunit beta-1 (LAMB1), laminin subunit gamma-1 (LAMC1), laminin subunit alpha-4 (LAMA4), and collagen alpha-1(XV) chain (COL15A1).[5] However, the expression of matrisome proteins that are responsible for cell migration and tumor invasion, including fibronectin (FN1), cartilage oligomeric matrix protein (COMP), cathepsin B (CTSB), and collagen alpha-1(XI) chain (COL11A1), are induced during tumorigenesis.[5]

In addition to the pathological conditions described above, in order for cancer to develop, neighboring cell populations around the tumor are also altered.[20] The cellular composition thus changes from normal tissue, consisting predominantly of endogenous cells with limited immune cell infiltration, to cancerous tissue with intense tumor-associated inflammation and a significant increase in leukocytes[5,20] (Figure 1).

2.2 | Cancer immunotherapy

Cancer immunotherapy is a form of cancer treatment that uses endogenous T cells to kill tumor cells[21] and is commonly utilized in both early- and advanced-stage patients, either as a first-line or

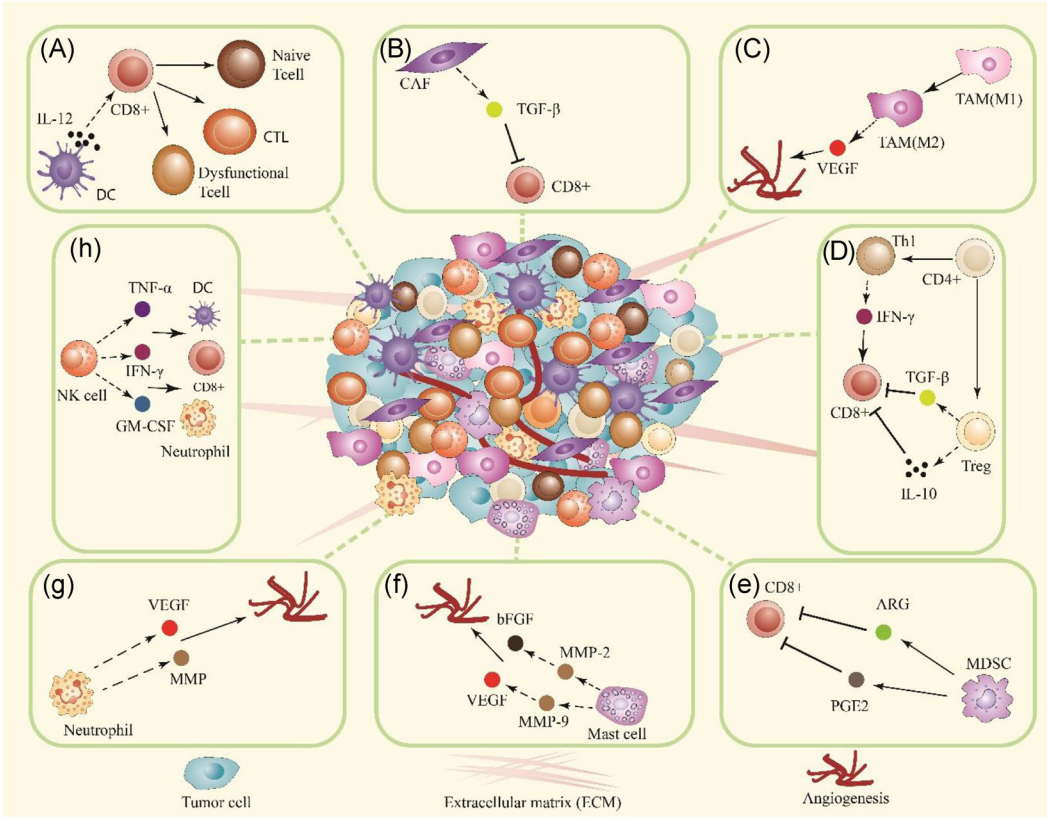

FIGURE 1 Different roles of TME-driven cell populations in cancer progression. Cell populations within the TME modulate immune-activating or immunosuppressive conditions to prevent or promote tumor growth. (A) T cell effector function and differentiation signals can be supported by the dendritic cell (DC) through the interleukin-12 (IL-12) secretion. (B) Cancer-associated fibroblast (CAF) plays an immunosuppressive function by inhibiting $CD8^+$ T cell infiltration and function via secreting transforming growth factor-beta (TGF-β). (C) Within the TME, tumor-associated macrophage (TAM) consists primarily of the M2 macrophage, which is tumorigenic. TAM (M2) contributes to angiogenesis through the secretion of vascular endothelial growth factor (VEGF). (D) $CD4^+$ T cell can play immunoactivative or immunosuppressive roles through the differentiation into T helper type 1 cell (Th1) and regulatory T cell (Treg), respectively. Th1 is able to induce $CD8^+$ T cell by releasing cell-activating cytokines such as interferon-gamma (IFN-γ); however, Treg has an immunosuppressive activity through the inhibition of $CD8^+$ T cell function via releasing IL-10 and TGF-β. (E) Myeloid-derived suppressor cell (MDSC) causes suppression of tumor-specific $CD8^+$ T cell response by increasing the levels of prostaglandin E2 (PGE2) and arginase (ARG). (F) Mast cell can promote tumor progression through angiogenesis development by secreting certain proteases, such as matrix metalloproteinase 2 (MMP-2) and MMP-9, and also by releasing VEGF and fibroblast growth factor (bFGF) from the extracellular matrix (ECM). (G) Similar to the mast cell, neutrophil is also capable of developing angiogenesis via secreting VEGF and MMPs. (H) Natural killer (NK) cell can induce innate and adaptive immune responses through the secretion of pro-inflammatory cytokines, including IFN-γ, tumor necrosis factor-alpha (TNF-α), and granulocyte/monocyte colony-stimulating factor (GM-CSF). TME, tumur microenvironment [Color figure can be viewed at wileyonlinelibrary.com]

after several lines of treatment.[22,23] There are currently two types of immunotherapy strategies that are widely used in clinical settings: ICIs and adoptive cellular therapy (ACT).[23,24] These approaches take advantage of cell surface molecules (such as PD-L1....), and host's immune cells (such as tumor-infiltrating T cells).[23,24]

2.2.1 | Immune checkpoint inhibitors

T cell recognizes peptide antigens by ligation of its surface receptors, and then becomes active against them.[25] Two signals are required to activate the T cell. One signal originates from the interaction between the heterodimeric T cell receptor (TCR) and MHC-presented antigens, and the second signal is induced by the ligation of T cell costimulatory surface receptor CD28 to its ligand CD80 or CD86, the so-called B7-1 and B7-2.[25] CD80 and CD86 are presented via professional antigen-presenting cells (APCs).[25] Once activation has occured, T cell expresses coinhibitory cell surface receptors, such as programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), to prevent continued activation.[25] PD-1 inhibits signaling downstream of the TCR by binding to its ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2), and subsequently block signal 1.[25] Also, the blockade of signal 2 occurs when CTLA-4 binds CD80 and CD86 with a higher affinity than CD28.[25] Given these functions, PD-1 and CTLA-4 are known as immune checkpoint proteins.[25]

The aim of immune checkpoint signaling pathways is to permit self-tolerance; nonetheless, tumor cells can exploit these pathways to escape from the immune system.[26,27] For example, tumor cells may be able to cause T cell exhaustion by upregulation of the PD-1 pathway, which, in turn, results in reduced T cell effector function and proliferation.[28] Thus, anti-PD-1/PD-L1 or anti-CTLA-4 antibodies, the so-called ICIs, have been developed to bind these immune checkpoint proteins and, consequently, to reinvigorate the immune system against cancer.[29] Also, in comparison to other anticancer therapies such as chemotherapeutic agents or targeted therapies, ICIs can cause a long-lasting response even after cessation of treatment in patients by inducing tumor-specific immunological memory.[1] For instance, patients with advanced-stage melanoma who received the anti-CTLA-4 and anti-PD-1 antibodies were shown to have more overall survival (OS) and to live longer.[30,31] The first FDA-approved ICI was ipilimumab which targeted CTLA-4 and used for the treatment of patients with advanced melanoma.[31] However, more ICIs have been developed to date for the treatment of cancer patients. These inhibitors include antibodies against PD-1 (pembrolizumab, nivolumab, cemiplimab, and tremelimumab), and PD-L1 (atezolizumab, avelumab, and durvalumab).[32] Currently, ICIs are being studied for the treatment of various types of cancers, such as melanoma, renal cell carcinoma (RCC), lung cancers, head and neck squamous cell carcinoma (HNSCC), gastric cancer, ovarian cancer, Hodgkin lymphoma, and tumors with mismatch repair (MMR) deficiency.[33] In addition to utilizing any of these antibodies as a single-agent treatment, researchers have evaluated the combination immunotherapy method by employing both anti-PD-1 and anti-CTLA-4 antibodies to target T cell activation signals 1 and 2 simultaneously.[31] The result has associated with higher overall response rates than those reported with monotherapy that contributed to the first FDA-approved combination immunotherapy for the treatment of patients with advanced-stage melanoma.[31,34]

Collectively, given the growing importance of ICIs, significant efforts have been made to identify predictive and prognostic biomarkers. Immunohistochemistry (IHC) assays for PD-L1 protein expression have been approved by the US FDA as companion diagnostic markers for anti-PD-L1 therapy in non-small cell lung carcinoma (NSCLC) patients.[22,35,36] The blueprint PD-L1 IHC assay comparison project was performed on 39 NSCLC tumors to offer information on the analytical and clinical comparability of four PD-L1 IHC assays (22C3, 28-8, SP142, and SP263).[22] This study reported that when the clones 22C3, 28-8, and SP263 were used, the percentage of PD-L1-stained tumor cells was comparable, while the SP142 assay had less total stained tumor cells.[22] The study found that although analytical performance of PD-L1 expression is similar between three assays, interchanging assays and cutoffs may result in some patients being "misclassified" for PD-L1 status.[22] Furthermore, the use of PD-L1 as a biomarker has been rigorously debated as it appears to be dynamically expressed.[37,38] Several investigations have shown that PD-L1 expression has increased the probability of response to ICIs[37,39]; however, a number of studies have mentioned that patients with no PD-L1 expression have also responded to ICIs.[40,41] This controversy can be a result of the cellular complex mechanisms that affect the PD-L1 expression.[24] For example, genomic aberrations, transcriptional and translational control mechanisms, RNA/protein stability, and host-microbiome immunoediting have been shown to play a significant role in PD-L1 expression.[24] A study by Rizvi et al.[42] evaluated

the impact of PD-L1 expression along with TMB (by targeted NGS (MSK-IMPACT)) in NSCLC patients who were treated with ICIs (anti-PD-1/PD-L1 therapy) to determine the immunotherapy response, and the relationship between these biomarkers. They observed that PD-L1 expression in 51% percent of patients had ≥1% expression which was associated with improved progression-free survival (PFS) and was consistent with previous studies.[42] Similarly, TMB was associated with improved benefit among ICI-treated patients.[42] However, there was no correlation between PD-L1 expression and tumor mutational burden.[42] The study reported that PD-L1 expression and TMB are independent variables associated with benefits; thus, a combination of these biomarkers may be most helpful in determining which patients are more likely to benefit from ICIs.[42]

3 | CELL TYPES IN THE TME

3.1 | Neutrophils

Neutrophils are the most common circulatory leukocytes in cancer patients.[43] There are two types of neutrophils in the blood circulation: circulating neutrophils, which circulate freely and are recruited into tumors; and marginated neutrophils, which are bound to the capillary endothelium.[43,44] Neutrophils play a fundamental role in inflammatory responses; however, their contribution to tumorigenesis is still controversial.[45] In the context of tumorigenesis, neutrophils have been found to affect tumor growth by releasing growth factors and cytokines into the TME.[46] Neutrophil-derived growth factors, such as VEGF, have been reported to promote tumor growth by regulating angiogenesis, while some cytokines, such as interferon-gamma (IFN-γ), have been shown to suppress tumor development by recruitment and activation of innate and adaptive immune cells.[46] Evidence indicates that when recruited to tumors, neutrophils secrete VEGF and matrix metalloproteinases (MMPs) thereby promoting angiogenesis and ECM reorganization, respectively.[47] Nevertheless, neutrophils also exhibit an antitumor function.[46] For instance, neutrophils isolated from patients with early stage lung cancer have been found to induce the release of IFN-γ by $CD4^+$ T cells, which in turn enhances the differentiation of $CD8^+$ T cells.[46] Building upon this, Zhang et al. investigated the impact of neutrophils on multiple murine models of triple-negative breast cancer (TNBC).[48] In the study, they have found that across all immune cell populations, tumor-infiltrating neutrophils (TINs), and tumor-infiltrating macrophages (TIMs) were the most dominant cell types in tumor models.[48] They also divided preclinical models into neutrophil-enriched subtypes (NESs) and macrophage-enriched subtypes (MESs) to examine their response to ICIs.[48] As a result, it was identified that MES-tumor-derived cell lines had a stronger response to ICIs, while NES-derived cell lines were resistant to therapies, suggesting an immunosuppressive microenvironment was created by TINs.[48] In addition to the mouse model, the team used published metastatic melanoma datasets to investigate the role of tumor-infiltrated neutrophils in response to ICIs.[48] They have found that patients with progressive disease (PD) or partial response (PR) had higher TIN scores, compared to patients with complete response (CR), implying neutrophils function in favor of tumor promotion.[48] Collectively, these studies showed that neutrophils are more likely to have tumor-promoting function than tumor suppression in the TME.[45,46]

3.1.1 | Mast cells

Mast cells (MCs) are the progeny of pluripotent bone marrow progenitor cells defined as positive for CD34, c-kit (CD117), and CD13, and are differentiated after being recruited into a given tissue.[49]

MCs are present throughout the body and have a wide range of activity in both physiological and pathophysiological conditions.[50] They utilize different classes of receptors, such as the high-affinity IgE receptor (FcεRI) and G-protein coupled receptors (e.g., C3a receptor) to detect inflammatory, immunological, and environmental

signals.[50] Upon stimulation, MCs release cytoplasmic granules containing, histamine, heparin, and granule-associated protease, and produce cytokines and leukotrienes.[50] These products have a variety of biological functions, including enhancement of vessel permeability, promotion of inflammation, and stimulation of peripheral nerves.[50] MCs also play significant roles in both innate and adaptive immune responses.[51] Inflammatory mediators, such as IL-1 and tumor necrosis factor-alpha (TNF-α), are released by MCs to recruit additional immune cells at the site of inflammation and to activate $CD8^+$ T cells, respectively.[51] Moreover, MCs have been found in various forms of cancer and have been shown to play protumorigenic roles.[52] MCs are able to express and secrete MMP2 and MMP9 to liberate VEGF and fibroblast growth factor (bFGF) from the ECM and thus to stimulate angiogenesis.[52] Also, it has been found that the interaction between MCs-secreted histamine and its receptor 1, H1 (H1HR), can drive cell-cycle progression, MMP2 production, and suppression of apoptosis.[53] In addition to tumor enhancing characteristics, studies have suggested that MCs also have immunomodulatory effects on cancer cells.[54] Numerous studies have been performed to investigate the relationship between MCs and other immune cells within the TME.[55] For example, Zhuang et al. conducted an in vitro/in vivo study on 114 tissue samples taken from gastric cancer (GC) patients to determine the role of MCs in modulating the TME.[54] As a result, it was shown that the percentage of MCs was significantly higher in patients with advanced stage of disease.[54] Also, MCs have been shown to have immunosuppressive activity in GC patients.[54] The researchers have found that MCs within GC tumors express a high level of immunosuppressive molecule PD-L1, an immune checkpoint protein.[54] Zhuang et al. have found a significant correlation between the levels of $PD\text{-}L1^+$ MCs and pro-inflammatory cytokine TNF-α in GC tumors.[54] In fact, it has been identified that tumor-derived TNF-α induces the expression of PD-L1 on MCs through the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways.[54] Also, they have shown that PD-L1 expressing MCs are able to modulate the cytotoxic function of T cells by suppressing the production of perforin (a pore-forming protein cytotoxic to tumor cells) and granzyme B (a proapoptotic protease that aids the destruction of tumor cells).[54,56] Considering these results, the study concluded that MCs may have immunosuppressive functions in cancer.[54]

3.1.2 | Myeloid-derived suppressor cells

Myelopoiesis is a process for the formation of myeloid cells resulting from the differentiation of oligopotent myeloid precursors.[57–59] However, this process may be disturbed by various conditions, such as chronic inflammation or cancer.[57] As a result, the amount of peripheral myeloid cells decreases and this reduction induces stronger myelopoiesis and increases the migration of undifferentiated cells to inflammatory or cancerous sites.[57] These cells have been shown to possess strong immunosuppressive properties and combined with their myeloid origin they are hence known as myeloid-derived suppressor cells (MDSCs).[57] MDSCs are now defined based on their phenotypic and morphological features; M-MDSC reflecting monocytic progenitor cells and PMN-MDSC for polymorphonuclear.[60] MDSCs exert their immunosuppressive role using several mechanisms, including the secretion of immunosuppressive cytokines, the production of reactive oxygen species (ROS), and the increase in prostaglandin E2 (PGE2) and arginase (ARG) levels.[61,62] To this end, it has been shown that PGE2 functions as a pro-inflammatory factor in tumor development and is a suppressor of host-antitumor immunity.[63] Also, arginase has been found to play a key role in impairing T cell functions by downregulation of T-cell receptor (TCR)-associated CD3ζ and ε chains expression.[64] In addition, it has been shown that tumor cells express a large amount of indoleamine 2,3-dioxygenase 1 (IDO1), a tryptophan-metabolizing enzyme that catalyzes the initial step of the kynurenine pathway (KP), to promote an immunosuppressive TME by the expansion and activation of MDSCs.[65] Considering this information, several studies have investigated the impact of MDSCs on cancer immunotherapy.[66] For example, Fultang et al. examined the effects of an anti-CD33 immunotoxin on MDSC population and chimeric-antigen receptor T (CAR-T) cell therapy in patients with different types of cancer.[66] They targeted MDSCs by anti-CD33 immunotoxin Gemtuzumab ozogamicin in peripheral blood and tissue samples.[66] It should be noted that

CD33 is a myeloid lineage cell surface marker.[66] As a result, the treatment of human MDSCs by Gemtuzumab ozogamicin resulted in the death of MDSCs, restoration of T cell proliferation, and increased CAR-T cell proliferation and cytotoxicity against solid tumors.[66] Therefore, they concluded that targeting MDSCs in cancer may have promising outcomes for patients.[66]

3.1.3 | Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are typically characterized by the expression of Thy1, a glycophosphatidylinositol (GPI)-anchored cell surface protein involved in cell–cell adhesion,[67] with subsets expressing smooth muscle actin (SMA) and fibroblast activation protein (FAP).[68] CAFs are the most common cells found in the TME[69] and contribute to tumor development through the nutritional and immunological mechanisms.[69] It has been noted that CAFs are capable of producing large amounts of growth factors, including VEGFA, fibroblast growth factor 2 (FGF2), and platelet-derived growth factor C (PDGFC), to enhance angiogenesis and thus promote tumor progression.[70] Also, considering their immunological role, CAFs have been shown to be a major source of transforming growth factor-beta (TGF-β) protein family members, in particular, TGF-β1.[71] During microenvironmental activation, TGF-β facilitates the establishment of desmoplastic stroma by promoting profibrotic signaling in the TME, which contributes to the sequestration of immune cells within the CAF- and collagen-rich tumor-stroma interface.[72,73] Consistent with the crucial role of TGF-β in promoting the immune-excluded phenotype (an immunological state in which T cells are unable to infiltrate tumor center) of certain tumors and their subsequent immunotherapy resistance, it has recently been shown that targeting TGF-β may sensitize different types of tumors to ICI therapy in preclinical settings.[74] These findings have indicated that TGF-β is a viable target for the development of novel immunotherapeutic regimens for the alteration of immune-excluded tumors to inflamed tumors, and suggest that TGF-β signaling indicators in CAFs may predict immune-excluded tumor response to immunotherapy.[74] In addition, it has recently been reported that CAFs may be able to deplete $CD8^+$ T cells from the TME.[75] In this context, Shield et al. have investigated the role of CAFs in the TME through an in vitro/in vivo study and have demonstrated that CAFs are capable of presenting antigens to $CD8^+$ T cells.[75] Also, CAFs have been shown to express large amounts of programmed cell death 1 ligand 2 (PD-L2) and first apoptosis signal receptor ligand (FasL).[75] PD-L2 has been shown to inhibit $CD8^+$ T cell activation by interacting with its receptor PD-1, and FasL has been indicated to drive apoptosis through interaction with its receptor Fas.[25,76] Thus, CAFs acquire and present tumor-derived antigens to $CD8^+$ T cells along with the lethal signals provided by PD-L2 and FasL.[75] These signals will lead to the inactivation and removal of $CD8^+$ T cells from the TME, thereby promoting cancer survival.[75] As such, this CAF-mediated mechanism may explain why CAFs are associated with poor prognosis in patients, and demonstrate the novel mechanism involved in T cell dysfunction and depletion within the TME.[75]

3.1.4 | Natural killer cells

Natural killer (NK) cells, as their name implies, are part of the innate immune cell population that kills any cell considered to be hazardous upon interaction and without any prior "education."[77,78] These cells are defined as $CD3^-$ $CD56^+$ cells and represent up to 15% of total circulating lymphocytes in human.[77,78] NK cells have been shown to play significant functions in the immune system and have been shown to act against tumor cells, by releasing perforin and GZMB or by inducing TNF-related apoptosis-inducing ligand (TRAIL)- and FasL-mediated apoptosis.[77,78] They are also capable of regulating other immune cell functions including controlling T cell expansion by killing activated T cells and promoting Th1 polarization via releasing IFN-γ.[77,78] It has also been indicated that, by producing chemotactic cytokines such as C-C motif chemokine 5 (CCL5) and lymphotactin

(XCL1), NK cells can attract effector lymphocytes and myeloid cells to inflamed tissues.[77,79] In the context of cancer, these cells contribute to the development of antitumor immunity and are associated with good prognosis in patients.[80] NK cells have been found to induce innate and adaptive immune responses through the activation of T cells and other immune cells such as DCs, macrophages, and neutrophils by secreting pro-inflammatory cytokines and chemokines, including IFN-γ, TNF-α, and granulocyte/monocyte colony-stimulating factor (GM-CSF).[77,79] Nevertheless, tumor cells may suppress or inhibit NK cell function using a variety of mechanisms, including the secretion of immunosuppressive metabolites and growth factors, such as adenosine[1] and TGF-β1.[82,83] It has been found that adenosine inhibits NK cell maturation, suppresses cytotoxic GZMB expression, and limits NK cell infiltration into the TME.[81] Similarly, TGF-β1 secreted by different cell types has been found to restrain NK cell activity,[82] by suppressing the mTOR (mammalian target of rapamycin) pathway which is a modulator of NK cell responsiveness.[84] TGF-β1 also suppresses NK cell priming and their subsequent cytotoxic activationand cytokine production.[85] Furthermore, evidence has shown that when human cells undergo neoplastic transformation, they begin to overexpress ligands capable of NK cell activation that induce NK cells to engage in tissue stress surveillance responses.[80,86] Activation of NK cell receptors via transformed cell ligands may be a key to innate antitumor immunity.[86] Further work by Sutherland et al. evaluated the antitumor functions of NK cells along with $CD8^+$ T cells in small cell lung cancer (SCLC) using samples from a cohort of SCLC patients and used an animal model to mimic metastasis.[87] They also used an anti-CD8 antibody to deplete $CD8^+$ T cells and took advantage of a genetic model for the depletion of NK cells in the mouse model.[87] Interestingly, they demonstrated that the depletion of $CD8^+$ T cells had no effect on distant metastasis, whereas NK cell depletion led to increased metastatic spread to the liver.[87] This finding suggest that $CD8^+$ T cells have a minimal function in controlling metastatic dissemination, whereas in contrast, NK cells play a key role in the immunosurveillance of SCLC dissemination.[87] Furthermore, Sutherland et al. investigated the impact of immunotherapy on the NK cell function and observed that treatment with anti-PD-L1 antibody resulted in the activation of peripheral blood NK cells, thereby concluding that NK cells could have additive antitumor functions in immunotherapy of SCLC patients.[87]

3.1.5 | Dendritic cells

Dendritic cells (DCs) are the main antigen presenting cells that play a part in providing antigens and secondary signals to the T cells of the adaptive immune system.[88] In the absence of various stimuli, such as bacteria, viruses, and other inducers, DCs express and secrete major histocompatibility complex (MHC) class II and cytokines at low levels, while their capacity for antigen uptake is high.[88] However, upon activation, these cells express and produce high amounts of costimulatory molecules and cytokines and their ability to capture antigens decreases.[88] DCs modulate T cell functions via four distinctive signals: the presentation of processed antigens as an antigen-presenting cell (APC); the provision of costimulatory signals that complement the TCR signal to ensure effective T cell activation; the provision of the differentiation signals to the T cells; and the induction of signals involved in T cell homing to specific tissues.[62] Conventional DCs (cDCs) exert their antitumor function by presenting tumor antigens and secreting cytokines that support T cell survival and effector functions.[89] cDCs are divided into at least two subsets: conventional type 1 dendritic cells (cDC1s) and conventional type 2 dendritic cells (cDC2s).[88] cDC1s play important roles within tumors by attracting, restimulating, and expanding tumor-specific $CD8^+$ T cells, as well as supporting T cell effector function by secreting interleukin (IL)-12,[80] while cDC2s are the initiator of $CD4^+$ T cell responses.[90] Although intratumoral cDC1s have not been extensively studied in human cancers, some studies have mentioned that their abundance in human melanoma is associated with T cell infiltration.[91] In addition, it has been found that the ratio of cDC1-selective transcripts over macrophage-restricted transcripts can be used as a prognostic marker for the survival of cancer patients.[91] Therefore, treatment regimens designed to enhance the abundance of cDC1s in tumors or to promote their activation may improve antitumor immunity and potentially increase patients' sensitivity to immunotherapy.[88,92] With regard to the role of DCs in antitumor immunity,

Williford et al. developed multiple tumor models to evaluate the recruitment of DCs for ICI efficacy.[93] As such, they employed a tumor stroma-targeting approach to deliver chemokine C-C motif chemokine 4 (CCL4), a chemokine that has been shown to induce the recruitment of DCs, into tumors.[93,94] For this, a fusion protein of CCL4 and the collagen-binding domain (CBD) of von Willebrand factor (VWF), a glycoprotein involved in hemostasis, was generated and injected into melanoma and breast cancer models.[93] As a result, the antitumor effect of ICI was improved in these tumor models following increased recruitment of DCs and $CD8^+$ T cells.[93] Thus, this result suggests that DC recruitment into TME may enhance the efficacy of ICIs.[93]

3.1.6 | Tumor-associated macrophages

While many immune cell populations have been reported to have a suppressive function in the TME, tumor-associated macrophages (TAMs) are the most commonly studied and well characterized.[95] There are two types of TAMs: M1 macrophages (tumoricidal) and M2 macrophages (tumorigenic).[95] M1 macrophages are classified as tumor suppressors, while M2 macrophages are known as tumor promoters.[95] M2 TAMs are thought to be the most dominant macrophages within the TME[95] and have been shown to promote angiogenesis and tumor development through the secretion of pro-inflammatory cytokines and growth factors such as TNF-α and VEGF.[96] Considering these characteristics, multiple studies have been designed to unveil the impact of TAMs on tumor immunotherapy.[97] Singhal et al.[97] examined TAM-mediated T cell responses in early-stage lung tumors where they investigated the effect of TAM-derived PD-L1 expression on $CD8^+$ T cell response. Exclusive M1 or M2 macrophages were not found in the tumors, but coexpressed both M1 and M2 markers indicating that the conventional M1/M2 paradigm may not apply to human TAMs within the TME.[97] Moreover, the study interestingly indicated that only tumor cell-derived PD-L1 was capable of inhibiting the effector function of T cells, and that there was no significant relationship between PD-L1 expression on TAMs and the suppression of T cells.[97] It was shown that PD-L1 on the surface of TAMs demonstrated its regulatory role only when these cells functioned as peptide presenting cells.[97] This may address the question as to why some patients with significant PD-L1 expression do not respond to PD-1/PD-L1 blockade, suggesting that the evaluation of total PD-L1 might not be a suitable diagnostic method.[97] This study has also demonstrated that the expression of PD-L1 on macrophages may have a protective function against $CD8^+$ T cells-mediated elimination.[97] This means that when macrophages express the cognate MHC class I/peptide complex, they are attacked and destroyed by $CD8^+$ T cells, but the expression of PD-L1 on the surface of these cells makes them less likely to be targeted by $CD8^+$ T cells.[97] Hence, based on these findings, Singhal et al.[97] concluded that anti-PD-L1 therapy could act as a two-edged sword. On the one hand, it can reinvigorate T cell function and improve the antitumor immunity, but on the other hand, it can lead to the blockade of PD-L1 expressed on APCs, which in turn, result in an inability to maintain stimulation of $CD8^+$ T cells in tumors.[97]

3.1.7 | $CD4^+$ T cells

$CD4^+$ T cells are lymphoid immune populations involved in the adaptive immune response.[98] $CD4^+$ T cells are activated in secondary lymphoid organs and function through the secretion of various chemokines.[99] They recognize antigens presented by MHC class II expressed on the surface of APCs.[99] As such, one of the major roles of $CD4^+$ T cells is to modulate the state and function of other immune cells.[99] They also play a role in autoimmunity and allergic responses, as well as tumor immunity.[99] It has been shown that $CD4^+$ T cells can inhibit or promote tumor cell growth, depending on whether they differentiate into immune-activating cells or immunosuppressive cells.[100] $CD4^+$ T cells have been shown to mediate antitumor response through several mechanisms, including CCR5 ligands which are central for CD4 and CD8 T cell activation.[98,101–103] T helper type 1 (Th1) cells are one of the most studied $CD4^+$ populations.[104] Th1 cells are responsible for immunity against intracellular pathogens by

increasing $CD8^+$ T cell response and activating macrophages to phagocytose intracellular pathogens.[104] However, in the context of cancer, Th1 cells have been found to induce $CD8^+$ T cell response by increasing the stimulatory capacity of the DCs.[105] A key signal required for functional maturation of the DCs is the interaction between CD40 ligand (CD40L) on Th1 cells and its receptor CD40 on the DCs.[105] DCs can attract, restimulate, and expand tumor-specific $CD8^+$ T cells.[80] Th1 cells have also been indicated to secrete pro-inflammatory cytokines, such as IFN-γ, not only to enhance $CD8^+$ T cell differentiation but to directly stop tumor cell growth by inducing senescence, a permanent state of cell cycle arrest, contributing to the decline of the cell regenerative potential.[105–107]

In addition to promoting antitumor immunity, $CD4^+$ T cells can function as immunosuppressive cells in the TME.[108] For this, $CD4^+$ T cells differentiate into T regulatory (Treg) cells that are characterized by the expression of forkhead box protein P3 (FoxP3) marker, a transcriptional repressor needed for maturation and immunosuppressive functionality.[108] Tregs are known to contribute to the prevention of excessive immune response against pathogens and inhibition of autoimmune diseases.[109] A number of studies have shown that Tregs promote cancer progression by impeding effective immunity.[109–111] As such, tumor-infiltrating Tregs have been found to express a large amount of cell surface molecules engaged in the inhibition of $CD8^+$ T cell activation, such as PD-L1 and PD-L2.[112] PD-L1 and PD-L2 expressed on Tregs interplay with their receptors PD-1 expressed on $CD8^+$ T cells and these interactions result in inhibition of signaling downstream of the T cell receptor (TCR), thereby preventing $CD8^+$ T cell activity.[112] Moreover, Tregs were found to secrete IL-10 and TGF-β to inhibit tumor-specific T cell infiltration and function, and therefore, to suppress the antitumor immunity.[105] A number of studies have been conducted on $CD4^+$ T cells to investigate how these immune cells affect the immunotherapy response in cancer patients.[98,99] Allison et al. assessed the underlying mechanisms by which anti-CTLA-4 and anti-PD-1 antibodies, known as ICIs, interact with both $CD4^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TILs).[113] For this, they employed mass cytometry and utilized human melanoma and murine tumor models. The study showed an increase in T cell population following treatment with anti-CTLA-4 and anti-PD-1 antibodies. This observation was followed by an increase in $CD8^+$/Treg ratio after both treatments, suggesting that these ICIs induced an effective immune response in the tumors. In the study, $TBET^+$ Th1-like $CD4^+$ effector subsets increased after exposure to anti-CTLA-4 across all effector $CD4^+$ T cells. It should be noted here that TBET is a transcription factor required for the induction of the Th1 cell phenotype.[114,115] Thus, the researchers suggested that only specific T cell subsets were targeted by ICIs.[113] Interestingly, these findings were found to be the same in both highly and poorly immunogenic tumors, indicating that the mechanisms underlying response to ICI were tumor agnostic. Taken together, this study showed that the response to ICIs is tumor-type independent and regulated by specific tumor-infiltrating T cell subsets.[113]

3.1.8 | $CD8^+$ T cells

$CD8^+$ T cells are the key components of the adaptive immune response.[116] They recognize antigenic peptides provided by MHC class I expressed on the surface of all nucleated cells via their T cell receptors (TCRs).[116] Having been exposed to antigenic peptides, naïve T cells become active, experience massive clonal expansion, and differentiate into potent effector cells.[117] These effector cells, known as cytotoxic T cells (CTLs), destroy target cells by releasing cytotoxic granules or by inducing FasL mediated-apoptosis.[118] Recent experiments using high-dimensional profiling technologies have revealed that T cells in human tumors are mostly capable of taking three functional states, naive, cytotoxic, and dysfunctional.[119–121] The relative abundance of these three states varies significantly between tumors, for example, dysfunctional T cell percentages of total T-cell infiltrate ranged from 5% to 80% in melanoma.[119–121] Gene expression studies have found that naive $CD8^+$ T cells mostly express C-C chemokine receptor type 7 (CCR7), transcription factor 7 (TCF7), lymphoid enhancer-binding factor 1 (LEF1), and L-selectin (SELL) which have roles in T cell homing to the lymph node,[122] T cell self-renewal and differentiation,[123] T cell identity,[124] and T cell adhesion to endothelial cells in the lymph node.[125] Cytotoxic T cells have been shown

to express target cell death inducers, such as perforin 1 (PRF1), granzyme A (GZMA), and granzyme B (GZMB).[56,100] Also, Dysfunctional T cells in human tumors, such as non-small cell lung cancer (NSCLC), basal cell carcinoma (BCC), colorectal cancer (CRC), and hepatocellular carcinoma (HCC), are characterized by the expression of PD-1, CTLA-4, lymphocyte activation gene 3 protein (LAG3), and T cell immunoglobulin mucin receptor 3 (TIM3).[100,126] These proteins were reported to be responsible for T cell exhaustion and inactivation.[100,126]

Studies have found that, within the same lesions, tumor-reactive T cells are more likely to differentiate toward a dysfunctional state than bystander cells.[100] However, T cells with the same specificity for tumor antigens may exhibit varying degrees of dysfunctionality (exhaustion), and the presence of tumor-reactive T cells with low dysfunctionality may be crucial to generating a long-lasting response to ICIs.[100] Additionally, the high rate of proliferation of pre-dysfunctional T cells, in particular early-dysfunctional cells, as well as the expression of CXC chemokine ligand 13 (CXCL13), a B cell attractant, by those cells that progress toward a dysfunctional axis contribute to these findings that T cells with varying rates of dysfunction are different in functional capacity.[100] Therefore, these results suggest that dysfunctional $CD8^+$ T cells in human TME should not be considered inert cells but should also be viewed as T cells with novel functions.[120] Allison et al. conducted a study to investigate the role of $CD8^+$ T cells in responding to anti-CTLA-4/PD-1 antibodies.[113] The study showed that only a few subsets of $CD8^+$ T cells were expanded after treatment with both antibodies. To this, a phenotypically exhausted PD-1^{high} $TIM3^+$ population was found to expand most across all $CD8^+$ T cell populations. Moreover, the study looked at the relationship between $CD8^+$ T subsets and tumor growth. It was demonstrated that only two of the four tumor-infiltrating $CD8^+$ T subsets found in tumor models had a negative correlation with tumor growth, suggesting that only specific populations of tumor-infiltrating $CD8^+$ T cells have mediated response to immune-checkpoint inhibitors. The researchers concluded that the quantification of these phenotypically defined T cell subsets, rather than the assessment of bulk compartments, may be more useful in improving the predictive value for response to therapy.[113]

4 | THERAPEUTIC STRATEGIES FROM UNDERSTANDING THE TME

It has been found that patients with the same histological tumor stage (TNM staging) have significantly different clinical outcomes.[127] Therefore, a better classification category is needed to take into account the TME cellular and molecular content as well as tumor characteristics. A number of studies have shown that better prognostic value can be achieved by T cell infiltration rather than by classic tumor invasion criteria (e.g., grade, stage, and metastatic status).[128,129] As a result, a strong definition of patient stratification was found with the observation of the type, density, and location of immune cells within the tumor site of CRC that was more accurate than the classical TNM system for predicting patient survival.[130] This definition led to the development and implementation of Immunoscore, a standardized scoring system based on $CD3^+$ T cells (a pan-T-cell marker) and $CD8^+$ T cells (cytotoxic T-cell marker) quantification at the tumor center and the invasive margin.[131–133] The Immunoscore ranges from I0 to I4. I0 defines the low density of both cell types in both regions and is known as "cold" tumor, and I4 shows a high density of both cell types in both locations and is known as "hot" tumor.[128] Moreover, as Immunoscore is dependent on the infiltration of T cells into tumors,[133] any mechanism that directly or indirectly influences this process could also have an effect on this scoring system.[134] For example, PD-L1 expression, genomic instability or neoantigen load, and the presence of pre-existing antitumor immunity are some of these mechanisms.[134]

In addition to hot and cold tumors, Camus et al.[135] termed the third type of tumors based on their Immunoscore, "altered tumors." As a result, tumors have been categorized as hot, altered, and cold in terms of their immune profiles.[135] These profiles have enabled the aforementioned classification according to the balance between tumor escape and immune coordination.[135,136] The altered phenotype was further divided into "excluded" and "immunosuppressed" phenotypes.[135] The "excluded" phenotype reflects the presence of T cells at the invasive margin without infiltration ability into tumor, and therefore this phenotype suggests that the host immune system

is capable of effectively mounting a T cell-mediated immune response, but the tumor cells hinder T cell infiltration physically.[135] In comparison, the "immunosuppressed" phenotype refers to poor, if not absent, infiltration of T cells, thus suggesting that although there are no physical barriers to T cell infiltration, there is an immunosuppressive environment that limits further recruitment.[135] Immunoscore-based stratification is a powerful prognostic tool that recapitulates the complex interaction of multiple immune and nonimmune factors within the TME.[133] Therefore, an Immunoscore could have promising advantages in the clinical settings alongside TNM staging, including better patient stratification in terms of immunotherapy.[136,137]

Studies have shown that TILs and other immune cells presented in the TME along with tumor intrinsic factors such as TMB and PD-1/PD-L1 expression are directly related to cancer treatment response[36,138,139] (Tables 1 and 2). It has been shown that prognostic biomarkers that are effective in predicting clinical outcomes are administered by immunohistochemistry and gene analysis of the immune cells surrounding primary tumors and their microenvironment.[154] Basic histological quantification of density, cytotoxicity, and memory of the T lymphocytes with respect to CD3, CD8, and CD45RO expression have shown that increased T lymphocyte infiltration is correlated with statistically significant patients' disease-free survival (DFS) and OS improvement.[154] Taken together, there are various types of immunogenic TME characteristics that have been shown to have substantially different responses to immunotherapy.[155]

4.1 | Tumor mutational burden

Genetic aberrations, in particular somatic mutations, are one of the most common causes of cancer initiation and progression.[156,157] Genetic clones carrying somatic mutations can be identified at different rates across normal tissues and are often dependent on multiple factors, such as tissue exposure to environmental mutagens, natural architecture, rate of proliferation, and microenvironment.[156,157] Some of these clones may develop as a result of genetic drift, and some may result from positive selection induced by certain somatic events, which may represent the early stages of tumorigenesis.[158] There are two types of mutations that are thought to contribute toward the development of cancer: driver mutations and passenger mutations.[159] Driver mutations provide somatic cells with a fitness advantage in their microenvironment, leading the cell lineage to cancer, while passenger mutations do not offer such a proliferative benefit.[160] Some mutations in the DNA can result in the formation of antigens that are recognized and targeted by the immune system, and so-called "neoantigens."[161] The identification of mutations, commonly termed "tumor mutation burden" (TMB) has been used as a predictive biomarker.[162] TMB has been shown to associate with outcomes to immunotherapy.[162] Studies have shown that in patients with higher TMB, experience more durable clinical benefits from the ICIs because of the increased rate of immunogenicity.[163] This finding has been found in a variety of solid tumor types, including patients with small cell and NSCLC, melanoma, bladder, and HNSCC.[25] Given the importance of TMB, Rizvi et al. have analyzed the clinical and genomic data of 1662 advanced cancer patients treated with ICIs, and also 5371 non-ICI-treated patients, whose tumors underwent targeted NGS (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets [MSK-IMPACT]).[139] They have reported that a higher somatic TMB (top 20% within each histology) is associated with an improved OS for all patients.[139] A high TMB cutoff for breast cancer is 5.9 mut/Mb, for HNSCC is 10.3 mut/Mb, for NSCLC is 13.8 mut/Mb, for melanoma is 30.7 mut/Mb, and for CRC is 52.2 mut/Mb.[139] Additionally, Mandal et al.[153] examined the impact of a higher rate of microsatellite instability (MSI) as a result of TMB on the response of patients to ICIs.[153] As a result, higher T cell infiltration was found to be associated with higher microsatellite instability (MSI-H).[153] It should be noted that MSI is considered to be a form of genetic mutation resulting from the DNA MMR mechanism defect, and was the first FDA-approved biomarker used in clinical settings to identify patients who could benefit from anti-PD-1 therapy in CRC.[164]

In addition to TMB, the quality of the mutation should also be taken into account. Certain forms of mutations are more likely to generate an immunogenic response.[153] For example, Mandal et al.[153] have found that insertion-

TABLE 1 Cell types in the tumor microenvironment associated with outcome to immunotherapy

Biomarker	Function	Patients number	Tumor type	Results/interpretation
Neutrophil	Tumor promoter	83	Squamous cell carcinoma of the lung	Patients with NLR-low tumors had a better RFS and OS compared to those with NLR-high tumors. Patients with both NLR-low and PD-L1-negative tumors showed a favorable prognosis. Conclusion: The status of NLR may influence the prognostic impact of tumor PD-L1 expression.[140]
Mast cell	Tumor promoter	304	NPC	Patients with low expression levels of PD-L1, CD163, CXCR5, and CD117 (markers for immune checkpoint protein, M2 macrophage, T helper cell, and mast cell, respectively) had improved DMFS and PFS compared to patients with high expression levels of the markers. Conclusion: high expression levels of these immune markers, in particular CD117, are associated with poorer prognosis in NPC patients.[141]
MDSC	Tumor promoter	92	Metastatic melanoma	Nivolumab treatment did not affect the suppression function of the MDSC. Conclusion: MDSCs are associated with poor PFS and OS in patients.[142]
CAF	Tumor promoter Tumor suppressor	117	Melanoma	The PFS and OS had positive associations with $Thy1^+$ and FAP^+ CAFs, as well as negative associations with SMA^+ CAFs. Conclusion: multiplex CAF analysis could be a promising companion diagnostic tool in immuno-oncology.[143]
NK cell	Tumor suppressor	67	NSCLC	CD56 (a surface marker of NK cells) was found to be highly expressed in the CD45 compartment (leukocytes) and associated with favorable clinical outcomes. Conclusion: $CD56^+$ immune cell (NK cell) counts in stroma could be a predictive biomarker for PFS and OS in patients undergoing PD-1 axis blockade therapy.[144]
DC	Tumor suppressor	56 and 92	RCC and NSCLC	DC gene signature was significantly associated with improved OS in atezolizumab-treated patients. Conclusion: PD-L1 blockade could reinvigorate DC activity to improve the antitumor function of T cells.[145]
Macrophage	Tumor suppressor	487	NSCLC	Among multiple immune cells, macrophages were found to be the predominant immune cells with higher PD-L1 expression in both tumor and stroma. Also, PD-L1 expression in macrophages was associated with PD-L1 expression in the tumor cells, $CD8^+$ T cell infiltration, and improved OS. Conclusion: the correlation of PD-L1 expression in macrophages with OS provides new insight into the clinical significance of the antitumor effect of macrophages in patients undergoing PD-L1 blockade.[146]

TABLE 1 (Continued)

Biomarker	Function	Patients number	Tumor type	Results/interpretation
Treg	Tumor promoter	196	NSCLC	The negative association between OS and increased FoxP3$^+$ TI/S ratio was established, while the association between OS and increased CD8$^+$ TI/S ratio was positive. Patients with high FoxP3$^+$ islet infiltration and high CD3$^+$ and CD8$^+$ stromal counts had worse survival, whereas patients with high CD8$^+$ islet and FoxP3$^+$ stromal counts had better survival. Conclusion: a high FoxP3$^+$ TI/S ratio is associated with worse survival, while a high CD8$^+$ TI/S ratio is associated with better clinical outcomes.[147]
CD8$^+$ T cell	Tumor suppressor	137	Colon cancer	High CD8$^+$ TILs were significantly associated with OS. Conclusion: measuring CD8$^+$ TILs could be a promising method for stratifying the prognosis of stage III colon cancer.[148]

Abbreviations: CAF, cancer-associated fibroblast; CD8+ TIL, CD8+ tumor infiltrating lymphocyte; DC, dendritic cell; DMFS, distant metastasis-free survival; FoxP3, forkhead box protein P3; MDSC, myeloid-derived suppressor cell; NK cell, natural killer cell; NLR, neutrophil-to-lymphocyte ratio; NPC, nasopharyngeal carcinoma; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RCC, renal cell carcinoma; RFS, recurrence-free survival; TI/S, tumor islet:stroma; Treg, T regulatory cell.

TABLE 2 Biomarkers associated with outcome to immunotherapy

Biomarker	Patients number	Tumor type	Results/interpretation
TMB	NM	Anal cancer Biliary cancer Cervical cancer Endometrial cancer Salivary cancer Thyroid cancer Vulvar carcinoma Mesothelioma Neuroendocrine tumor SCLC	Based on the KEYNOTE-158 study, patients with TMB-H (≥10 mut/Mb) had a higher overall survival response rate compared to patients with TMB < 10 mut/Mb, following treatment with KEYTRUDA (pembrolizumab), Merck's anti-PD-1 therapy. The measurement of TMB was also carried out by FoundationOne assay. Conclusion: TMB-H patients with unresectable or metastatic solid tumors could benefit from KEYTRUDA, regardless of solid tumor type.[149]
Microbiome	112	Melanoma	Patients who responded to anti-PD-1 therapy were enriched with Clostridiales order and Ruminococcaceae family, while nonresponders were enriched with Bacteroidales family. In addition, patients with a high abundance of *Faecalibacterium* had prolonged PFS in comparison with patients with a low abundance. Also, patients with a high Bacteroidales abundance had a shortened PFS versus those with a low abundance. Conclusion: differentially abundant bacteria can stratify responders from nonresponders for ICIs. Also, the bacterial taxa within the gut microbiome of patients could be associated with the treatment outcomes.[150]
PD-L1 expression	386	HCC	There was a negative correlation between EZH2, an epigenetic modifier, with PD-L1 expression. The combination of EZH2 and PD-L1 expression had a prognostic role in both RFS and OS. Conclusion: EZH2 can directly suppress PD-L1 expression by upregulating H3K27me3 levels on the promoter of CD274 (encoding PD-L1) and IRF1, a transcription factor required for PD-L1 expression.[151]
Chromosome instability	100	NSCLC	Multiple genomic aberrations, including point mutations, chromosome deletions/duplications, and whole-genome doubling, were reported in patients involved in the TRACERx project. Conclusion: chromosome instability could be one of the potential drivers of tumor cells escaping from immune system detection.[152]
MSI	NM	Uterine/endometrial carcinomas Stomach adenocarcinoma CRC	There was a general trend toward increased immune infiltration in MSI-high tumors compared to MSI-low tumors. Conclusion: the genome-wide intensity of MSI and resultant tumor mutational load influence response to immunotherapy and tumor evolution in MMR-deficient tumors. [153]

Abbreviations: EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; H3K27me3, histone 3 lysine 27; HCC, hepatocellular carcinoma; IRF1, interferon regulatory factor 1; MMR, mismatch repair; MSI, microsatellite instability; mut/Mb, mutation/megabase; NM, not mentioned; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; SCLC, small cell lung cancer; TMB, tumor mutational burden; TMB-H, TMB-High; TRACERx, tracking cancer evolution through therapy.

deletion (indel) mutations cause a higher immunogenic response compared with missense mutations. There was an association between clinical response and indel mutational load in MMR-deficient gastrointestinal tumor patients, while there was no such association for missense mutational load.[153] For this, mutations in the MHC class-1-coding loci, as well as β2 microglobulin (B2M) and caspase-8 (CASP8) genes, have been shown to contribute to a better response to immunotherapy, while mutations in the interferon-gamma receptor (IFNGR) signaling pathway, such as tyrosine-protein kinase JAK1 (JAK1), JAK2, and apelin receptor (APLNR), have been found to lead to resistance to ICI therapy.[74] Also, specific HLA-I serotypes can also play a key role in response to therapy.[165] As an instance, Chowell et al.[165] have mentioned that HLA B44 and B62 serotypes have a significant association with the OS in ICI-treated melanoma patients. They have shown that patients with HLA B44 serotype had improved survival, while patients with HLA B62 serotype had worse survival.[165]

Overall, TMB has been shown to stratify patients receiving immunotherapies by response rate and survival.[166] Nevertheless, further research is required on the role of TMB as a predictive biomarker, and it is very important to identify mutations that contribute to the development of tumor-specific neoantigens and stimulation of the immune response.[32]

4.2 | Adoptive cellular therapy

In addition to ICIs, there is another immunotherapy approach used in clinical settings, known as ACT, which is cell-based therapy.[167] ACT is typically based on two forms: tumor-infiltrating T cells and chimeric antigen receptors (CARs).[167] In the first form, tumor-infiltrating T cells are isolated from the patient's tumor, expanded ex vivo, and then reinfused with T cell activating factors to increase the activity and survival of effector T cells.[167] This approach is widely used in patients after lymphodepletion to decrease immune suppression.[167] In the second form, autologous T cells are isolated and genetically engineered to express the intracellular domain of a T cell receptor fused to the antigen-binding domain of a B cell receptor.[168] The efficacy of this approach has been significant in hematological malignancy, but it remains to be fully understood in solid tumors.[168,169]

4.3 | TME: Inflammation gene signature

This type of TME contains TILs along with immunosuppressive immune cell populations and is likely to include some pancreatic, prostate, and BRCA-proficient breast tumors.[23] Tumors with this TME type present an environment in which the immunosuppressive interaction of PD-1–PD-L1 is less dominant, however, their response rate is not currently established.[170] For these tumors, an inflammatory TME promotes tumor growth and progression.[171] Nonetheless, two kinds of strategies will be effective for the treatment of patients with these tumor types: activate effector immune cells and alleviate immunosuppression induced by MDSCs, TAMs, and Tregs.[23,172,173]

4.3.1 | TME with the inflammation gene signature and elevated PD-L1 expression

Tumor cells that are surrounded by the TME with an inflammation gene signature and elevated PD-L1 expression are more likely to respond to ICIs.[174] These tumor cells are more common among melanomas and carcinomas of the lung, uterus, stomach, and cervix.[175] However, these tumor cells are likely to take advantage of the immune-evasive or immunosuppressive TME signaling pathways related to adaptive immune resistance, such as loss of tumor antigen expression, insensitivity to interferons or metabolites, and cytokine dysregulation, to reduce treatment efficacy; thus, additional possible targets for the treatment of these tumors are required.[23] In the

process of adaptive immune resistance, the expression of PD-L1 is increased in response to IFN-γ secretion in tumor cells and tumor-infiltrating immune cells.[170] Therefore, after cumulative interactions between PD-1 and PD-L1, a T cell dysfunction state is formed which is also referred to as T cell exhaustion.[176,177] Together, tumors with a TME highly enriched in $CD8^+$ T cells plus high levels of PD-L1 expression are more likely to respond to inhibition of PD-1/PD-L1.[23,24]

4.3.2 | TME without the inflammation gene signature

Tumor cells that are able to grow rapidly often develop hypoxic regions that contribute to aberrant angiogenesis and thus disrupt lymphocyte infiltration.[178] This event is the result of abnormalities in the distribution, branching and blood flow of the tumor vasculature, which can lead to heterogeneity depending on the availability of nutrients and oxygen across the tumor regions.[179] Additionally, tumor cells have the ability to modulate adhesive and chemotactic signaling pathways in the blood vessels to exclude T cells from their tissues,[23,180] TME with this characteristic can be resistant to cancer immunotherapy.[181] However, therapeutic combinations using T cells and NK cells are likely to have positive outcomes in these tumor types.[23]

4.3.3 | TME with low TMB and lack of the inflammation gene signature

Some tumors are characterized by low TMB and lack an inflammatory gene signature. This class can include tumors with either an immunosuppressed or an immune-excluded phenotype.[180] Pancreatic carcinomas, ovarian carcinomas, and microsatellite-stable (MSS) CRCs are specific to this type of TME.[23] The lack of a detectable immune response in these tumors can imply the inefficient antigen presentation and priming of an adaptive immune response.[94,170] Initiating an adaptive immune response to tumor antigens demands that they are taken up by APCs and introduced to naive T cells with cognate T cell receptors.[182] Hence, tumors with this microenvironment are likely to have defects related to infiltration of APCs into the tumor tissue.[23] As such, patients with these types of tumors are likely to experience the poorest outcomes when undergoing immunotherapy.[23]

5 | CONCLUSION

Immunotherapies have been hailed as a “game-changer” over the last number of years. However, only a subset of patients appears to respond to therapy. Tumors are complex ecosystems consisting of various types of cells that communicate with each other. One of the challenges in the field has been identifying predictive biomarkers of response to therapy. An intricate understanding of the TME may aid in a greater understanding of the tumor–immune cell interactions which in turn can predict response to immunotherapy. Distinct sets of stroma, epithelium, and immune cell types provide almost a myriad of ways to interpret TME, however, it remains unclear how many cellular combinations help a rapidly growing tumor to proliferate.

TME originates primarily from different signaling pathways, either cytoprotective or cytotoxic, in malignant stroma, endothelial, and immune cells. Multiple factors (e.g., tumor types, TMB, and immune cell infiltration) have been shown to play a significant role in the heterogeneity of the TME and therefore the immune system response. Consideration of the relationship between both these patients' intrinsic and tumor-dependent effects at different levels will be critical to improving the efficacy of existing immunotherapeutic approaches. Thus, it is of great value to have a deep insight into the heterogeneity of TME within each type of cancer, as well as to understand the functions of the different factors involved in the biological crosstalk of the tumor–the host interface. Such knowledge will contribute to the development of better therapeutic strategies, not to mention the prediction of

clinical response to treatment. In this regard, multiplexed sequencing and imaging platforms that provide in-situ and spatial information on various immune and nonimmune factors within the TME could bring us closer to the goal of developing more effective cancer immunotherapy.

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