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REVIEW

Understanding the tumor microenvironment in head and neck squamous cell carcinoma

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Abstract

Head and neck squamous cell carcinoma (HNSCC) represents a heterogeneous group of tumors. While significant progress has been made using multimodal treatment, the 5-year survival remains at 50%. Developing effective therapies, such as immunotherapy, will likely lead to better treatment of primary and metastatic disease. However, not all HNSCC tumors respond to immune checkpoint blockade therapy. Understanding the complex cellular composition and interactions of the tumor microenvironment is likely to lead to new knowledge for effective therapies and treatment resistance. In this review, we discuss HNSCC characteristics, predictive biomarkers, factors influencing immunotherapy response, with a focus on the tumor microenvironment.

Keywords: biomarkers, head and neck squamous cell carcinoma, human papillomavirus, immune checkpoint inhibitors, immunotherapy, tumor microenvironment

INTRODUCTION

Head and neck cancer squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) is the 7th most common cancer in the world, and it accounts for more than 1.5% of cancer deaths in the United States.[1,2] HNSCC tumors are found in the oral/nasal cavity, paranasal sinuses, nasopharynx, larynx and oropharynx. Tobacco, alcohol consumption, both independently and synergistically, and human papillomavirus (HPV) are known risk factors.[3–6] HPV-positive oropharyngeal SCC (OPSCC) is often susceptible to therapy, leading to a better prognosis, whereas HPV-negative HNSCC tends to have unfavorable prognosis.[7] Surgery and chemoradiotherapy are the standard treatment modalities for HNSCC. However, with locoregional or distant metastatic disease, the prognosis is often poor. Immunotherapies have shown promise for recurrent and metastatic HNSCC. However, determining which patients are likely to respond

to immune checkpoint blockade therapy remains a challenge.

In a study evaluating the efficacy of standard fractionated radiotherapy with cisplatin for locally advanced HNSCC patients, patients with HPV-positive OPSCC had smaller primary tumors and better survival than patients with HPV-negative tumors.[8] Phase III clinical trials comparing an epidermal growth factor receptor (EGFR) inhibitor, cetuximab/radiotherapy and cisplatin/radiotherapy, revealed that in HPV-positive tumors, cisplatin/radiotherapy had better treatment outcomes and improved patient survival than cetuximab/radiotherapy. Transoral surgery has also achieved good outcomes and is standard of care for appropriate HPV-positive tumors.[9] In the tumor microenvironment (TME), HPV-positive tumors demonstrate an increased number of infiltrated natural killer (NK) cells[10] and HPV-positive OPSCC have shown a higher degree of infiltrating $CD3^+$ and $CD8^+$ T cells than HPV-negative tumors.[11]

TUMOR MICROENVIRONMENT (TME)

The TME is a heterogeneous milieu of cell types, including immune and non-immune cells, that surrounds the tumor[12] (Figure 1). There are broadly three types of tumor phenotypes, characterised by the TME, and defined by the cell type, density and location: inflamed, immune-excluded and immune-desert tumors[13] (Figure 2). Inflamed tumors are defined when immune cells infiltrate the tumor and the stroma.[14] Immune-excluded is a phenotype that occurs when immune cells are restricted to the stroma and are unable to infiltrate the tumor. The immune-desert phenotype arises when immune cells, specifically $CD8^+$ T cells, are incapable of infiltrating neither the tumor nor the stroma.[15] In non-inflamed tumors, tumor immune escape originates from the exclusion of T cells by various oncogenic pathways such as p53 inactivation, NOTCH1 inactivation and epigenetic regulations[16] (Table 1). In patients with HPV-positive tumors, those with high levels of tumor-infiltrating lymphocytes (TILs) have better outcomes.[17] Higher infiltration rate of TILs correlates with greater production of interleukin (IL)-10, C-C motif chemokine 21 (CCL21), IL-17, CCL17, tumor necrosis factor alpha (TNF-α), IL-21 and interferon gamma (IFN-γ), hence revealing an HPV-specific T-cell response that enables better overall survival (OS) in HPV-positive HNSCCs.[18] The balance of antitumor cells versus immunosuppressive cells within the TME is key for treatment outcomes and survival. A TME with high infiltration of cytotoxic T cells and NK cells results in better therapy outcomes, whereas TME with regulatory T cells (Tregs), M2 macrophages and myeloid-derived suppressor cells (MDSCs) results in poorer therapy outcomes.[19] Studies have shown that the composition and abundance of immune cells differ between HPV-positive and HPV-negative tumors.[20,21]

CELLULAR FACTORS IN THE TME

Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are activated fibroblasts within the TME.[22] Fibroblast activation protein-alpha (FAP-α) and alpha-smooth muscle chain (α-SMA) are specific markers to purify CAFs.[23] CAFs play a critical role in tumor evolution by producing collagen fibrils in the extracellular matrix (ECM), eventually increasing invasiveness of HNSCC tumor cells.[24] Within the TME, CAFs are stimulated by angiotensin II (Ang-II) via their receptor, angiotensin II receptor type I (ATR1), to proliferate and secrete immunosuppressive factors.[25] In addition, CAFs have the ability to suppress $CD8^+$ T-cell function by increasing the expression levels of tumor growth factor β (TGF β).[26]

Myeloid-derived suppressor cells (MDSCs)

Myeloid-derived suppressor cells (MDSCs) are activated and expanded immature myeloid cells detected in pathologic conditions like cancer, autoimmune diseases, chronic inflammation and trauma.[27] MDSCs are categorised into two groups including polymorphonuclear MDSCs, and monocytic MDSCs which morphologically bear striking resemblance to neutrophils and monocytes.[27] MDSCs could promote the formation of CAFs, tumor-associated macrophages (TAMs) and Tregs within the TME. Having been formed, Tregs produce transforming growth factor beta (TGF-β), IL-10 and adenosine, thereby suppressing T cells (helper and cytotoxic).[22] MDSCs can also suppress $CD8^+$ T cells by secreting prostaglandin E2 (PGE2) and arginase (ARG). MDSCs were found to be related to a tolerogenic tumor immune landscape. In this regard, IL-1, IL-6 and granulocyte/monocyte colony-stimulating factor

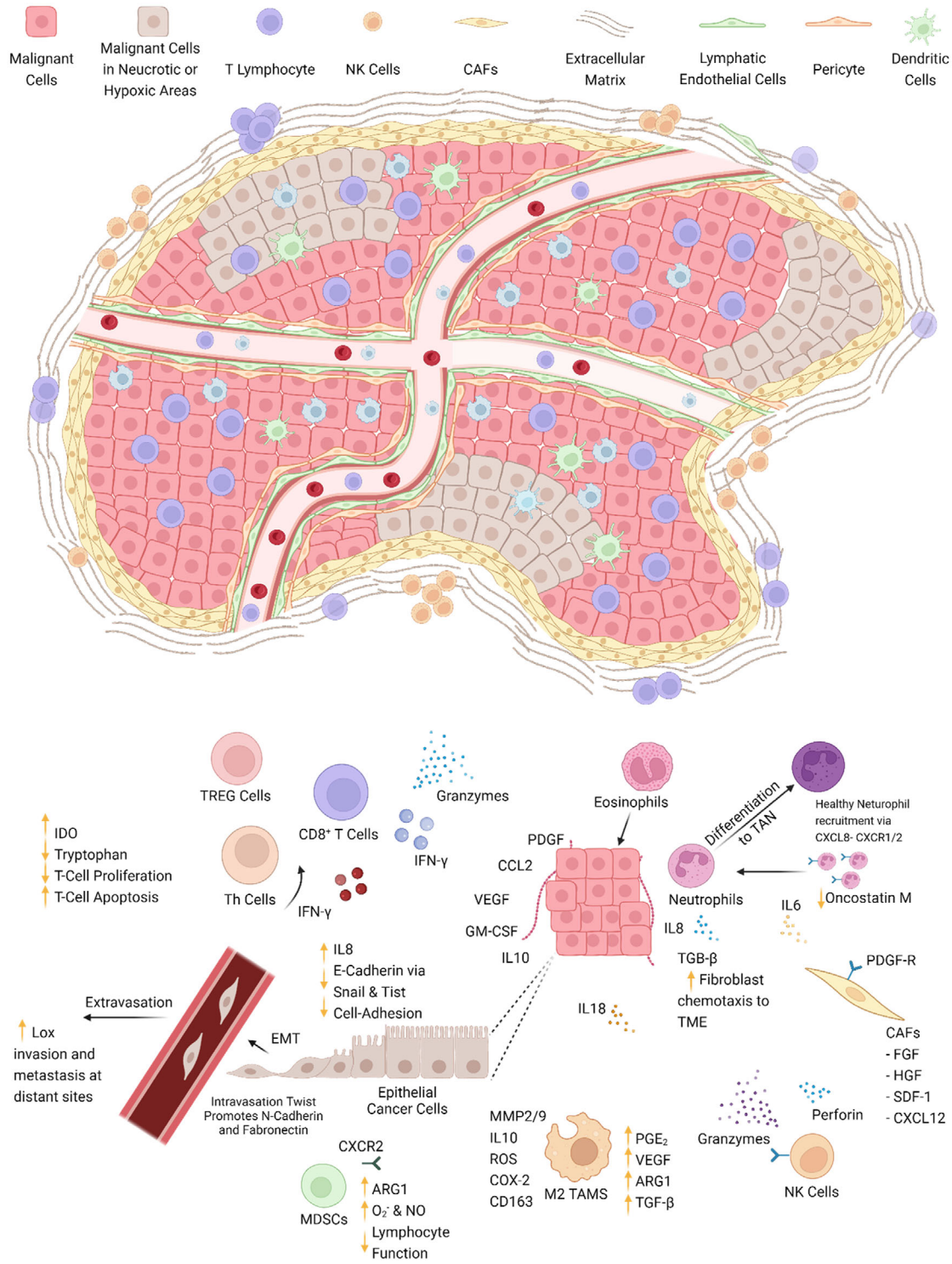

Figure 1. Immune cells in the tumor microenvironment and their interactions. Cell populations within the TME promote or suppress tumor growth by secreting various cytokines and chemokines. $CD4^+$ T cells differentiate into Th cells, which act as tumor suppressors, and Tregs, which act as tumor promoters. TANs promote tumor growth by secreting ECM remodelling enzymes and angiogenic factors. CAFs play an immunosuppressive role by limiting $CD8^+$ T-cell function via TGF secretion. NK cells have tumor suppressing functions by producing perforin and granzymes. TAMs promote tumor growth via increasing the levels of MMPs. By secreting ARG1, MDSCs suppress tumor specific $CD8^+$ T-cell response. Adapted from Barriga *et al.*[130] and Balkwill *et al.*[131]

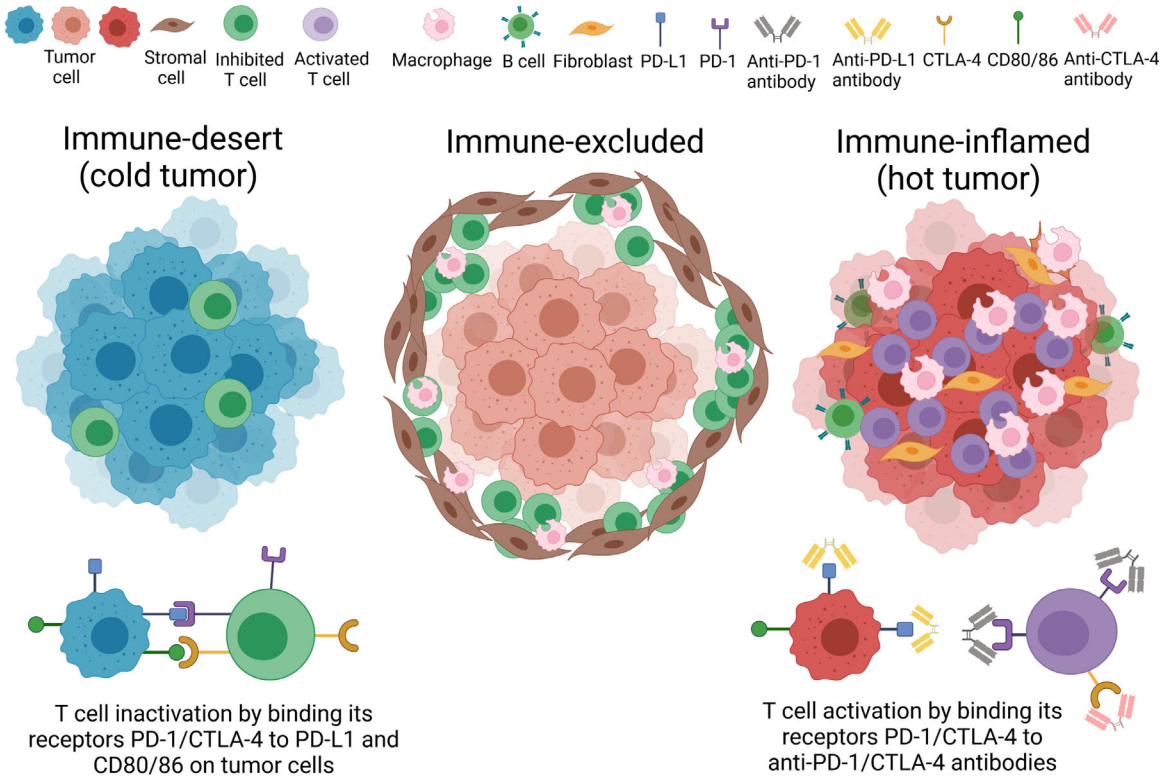

Figure 2. The characteristics of different types of tumor microenvironments. There are three types of the TMEs, including immune-desert, immune-excluded and immune-inflamed. In the immune-desert TME, T cells are not able to infiltrate neither the tumor nor the stroma, and are inactivated by binding their inhibitory cell surface receptors PD-1 and CTLA-4 to ligands CD80 and CD86 on the tumor cells, this environment is referred to as a 'cold tumor'. Immune-excluded TME occurs when immune cells, specifically T cells, can be found in the stroma but are unable to infiltrate the tumor. In immune-inflamed TME, various types of immune cells, particularly activated T cells, can infiltrate the tumor, creating a so-called 'hot tumor' environment. Immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1/CTLA-4, block the connection between T and tumor cells, causing T cells to reactivate.

Table 1. The most common genes involved in HNSCC

Gene	Cytogenetic location	Mutation type	Function in	Role
TP53	17p13.1	MissenseAllelic loss	DNA damage	TSG
NOTCH1	9q34.3	Inactivating mutation	Signal transduction pathways	TSG
PIK3CA	3q26.32	AmplificationActivating mutation	Signal transduction pathways	Oncogene
FAT1	4q35.2	Inactivating mutationDeletion	Cell-cell connectionActin dynamics	TSG
HRAS	11p15.5	Activating mutation	Signal transduction pathways	Oncogene
CDKN2A	9p21.3	Loss of function	Cell cycle	TSG
NSD1	5q35.3	Inactivating mutation	Epigenetic regulation	TSG
KMT2D	12q13.12	Inactivating mutation	Epigenetic regulation	TSG

CDKN2A, cyclin-dependent kinase inhibitor 2A; FAT1, FAT atypical cadherin 1; HRAS, HRas proto-oncogene, GTPase; KMT2D, lysine methyltransferase 2D; NOTCH1, notch receptor 1; NSD1, nuclear receptor binding SET domain protein 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TP53, tumor protein p53; TSG, tumor suppressor gene.
Data from Cancer Genome Atlas Network,[122] India Project Team of the International Cancer Genome Consortium,[123] Leemans *et al.*[124] and Chai *et al.*[125]

(GM-CSF) expression by M2 TAMs, TANs and tumor cells resulted in the recruitment of MDSCs into the TME in HNSCC.[28]

Tumor-associated macrophages (TAMs)

Macrophages play a part in both innate and adaptive immunity, dividing into two groups

including M1-like and M2-like macrophages.[29,30] TNF-α and lipopolysaccharides (LPS) induce forming M1 macrophages, showing antitumoral effects by producing IL-1β, IL-6 and C-X-C motif chemokine 10 (CXCL10).[29,31] The M2 phenotype, however, could induce inflammation and also promote tumor growth and angiogenesis through producing TGFβ, matrix metalloproteinases (MMPs), vascular endothelial growth factors (VEGFs) and interleukins such as IL-4 and IL-10.[29,32] Both TAMs M1 and M2 can be detected using CD68 immunostaining. Despite this, Singhal *et al*. found no exclusive M1 or M2 macrophage markers in lung cancer. Instead, the study found that the tumors expressed both M1 and M2 macrophage markers, implying that macrophage differentiation is a continuum rather than two distinct states.[33] Chen *et al*. discovered two types of TME in HNSCC: one with the presence of B cells and M1 macrophages, which was linked to better immunotherapy outcomes, and the other with WNT/TGF-signalling activation and the presence of M2 TAMs, which was linked to tumor development.[34,35]

$CD8^+$ T cells

$CD8^+$ T cells, a key player in the acquired immune system, express T-cell receptors (TCRs), allowing them to recognise peptides presented by major histocompatibility complex 1 (MHC-I).[36] After being exposed to an antigenic peptide, naïve T cells undergo massive clonal expansion and differentiation to become potent effector cells, also known as cytotoxic T cells (CTLs).[37] CTLs kill tumor cells either through the release of cytotoxic mediators or stimulation of first apoptosis signal receptor ligand (FasL)-mediated apoptosis.[38] Three different functional states including naïve, cytotoxic and dysfunctional of tumor-infiltrating lymphocytes (TILs) have been revealed using high dimensional profiling technologies.[39–41] These cells may show various degrees of exhaustion; however, TILs with decreased effector function may play a key role in providing long-lasting immune responses to ICIs.[42] Higher $CD8^+$ T-cell infiltration was found to be associated with a better response to anti-PD-1/PD-L1 antibodies in patients with cutaneous head and neck cancer.[43] It was found that OS and relapse-free survival (RFS) have a positive correlation with higher numbers of $CD4^+$ and $CD8^+$ TILs.[44] Studies showed the effect of TILs on patient survival. Vassilakopoulou *et al*. reported that stromal TILs were associated with OS, whereas Badr *et al*. found that intraepithelial TILs were correlated with clinical outcomes.[45,46]

Tissue-resident memory T cells

The tumor infiltration of tissue-resident memory T cells (Trm), as detected by $CD103^+CD8^+$ T cells, has a positive correlation with a favorable prognosis in patients with various types of cancer.[20,47] Patients with Trms infiltration into TME had better responses to immunotherapy.[47] Ida *et al*. investigated the biological and clinical significance of Trm in head and neck cancer using RNA-seq data from The Cancer Genome Atlas (TCGA) and blood samples taken from patients. The team found that Trm-enriched tumors overexpressed immune checkpoint molecules and had a correlation with HPV-positive status. Also, patients with Trm-enriched tumors had a better prognosis.[47]

Regulatory T cells (Tregs)

$CD4^+$ T cells are key players in the adaptive immune response through secreting various chemokines.[48,49] MHC-II on the surface of antigen-presenting cells (APCs) mediates antigen presentation and $CD4^+$ T-cell activation, leading to a role in allergy, autoimmunity and cancer, as well as an immunosuppressive environment in the TME.[49,50] $CD4^+$ T cells can turn into Tregs, expressing the forkhead box P3 (FOXP3) protein, which is required for development and immunosuppressive function of Tregs. Tregs contribute to tumor growth thanks to their inhibitory role.[50,51] It was reported that tumor-infiltrating Tregs can express surface molecules such as PD-L1 and PD-L2 in order to bind their receptors on the surface of $CD8^+$ T cells, inhibiting $CD8^+$ T-cell activation.[52] In addition, Tregs could also suppress tumor specific T-cell infiltration and function by secreting IL-10 and TGF-β, leading to inhibition of antitumor immune responses.[53] A higher amount of TGF-β was reported during the latter phase of HNSCC progression, indicating a disruption in the T-helper (Th)-17 vs Treg ratio.[54] The disruption enhances Treg differentiation as well as IL-10 production in HNSCCs.[55,56] However, increased $FoxP3^+$ Treg infiltration in HNSCC was related to improved RFS and OS, implying an antitumor immune response that could result in tumor progression suppression.[57,58]

Tumor-associated neutrophils (TANs)

Neutrophils make up the majority of white blood cells and are recruited to the TME by a variety of cytokines and chemokines.[59] These cells are categorised into two phenotypes: antitumor (N1) and pro-tumor (N2).[59] N1 TANs are characterised by the upregulation of TNF-α, CD54 and CCL3, whereas N2 TANs show increased levels of CCL2, 3, 4, 8, 12 and 17 as well as CXCL2, 8 and 16.[59] Functionally, N1 TANs have antitumor functions through direct cytotoxicity or stimulating innate and adaptive immune cells such as B and T cells, NK cells and dendritic cells (DCs), thereby inhibiting tumor growth.[59] Furthermore, by increasing NADPH oxidase, these cells produce reactive oxygen species (ROS), which are potentially toxic to tumor cells.[59] N2 TANs produce ECM remodelling enzymes and angiogenic factors, promoting tumor growth.[59]

Natural killer (NK) cells

NK cells are characterised as $CD3^-/CD56^+$ cells.[60,61] NK cells function as an antitumor immune system by either producing perforin/granzyme B (GZMB) or inducing FasL/TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.[60,61] These cells regulate immune responses, including T-cell expansion and Th1 polarisation, via targeting activated T cells and secreting IFN-γ.[60,61] Additionally, NK cells play a variety of roles in both innate and adaptive immune responses through the activation of DCs, macrophages, neutrophils and T cells by secreting a wide range of cytokines and chemokines, such as IFN-γ, TNF-α and GM-CSF.[60,62] HNSCC-infiltrating NK cells were found to express significantly less killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and KIR2DL1/2/3 than circulating NK cells.[63] Furthermore, it was shown that, while mature CD56 dim NK cells constitute the majority of NK cells in patients with head and neck cancer, an immature CD56 bright, CD16dim/negative subset lacking CD57 expression is also found in HNSCC tumors.[63–65] However, NK cell infiltration, in particular CD56dim, was reported to improve disease-free survival (DFS) and OS regardless of HPV status.[21,66]

Dendritic cells (DCs)

DCs are antigen-presenting cells that regulate T-cell functions by sending four distinct signals: primary signals to initiate T-cell activation, secondary signals to complete T-cell activation, T-cell differentiation signals and activating signals for T-cell homing to specific tissues.[67,68] Conventional DCs (cDCs) activate antitumor immune responses as either a tumor antigen-presenting cell or a cytokine secretor.[69] There are two types of cDCs, cDC1s and cDC2s.[67] In the TME, cDC1s recruit and stimulate $CD8^+$ T cells to fight tumor cells. cDC1 also secrete IL-12 to support T-cell function.[70] Several studies have found that the cDC1 signature in the TME is associated with a higher tumor-infiltrating lymphocyte quantitation score and improved patient survival.[71–73] Bottcher *et al*. reported that a cDC1 signature composed of four genes, including C-type lectin domain containing 9A (CLEC9A), X-C motif chemokine receptor 1 (XCR1), cytokine-dependent hematopoietic cell linker (CLNK) and basic leucine zipper ATF-like transcription factor 3 (BATF3), was linked to improved survival in patients with head and neck, breast, lung and metastatic melanoma.[73]

NON-CELLULAR FACTORS IN THE TME

Interferon-gamma (IFN-γ)

In the TME, a variety of immune cells such as activated lymphocytes, CD4 T helper type (TH1), CD8 and NK cells secrete IFN-γ. It was found that all nucleated cells respond to IFN-γ because they express IFN-γ receptor (IFNGR1).[74] However, IFN-γ can act as a double-edged sword in the TME because of its anti- and pro-tumorigenic effects, depending on the balance of antitumor and pro-tumor IFN signalling.[74] The KEYNOTE-012 HNSCC trial examined a six-gene IFN-γ signature (including CXCL9, CXCL10, signal transducer and activator of transcription 1 (STAT1), human leukocyte antigens, DR alpha (HLA-DRA), IFN-γ and indoleamine 2,3-dioxygenase 1 (IDO1) gene expression) in pretreatment biopsies to assess the relationship between interferons and response to ICIs.[75] A significant association was identified between IFN-γ gene signature and best overall response (BOR) and progression-free survival (PFS).[75]

The antitumorigenic effects of IFN-γ contribute to the recruitment of various immune cells in the TME via transcriptional regulation of CXCL 9, CXCL10 and CXCL11, and their receptor CXCR3.[76] Immunotherapy was indicated to induce IFN-γ expression and thus promote the expansion of

effector and memory $CD8^+$ T cells.[77,78] IFN-γ drives CTL chemotaxis and motility within the TME, increasing CTL cytotoxicity and limiting tumor growth.[79] IFN-γ promotes tumorigenesis by inducing the expression of IDO1, inducible nitric oxide synthases (iNOS), PD-L1 and the FasL.[74] IFN-γ triggers IDO1 expression, which contributes to T-cell apoptosis, as IDO1 has been shown to play an important role in catalysing the kynurenine pathway and, as a result, activating caspase 8, as well as releasing mitochondrial cytochrome C.[80] In terms of iNOS, it was found that tumor-derived iNOS triggers tumor cell angiogenesis and vascularisation, resulting in tumor growth.[81] Furthermore, tumor cells have been found to suppress immune response via FASL-mediated apoptosis of immune effector cells.[74]

Hypoxia

When tumor cells proliferate, they gradually deplete oxygen and other nutrients, resulting in tumor hypoxia.[82] By upregulating hypoxia-inducible factors (HIFs) like VEGF, tumor cells overcome this challenge.[83] Tumor angiogenesis and neovascularisation differ structurally and functionally from normal angiogenesis, with tumor vessels having blunt ends and poor perfusion.[12] Tumor endothelial cells have numerous gaps, which contribute to vascular leakage, blood clots and tissue oedema when compared to normal endothelial cells.[82] Overexpression of hypoxic pathway mediators, such as HIF-α and HIF1β, has been related to the tumor progression because these mediators bind hypoxia response elements engaged in tumor angiogenesis.[12] Tumor hypoxia is a common feature of locally advanced HNSCC that is considered as a negative prognostic factor, leading to decreasing radiotherapy efficacy.[84,85] This means that a hypoxic environment reduces the production of ROS, which reduces radiation-induced DNA damage and makes these cells resistant to radiotherapy.[86]

Adrenergic neurons

The underlying mechanisms of tumor-neuron interaction are not clearly comprehended; however, this may drive tumor innervation and invasion in the TME of various solid tumors.[87] Trp53 knockout mice demonstrated increased nerve density (neuritogenesis) in mucosal oral cavity squamous cell carcinoma (OCSCC) tumors caused by loss of p53 expression and regulated by tumor-derived microRNA-laden extracellular vesicles.[88] Extracellular vesicle-delivered miR-21 and miR-324 were found to induce neuritogenesis, whereas extracellular vesicle-delivered miR-34a suppressed neuritogenesis.[88] It has been found that neurons innervating p53-deficient OCSCC tumors arises by trans-differentiation of trigeminal sensory nerve fibres to adrenergic nerve fibres, which is associated with higher expression of neuron reprogramming transcription factors such as achaete-scute homolog 1 (ASCL1), kruppel-like factor 4 (KLF4) and POU domain, class 5, transcription factor 1 (POU5F1).[88] Markers of adrenergic neuron in OCSCC samples are heavily linked to poor outcomes, highlighting the relevance of these results to cancer. Understanding the adrenergic nature of neurons that drives tumor growth gives patients with OCSCCs hope for treatment options. Available beta-adrenergic blockers have already been approved to treat patients with migraines, angina, heart arrhythmias and hypertension.[87] According to clinical research data, anti-adrenergic agents could be considered as therapeutic options for patients with breast cancer and hepatocellular carcinoma.[89,90] Amit *et al*.[88] showed that Carvedilol, an α1, β1 and β2 adrenergic receptor blocker, significantly decreased tumor progression and proliferation. Therefore, more emphasis might be placed on anti-adrenergic approaches in the treatment of OCSCC.

IMMUNOTHERAPY

Immunotherapy reinvigorates patients' immune responses against tumor cells, causing them to regress[12] (Table 2). Immune checkpoint inhibitors (ICIs) and adoptive cellular therapy (ACT) are the most common immunotherapy approaches used in clinical studies. ICI makes use of cell surface molecules like anti-programmed cell death 1 (PD-1), while ACT employs host immune cells such as tumor-infiltrating T cells.[91] The development of immunotherapy has significantly improved the treatment of HNSCC. For example, anti-PD-1 antibodies (pembrolizumab and nivolumab), have demonstrated long-term responses. These immunotherapeutic agents were found to provide durable responses and improved survival in recurrent/metastatic (R/M) HNSCC patients who had previously received platinum-based

Table 2. Predictive biomarkers of response to immunotherapy

Biomarkers	Type	Therapy	Significance	Ref.
PD-L1 expression	Staining assays	Immunotherapy	indicator of response to ICIs	126
TMB	WES	Immunotherapy	Plays a role in T-cell activation	43
GEP (IFN-γ gene expression profile)	WES	Immunotherapy	Is predictive of response to pembrolizumab	127
MSI	DNA (PCR)	Immunotherapy	It is related to durable complete response to PD-L1 inhibitor	128
Microbiota	NGS	Immunotherapy	It is associated with the efficacy of CTLA-4 blockade	129
ML	WES	Immunotherapy	Is predictive of response to pembrolizumab	127

GFP, gene expression profile; ICIs, immune checkpoint inhibitors; ML, mutation loads; MSI, microsatellite instability; NGS, next-generation sequencing; PD-L1, programmed cell death ligand 1; WES, whole exome sequencing.

chemotherapy, and were approved by the US Food and Drug Administration (FDA) in 2016.[75,92,93] The long-term follow-up studies confirmed pembrolizumab's safety, durability, efficacy and improvement in survival for R/M HNSCC.[94,95] Biomarkers of response to immunotherapy, including PD-L1 expression, tumor mutational burden (TMB) and T-cell inflammatory gene signature, have been discovered through the analysis of combined tissue samples from the KEYNOTE-012 and KEYNOTE-055 trials.[92,96] Each of these biomarkers independently predicted response to Pembrolizumab, which led to its approval as standard first-line treatment. The FDA, also, approved Nivolumab for HNSCC patients based on the findings of the CHECKMATE 141 trial.[92,97] Nivolumab was found to improve response rate, OS and 6-month PFS in platinum-pretreated patients.[97] Despite these promising results, response rates to ICIs in HNSCC patients were reported to be between 13 and 20%, indicating the need for novel predictive biomarkers of response to immunotherapy for these patients.[98]

PD-L1 expression

PD-1 checkpoint receptor expressed on the surface of activated T cells was found to have an immunosuppressive role when interacting with its ligands (PD-L1 and PD-L2) expressed on the surface of tumor- and immune-infiltrating cells.[99–101] The expression of PD-L1 on the surface of immune cells in pretreatment tumor biopsies was found to be associated with a better antitumor adaptive immune response and, as a result, better treatment outcomes.[102,103] Anti-PD-1/PD-L1 antibodies are used to block the interaction of PD-1/PD-L1 and thus promote the immune response against tumor growth.[104,105] Studies showed that the expression of PD-L1 on the surface of tumor cells, also known as tumor proportional score (TPS), was linked to better clinical outcomes and improved survival in patients who received an anti-PD-1 antibody.[97,106] However, it was found that the assessment of PD-L1 expression on both tumor and immune cells (lymphocytes and macrophages) together, known as the combined positive score (CPS), could be a better predictor of immunotherapy response than the TPS.[107] A study comparing the efficacy of first-line pembrolizumab to CPS < 1, 1–19 and ≥ 20 in R/M HNSCC patients discovered a link between increased efficacy and increased PD-L1 expression.[108] However, there are some concerns about PD-L1 expression as a predictive biomarker of response, such as inter-/intra-tumor heterogeneity, differences in 'cut-offs', and the antibody clones used for staining.[107] A study on 28 HNSCC patients found that the 1% cut-off had 36% and 52% concordance with TPS and CPS, while the 50% cut-off had 70% and 55% concordance with TPS and CPS, respectively.[109] In another study comparing the differences between three different PD-L1 staining assays (the Ventana SP263 assay used for Durvalumab (anti-PD-L1) trials, the Dako 28–8 assay used for Nivolumab (Opdivo®) trials, and the Dako 22C3 assay used for Pembrolizumab (Keytruda®) trials), it was shown that the overall per cent agreement was $> 90\%$.[110]

Tumor mutation burden (TMB)

Neo-epitopes caused by non-synonymous mutations in tumor cells' DNA, known as 'tumor mutation burden' (TMB), were found to have a significant impact on the immune system recognition and, in particular, T-cell activation.[111,112] Although tumors with a high frequency of missense mutations are more likely to respond to immunotherapy because of an increased number of infiltrating $CD8^+$ T cells, only a small number of these mutations contribute to

neo-antigen production, and only a small proportion of those neo-antigens may result in T-cell recognition and reactivity.[113,114] However, evidence suggests that only immunogenic mutations, rather than all mutational load, are associated with improved survival and increased immune exhaustion marker expression.[115] TMB was found to be a promising predictive biomarker of response to immunotherapy. The association of TMB with improved response to PD-1 blockade and anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) antibody, as well as a better clinical outcome, was reported in patients with non-small cell lung cancer (NSCLC), and patients with melanoma.[116–118] In the case of HNSCC, the KEYNOTE-012 study found a link between the total mutational load and response to immunotherapy.[75,119] In a study of 126 HNSCC patients treated with anti-PD-1/PD-L1 antibodies, responders had higher TMB and microsatellite instability (MSI) than non-responders.[43] Rizvi *et al*. investigated the relationship between TMB and clinical outcomes in 1662 patients with various tumor types using NGS, Memorial Sloan Kettering Cancer Center-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT).[120] According to the study, patients with higher TMB (top 20% within each histology) had a better OS. The study, also, showed that different tumor types had different TMB cut-offs. A high TMB cut-off was defined as 10.3 mutations per megabase (mut/Mb) for HNSCC, 5.9 mut/Mb for breast cancer, 13.8 mut/Mb for NSCLC, 30.7 mut/Mb for melanoma and 52.2 mut/Mb for colorectal cancer (CRC).[120]

FUTURE PERSPECTIVES

While immunotherapies has been found to be effective in a number of solid malignancies, only a subset of patients may benefit from therapy. It has been found that the TME, which includes distinct cell types and originates from different signalling pathways, plays an important role in tumor immune cell interactions. The TME is established when both immune and non-immune cells congregate around tumor cells to help with tumor growth, proliferation and development. Although the presence of tumor-infiltrating lymphocytes may be a prognostic factor for cancer treatment outcome, different subgroups of other immune and non-immune cells perform important functions. The absence or decreased expression of CD3, failure to destroy tumor cells, imbalances in cytokine production, and decreased responses to mitogens are some key features of the TME resident immune cells. There are several other factors in the TME, including PD-L1 expression on tumor and immune cells, tumor mutation burden and immune cell infiltration, as well as microbiome and adrenergic neurons, which play significant roles in the prediction of response to immunotherapy. Therefore, gaining a better understanding of immune-tumor cell interactions, as well as gene and protein expression within the TME, paves the way for the development of more effective immunotherapeutic strategies. Moreover, a deeper insight into the TME heterogeneity in each tumor type, as well as tumor-host biological crosstalk, will lead to more precise personalised medicine.[121] A multi-omic genomic/proteomic readout combined with spatial phenotyping yield from spatial profiling technologies may aid in understanding TME phenotypes associated with therapy response.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Habib Sadeghi Rad: Conceptualization; Investigation; Visualization; Writing – original draft; Writing – review & editing. **Yavar Shiravand:** Writing – original draft. **Payar Radfar:** Visualization; Writing – original draft. **Rahul Ladwa:** Conceptualization; Writing – review & editing. **Chris Perry:** Conceptualization; Writing – review & editing. **Xiaoyuan Han:** Writing – review & editing. **Majid Ebrahimi Warkiani:** Conceptualization; Writing – review & editing. **Mark N Adams:** Conceptualization; Writing – review & editing. **Brett GM Hughes:** Conceptualization; Writing – review & editing. **Ken O'Byrne:** Conceptualization; Writing – review & editing. **Arutha Kulasinghe:** Conceptualization; Funding acquisition; Investigation; Project administration; Writing – original draft; Writing – review & editing.

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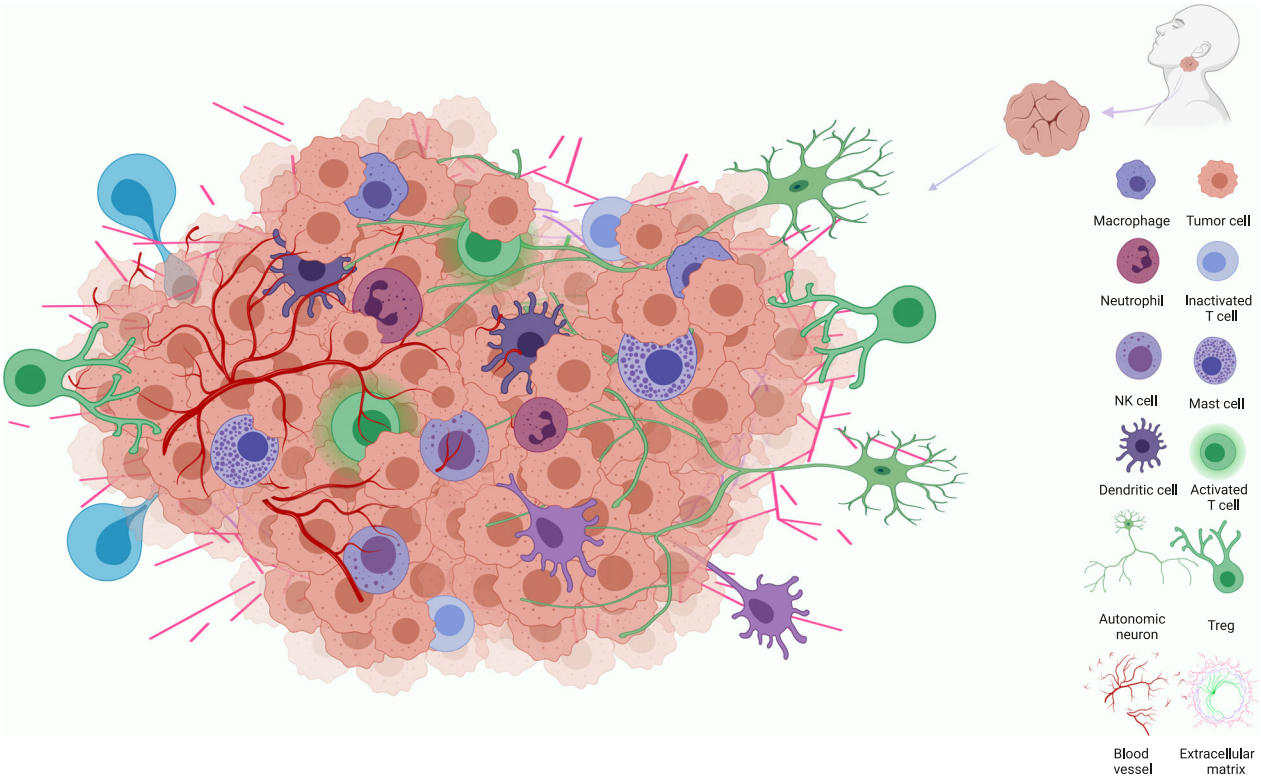
Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main.



This manuscript describes the tumor microenvironment of head and neck cancers that is integral for understanding effective therapies.