Understanding the tumor microenvironment in head and neck squamous cell carcinoma

Habib Sadeghi Rad1, Yavar Shiravand2, Payar Radfar3, Rahul Ladwa1,4, Chris Perry1,4, Xiaoyuan Han5, Majid Ebrahimi Warkiani3,6, Mark N Adams7, Brett GM Hughes1,8, Ken O’Byrne4,7 & Arutha Kulasinghe1

1University of Queensland Diamantina Institute, the University of Queensland, Brisbane, QLD, Australia
2Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy
3School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW, Australia
4Princess Alexandra Hospital, Brisbane, QLD, Australia
5Department of Biomedical Science, University of the Pacific, Arthur A. Dugoni School of Dentistry, Stockton, CA, USA
6Institute of Molecular Medicine, Sechenov First Moscow State University, Moscow, Russia
7Centre for Genomics and Personalised Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
8Royal Brisbane and Women’s Hospital, Brisbane, QLD, Australia

Correspondence
A Kulasinghe, Translational Research Institute, University of Queensland, 37 Kent Street, Woolloongabba, QLD 4102, Australia.
E-mail: arutha.kulasinghe@uq.edu.au

Received 6 January 2022; Revised 11 March and 5 May 2022; Accepted 19 May 2022
doi: 10.1002/cti2.1397

Clinical & Translational Immunology 2022; 11: e1397

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) cells10 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 tumor12 (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 tumors13 (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 regulations16 (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.\textsuperscript{130} and Balkwill et al.\textsuperscript{131}
GM-CSF expression by M2 TAMs, TANs and tumor cells resulted in the recruitment of MDSCs into the TME in HNSCC. 28

Tumor microenvironment of head and neck cancer

Table 1. The most common genes involved in HNSCC

| Gene     | Cytogenetic location | Mutation type      | Function in                           | Role            |
|----------|----------------------|--------------------|---------------------------------------|-----------------|
| TP53     | 17p13.1              | Missense Allelic 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

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 reanimate.

Tumor-associated macrophages (TAMs)
Macrophages play a part in both innate and adaptive immunity, dividing into two groups

(GM-CSF) expression by M2 TAMs, TANs and tumor cells resulted in the recruitment of MDSCs into the TME in HNSCC. 28
including M1-like and M2-like macrophages.\textsuperscript{29,30} TNF-\(\alpha\) and lipopolysaccharides (LPS) induce forming M1 macrophages, showing antitumoral effects by producing IL-1\(\beta\), IL-6 and C-X-C motif chemokine 10 (CXCL10).\textsuperscript{29,31} The M2 phenotype, however, could induce inflammation and also promote tumor growth and angiogenesis through producing TGF\(\beta\), matrix metalloproteinases (MMPs), vascular endothelial growth factors (VEGFs) and interleukins such as IL-4 and IL-10.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).\textsuperscript{36} After being exposed to an antigenic peptide, naive T cells undergo massive clonal expansion and differentiation to become potent effector cells, also known as cytotoxic T cells (CTLs).\textsuperscript{37} CTLs kill tumor cells either through the release of cytotoxic mediators or stimulation of first apoptosis signal receptor ligand (FasL)-mediated apoptosis.\textsuperscript{38} Three different functional states including naive, cytotoxic and dysfunctional of tumor-infiltrating lymphocytes (TILs) have been revealed using high dimensional profiling technologies.\textsuperscript{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 ICs.\textsuperscript{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.\textsuperscript{43} It was found that OS and relapse-free survival (RFS) have a positive correlation with higher numbers of CD4\(^+\) and CD8\(^+\) TILs.\textsuperscript{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.\textsuperscript{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.\textsuperscript{20,47} Patients with Trms infiltration into TME had better responses to immunotherapy.\textsuperscript{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.\textsuperscript{47}

**Regulatory T cells (Tregs)**

CD4\(^-\) T cells are key players in the adaptive immune response through secreting various chemokines.\textsuperscript{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.\textsuperscript{49,50} CD4\(^+\) T cells can turn into Tregs, expressing the forkehead box P3 (FOXP3) protein, which is required for development and immunosuppressive function of Tregs. Tregs contribute to tumor growth thanks to their inhibitory role.\textsuperscript{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.\textsuperscript{52} In addition, Tregs could also suppress tumor specific T-cell infiltration and function by secreting IL-10 and TGF-\(\beta\), leading to inhibition of antitumor immune responses.\textsuperscript{53} A higher amount of TGF-\(\beta\) was reported during the latter phase of HNSCC progression, indicating a disruption in the T-helper (Th)-17 vs Treg ratio.\textsuperscript{54} The disruption enhances Treg differentiation as well as IL-10 production in HNSCCs.\textsuperscript{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.\textsuperscript{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. These cells are categorised into two phenotypes: antitumor (N1) and pro-tumor (N2). 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 ICI.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 CXCL9, 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. IFN-γ drives CTL chemotaxis and motility within the TME, increasing CTL cytotoxicity and limiting tumor growth. IFN-γ promotes tumorigenesis by inducing the expression of IDO1, inducible nitric oxide synthases (iNOS), PD-L1 and the FasL. 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. In terms of iNOS, it was found that tumor-derived iNOS triggers tumor cell angiogenesis and vascularisation, resulting in tumor growth. Furthermore, tumor cells have been found to suppress immune response via FASL-mediated apoptosis of immune effector cells.

Hypoxia

When tumor cells proliferate, they gradually deplete oxygen and other nutrients, resulting in tumor hypoxia. By upregulating hypoxia-inducible factors (HIFs) like VEGF, tumor cells overcome this challenge. Tumor angiogenesis and neovascularisation differ structurally and functionally from normal angiogenesis, with tumor vessels having blunt ends and poor perfusion. Tumor endothelial cells have numerous gaps, which contribute to vascular leakage, blood clots and tissue oedema when compared to normal endothelial cells. 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. Tumor hypoxia is a common feature of locally advanced HNSCC that is considered as a negative prognostic factor, leading to decreasing radiotherapy efficacy. This means that a hypoxic environment reduces the production of ROS, which reduces radiation-induced DNA damage and makes these cells resistant to radiotherapy.

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. 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. Extracellular vesicle-delivered miR-21 and miR-324 were found to induce neuritogenesis, whereas extracellular vesicle-delivered miR-34a suppressed neuritogenesis. 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). 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. According to clinical research data, anti-adrenergic agents could be considered as therapeutic options for patients with breast cancer and hepatocellular carcinoma. Amit et al. 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 (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. 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. However, evidence suggests that only immunogenic mutations, rather than all mutational load, are associated with improved survival and increased immune exhaustion marker expression. 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. In the case of HNSCC, the KEYNOTE-012 study found a link between the total mutational load and response to immunotherapy. 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, and 30.7 mut/Mb for melanoma and 52.2 mut/Mb for colorectal cancer (CRC). 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.

ACKNOWLEDGMENTS
AK is supported by an NHMRC Fellowship (APP1157741) and Cure Cancer (APP1182179). KOB is supported by the Princess Alexandra Hospital Foundation (PARF).

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. Anutha Kulasinghe: Conceptualization; Funding acquisition; Investigation; Project administration; Writing – original draft; Writing – review & editing.

REFERENCES
1. Siegel R, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2018; 2018: 7–30.
2. Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 2020; 313.
3. Mourad M, Jetmore T, Jategaonkar AA, Moubayed S, Mosher E, Urken ML. Epidemiological trends of head and neck cancer in the United States: a SEER population study. J Oral Maxillofac Surg 2017; 75: 2562–2572.

4. Fitzmaurice C, Allen C, Barber RM et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol 2017; 3: 524–548.

5. Mohebbi E, Nooromohamadi Z, Sadeghi-Rad H et al. Low viral load of Merkel cell polyomavirus in Iranian patients with head and neck squamous cell carcinoma: is it clinically important? J Med Virol 2018; 90: 344–350.

6. Kulasinghe A, Perry C, Jovanovic L, Nelson C, Punyadeera C. Circulating tumour cells in metastatic head and neck cancers. Int J Cancer 2015; 136: 2515–2523.

7. Sabatini ME, Chiocca S. Human papillomavirus as a driver of head and neck cancers. Br J Cancer 2020; 122: 306–314.

8. Nguyen-Tan PF, Zhang Q, Ang KK et al. Randomized phase III trial to test accelerated versus standard fractionation in combination with concurrent cisplatin for head and neck carcinomas in the Radiation Therapy Oncology Group 0129 trial: long-term report of efficacy and toxicity. J Clin Oncol 2014; 32: 3858.

9. Kim C, Martinez E, Kulich M, Swanson MS. Surgeon practice patterns in transoral robotic surgery for HPV-related oropharyngeal cancer. Oral Oncol 2021; 121: 105460.

10. Charap AJ, Enokida T, Brody R et al. Landscape of natural killer cell activity in head and neck squamous cell carcinoma. J Immunother Cancer 2020; 8: e001523.

11. Koneva LA, Zhang Y, Virani S et al. HPV integration in HNSCC correlates with survival outcomes, immune response signatures, and candidate drivers. Mol Cancer Res 2018; 16: 90–102.

12. Sadeghi Rad H, Monkman J, Warkiani ME et al. Understanding the tumor microenvironment for effective immunotherapy. Med Res Rev 2021; 41: 1474–1498.

13. Rad HS, Rad HS, Shiravand Y et al. The Pandora’s box of novel technologies that may revolutionize lung cancer. Lung Cancer 2021; 159: 34–41.

14. Kalita-de Croft P, Sadeghi Rad H, GASPER H, O’Byrne K, LAKHANI SR, Kulasinghe A. Spatial profiling technologies and applications for brain cancers. Expert Rev Mol Diagn 2021; 21: 323–332.

15. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017; 541: 321–330.

16. Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumour immune responses. Nat Rev Cancer 2018; 18: 139–147.

17. Ward MJ, Thirdborough SM, Mellows T et al. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. Br J Cancer 2014; 110: 489–506.

18. Bhat AA, Yousuf P, Wani NA et al. Tumor microenvironment: an evil nexus promoting aggressive head and neck squamous cell carcinoma and avenue for targeted therapy. Signal Transduct Target Ther 2021; 6: e12.

19. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. Mol Cancer 2019; 18: 63.

20. Partlová S, Bouček J, Kloudová K et al. Distinct patterns of intratumoral immune cell infiltrates in patients with HPV-associated compared to non-virally induced head and neck squamous cell carcinoma. Oncoimmunology 2015; 4: e965570.

21. Mandal R, Şenbabaoğlu Y, Desrichard A et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight 2016; 1: e98929.

22. Denaro N, Merlano MC, Lo Nigro C. Further understanding of the immune microenvironment in head and neck squamous cell carcinoma: implications for prognosis. Cancer Manag Res 2021; 13: 3973.

23. Mishra PJ, Mishra PJ, Humeniuk R et al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res 2008; 68: 4331–4339.

24. Ortiz-Otero N, Clinch AB, Hope J, Wang W, Reinhart-King CA, King MR. Cancer associated fibroblasts confer shear resistance to circulating tumor cells during prostate cancer metastatic progression. Oncotarget 2020; 11: 1037.

25. Xie G, Cheng T, Lin J et al. Local angiostatin II contributes to tumor resistance to checkpoint immunotherapy. J Immunother Cancer 2018; 6: e88.

26. Chen W, Ten Dijke P. Immunoregulation by members of the TGFβ superfamily. Nat Rev Immunol 2016; 16: 723–740.

27. Yang Y, Li C, Liu T, Dai X, Bazhin AV. Myeloid-derived suppressor cells in tumors: from mechanisms to antigen specificity and microenvironmental regulation. Front Immunol 2020; 11: 1371.

28. Allen CT, Judd NP, Bui JD, Uppaluri R. The clinical implications of antitumor immunity in head and neck cancer. Laryngoscope 2012; 122: 144–157.

29. Evrard D, Szurz P, Tijeras-Raballand A et al. Macrophages in the microenvironment of head and neck cancer: potential targets for cancer therapy. Oral Oncol 2019; 88: 29–38.

30. Kumar AT, Knops A, Swendsen B et al. Prognostic significance of tumor-associated macrophage content in head and neck squamous cell carcinoma: a meta-analysis. Front Oncol 2019; 9: 656.

31. Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application. J Hematol Oncol 2017; 10: e58.

32. Pathria P, Louis TL, Varner JA. Targeting tumor-associated macrophages in cancer. Trends Immunol 2019; 40: 310–327.

33. Singhal S, Stadanlick J, Annunziata MJ et al. Human tumor-associated monocytes/macrophages and their regulation of T cell responses in early-stage lung cancer. Sci Transl Med 2019; 11: eaat1500.

34. Chen Y-P, Wang Y-Q, Lv J-W et al. Identification and validation of novel microenvironment-based immune molecular subgroups of head and neck squamous cell carcinoma: implications for immunotherapy. Ann Oncol 2019; 30: 68–75.
35. Gavrielatou N, Doumas S, Economidou P, Foukas PG, Psyrri A. Biomarkers for immunotherapy response in head and neck cancer. Cancer Treat Rev 2020; 84: 101977.

36. Hashimoto M, Kamphorst AO, Im SJ et al. CD8 T cell exhaustion in chronic infection and cancer: opportunities for interventions. Annu Rev Med 2018; 69: 301–318.

37. Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. J Virol 2004; 78: 5535–5545.

38. Williams MA, Bevan MJ. Effector and memory CTL differentiation. Annu Rev Immunol 2007; 25: 171–192.

39. Tirosi I, Ikar B, Prakadan SM et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science 2016; 352: 189–196.

40. Li H, van der Leun AM, Yofe I et al. Dysfunctional CD8 T cells form a proliferative, dynamically regulated compartment within human melanoma. Cell 2019; 176: 775–789 e718.

41. Sade-Feldman M, Yizhak K, Bjorgaard SL et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. Cell 2018; 175: 998–1013 e1020.

42. Van der Leun AM, Thommen DS, Schumacher TN. CD8 T cell states in human cancer: insights from single-cell analysis. Nat Rev Cancer 2020; 20: 218–232.

43. Hanna GJ, Lizotte P, Cavanaugh M et al. Frameshift events predict anti-PD-1/L1 response in head and neck cancer. JCI Insight 2018; 3: e98811.

44. Nguyen N, Bellille E, Thomas D et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. Head Neck 2016; 38: 1074–1084.

45. Vassilakopoulou M, Avgeris M, Velchiti V et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. Clin Cancer Res 2016; 22: 704–713.

46. Badr M, Jöhnrens K, Allgäuer M et al. Morphomolecular analysis of the immune tumor microenvironment in human head and neck cancer. Cancer Immunol Immunother 2019; 68: 1443–1454.

47. Ida S, Takahashi H, Kawabata-Iwakawa R, Mito I, Tada H, Chikamatsu K. Tissue-resident memory T cells correlate with the inflammatory tumor microenvironment and improved prognosis in head and neck squamous cell carcinoma. Oral Oncol 2021; 122: 105508.

48. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy—new insights into old paradigms. Cancer Gene Ther 2021; 28: 5–17.

49. Borst J, Ahrends T, Babala N, Melief CJ, Kastenmüller W. CD4+ T cell help in cancer immunology and immunotherapy. Nat Rev Immunol 2018; 18: 635–647.

50. Konopacki C, Pritykin Y, Rubtsov Y, Leslie CS, Rudensky AY. Transcription factor Foxp1 regulates Foxp3 chromatin binding and coordinates regulatory T cell function. Nat Immunol 2019; 20: 232–242.

51. Marshall EA, Ng KW, Kung SHY et al. Emerging roles of T helper 17 and regulatory T cells in lung cancer progression and metastasis. Mol Cancer 2016; 15: e67.

52. De Simone M, Arrigoni A, Rossetti G et al. Transcriptional landscape of human tissue lymphocytes unveils uniqueness of tumor-infiltrating T regulatory cells. Immunity 2016; 45: 1135–1147.

53. Ahrends T, Borst J. The opposing roles of CD 4+ T cells in anti-tumour immunity. Immunology 2018; 154: 582–592.

54. Maggioni D, Pignataro L, Garavello W. T-helper and T-regulatory cells modulation in head and neck squamous cell carcinoma. Oncoimmunology 2017; 6: e1325066.

55. Yu L, Yang F, Zhang F et al. CD69 enhances immunosuppressive function of regulatory T-cells and attenuates colitis by prompting IL-10 production. Cell Death Dis 2018; 9: e905.

56. de Medeiros MC, Baerjee R, Liu M, Anovazzi G, D’Silva NJ, Junior CR. HNSCC subverts PBMCs to secrete soluble products that promote tumor cell proliferation. Oncotarget 2017; 8: 60860–60874.

57. de Ruiter EJ, Ooft ML, Devries LA, Willems SM. The prognostic role of tumor infiltrating T-lymphocytes in squamous cell carcinoma of the head and neck: a systematic review and meta-analysis. Oncoimmunology 2017; 6: e1356148.

58. Seminerio I, Descamps G, Dupont S et al. Infiltration of FoxP3+ regulatory T cells is a strong and independent prognostic factor in head and neck squamous cell carcinoma. Cancers (Basel) 2019; 11: 227.

59. Masucci MT, Minopoli M, Carriero MV. Tumor associated neutrophils. Their role in tumorigenesis, metastasis, prognosis and therapy. Front Oncol 2019; 9: 1146.

60. Chioussone L, Dumas P-Y, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol 2018; 18: 671–688.

61. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. Nat Immunol 2016; 17: 1025–1036.

62. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. Front Immunol 2018; 9: 1869.

63. Habif G, Crinier A, André P, Vivier E, Narni-Mancinelli E. Targeting natural killer cells in solid tumors. Cell Mol Immunol 2019; 16: 415–422.

64. Wagner S, Witekkindt C, Reuschenbach M et al. CD56-positive lymphocyte infiltration in relation to human papillomavirus association and prognostic significance in oropharyngeal squamous cell carcinoma. Int J Cancer 2016; 138: 2263–2273.

65. Weil S, Memmer S, Lechner A et al. Natural killer group 2D ligand depletion reconstitutes natural killer cell immunosurveillance of head and neck squamous cell carcinoma. Front Immunol 2017; 8: 387.

66. Lu J, Chen X-M, Huang H-R et al. Detailed analysis of inflammatory cell infiltration and the prognostic impact on nasopharyngeal carcinoma. Head Neck 2018; 40: 1245–1253.

67. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krmähl MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol 2020; 20: 7–24.

68. Kalinski P, Talmadge JE. Tumor immuno-environment in cancer progression and therapy. Tumor immune microenvironment in cancer progression and cancer therapy. Adv Exp Med Biol 2017; 1036: 1–18.
69. Binnewies M, Mujal AM, Pollack JL et al. Unleashing type-2 dendritic cells to drive protective antitumor CD4+ T cell immunity. Cell 2019; 177: 556–571 e516.

70. Krishnaswamy JK, Gowthaman U, Zhang B et al. Migratory CD11b+ conventional dendritic cells induce T follicular helper cell-dependent antibody responses. Sci Immunol 2017; 2: eaam9169.

71. Verneau J, Sauté-Fridman C, Sun C-M. Dendritic cells in the tumor microenvironment: prognostic and thanaromic impact. Semin Immunol 2020; 48: e101410.

72. Broz M, Binnewies M, Boldajipour B et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell 2014; 26: 638–652.

73. Böttcher JP, Bonavita E, Chakravarty P et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. Cell 2018; 172: 1022–1037 e1014.

74. Gocher AM, Workman CJ, Vignali DA. Interferon-γ: teammate or opponent in the tumour microenvironment? Nat Rev Immunol 2021; 22: 158–172.

75. Seiwert TY, Burtness B, Mehra R et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase Ib 1b trial. Lancet Oncol 2016; 17: 956–965.

76. Colvin RA, Campanella GS, Sun J, Luster AD. Intracellular domains of CXCR3 that mediate CXCL9, CXCL10, and CXCL11 function. J Biol Chem 2004; 279: 30219–30227.

77. Stoycheva D, Deiser K, Stoycheva D, et al. Inhibition of IFN-γ mediates CD8+ T-cell differentiation and survival in response to weak, but not strong, TCR signals. J Immunol 2015; 194: 553–559.

78. Pedicord VA, Montalvo W, Leiner IM, Allison JP. Single dose of anti-CTLA-4 enhances CD8+ T-cell memory formation, function, and maintenance. Proc Natl Acad Sci USA 2011; 108: 266–271.

79. Bhat P, Leggatt G, Waterhouse N, Frazer IH. Interferon-γ derived from cytotoxic lymphocytes directly enhances their motility and cytotoxicity. Cell Death Dis 2017; 8: e2836.

80. Fallarino F, Grohmann U, Vacca C et al. T cell apoptosis by tryptophan catabolism. Cell Death Differ 2002; 9: 1069–1077.

81. Park S-Y, Seol J-W, Lee Y-J et al. IFN-γ enhances TRAIL-induced apoptosis through IRF-1. Eur J Biochem 2004; 271: 4222–4228.

82. Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. Nat Rev Mol Cell Biol 2020; 21: 268–283.

83. Barr MP, Gray SG, Gately K et al. Vascular endothelial growth factor is an autocrine growth factor, signaling through neuropilin-1 in non-small cell lung cancer. Mol Cancer 2015; 14: e45.

84. Mihayachi S; Kim SS, Pang J et al. Immune modulation of head and neck squamous cell carcinoma and the tumor microenvironment by conventional therapeutics. Clin Cancer Res 2019; 25: 4211–4223.

85. Nordmark M, Bentzen SM, Rudat V et al. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 2005; 77: 18–24.

86. Bristow RG, Hill RP. Hypoxia and metabolism: hypoxia, DNA repair and genetic instability. Nat Rev Cancer 2008; 8: 180–192.

87. Hunt PJ, Amit M. Head and neck cancer exosomes drive microRNA-mediated reprogramming of local neurons. Extracell Vesicles Circ Nucl Acids 2020; 1: 57.

88. Amit M, Takahashi H, Dragomir MP et al. Loss of p53 drives neuron reprogramming in head and neck cancer. Nature 2020; 578: 449-454.

89. Childers WK, Hollenbeak CS, Cheriyath P. β-blockers reduce breast cancer recurrence and breast cancer death: a meta-analysis. Clin Breast Cancer 2015; 15: 426–431.

90. Grytli HH, Fagerland MW, Fossa SD, Taskén KA. Association between use of β-blockers and prostate cancer-specific survival: a cohort study of 3561 prostate cancer patients with high risk or metastatic disease. Eur Urol 2014; 65: 635–641.

91. Hirsch FR, McElhinny A, Stanforth D et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. J Thorac Oncol 2017; 12: 208–222.

92. Chow LQ. Head and neck cancer. N Engl J Med 2020; 382: 60–72.

93. Chow LQM, Haddad R, Gupta S et al. Antitumor activity of pembrolizumab in biomarker-unselected patients with recurrent and/or metastatic head and neck squamous cell carcinoma: results from the phase Ib KEYNOTE-012 expansion cohort. J Clin Oncol 2016; 34: 3838.

94. Mehra R, Seiwert TY, Gupta S et al. Efficacy and safety of pembrolizumab in recurrent/metastatic head and neck squamous cell carcinoma: results from a single-arm, phase II study. J Clin Oncol 2017; 35: 1542.

95. Baum J, Seiwert TY, Pfister DG et al. Pembrolizumab for platinum-and cetuximab-refractory head and neck cancer: results from a single-arm, phase II Study. J Clin Oncol 2017; 35: 1542.

96. Seiwert TY, Haddad R, Baum J et al. Pembrolizumab for head and neck squamous cell carcinoma. Cancer Res 2018; 78(13 suppl): LB339. https://doi.org/10.1158/1538-7445.AM2018-LB-339.

97. Ferris RL, Blumenschein G, Fayette J et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016; 375: 1856–1867.

98. Sim F, Leidner R, Bell RB. Immunotherapy for head and neck cancer. Hematol Oncol Clin North Am 2019; 33: 301–321.

99. Freeman GJ, Long AJ, Iwai Y et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000; 192: 1027–1034.

100. Dong H, Strome SE, Salomao DR et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793–800.
Tumor microenvironment of head and neck cancer

101. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/PD-L1 pathway to activate anti-tumor immunity. *Cur Opin Immunol* 2012; 24: 207–212.

102. Tumeh PC, Harvie CL, Yearley JH et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; 515: 568–571.

103. Herbst RS, Soria J-C, Kowanetz M et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; 515: 563–567.

104. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; 366: 2443–2454.

105. Brahmer JR, Tykodi SS, Chow LQM et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; 366: 2455–2465.

106. Cohen EEW, Soulières D, Le Tourneau C et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. *Lancet* 2019; 393: 156–167.

107. Cohen EEW, Bell RB, Bifulco CB et al. The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of squamous cell carcinoma of the head and neck (HNSCC). *J Immunother Cancer* 2019; 7: e184.

108. Burtness L, Rischin D, Greil R et al. Abstract LB-258: Efficacy of first-line (1L) pembrolizumab by PD-L1 combined positive score (<1, 1–19, and ≥20 in recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC): KEYNOTE-048 subgroup analysis. *Cancer Res* 2020. https://doi.org/10.1158/1538-7445.AM2020-LB-258

109. Rasmussen JH, Lelkaitis G, Hapkisson K et al. Intradatum heterogeneity of PD-L1 expression in head and neck squamous cell carcinoma. *Br J Cancer* 2019; 120: 1003–1006.

110. Ratcliffe MJ, Sharpe A, Rebelatto M et al. A comparative study of PD-L1 diagnostic assays in squamous cell carcinoma of the head and neck (SCCHN). *Ann Oncol* 2016; 27: vi330.

111. Robbins PF, Lu Y-C, El-Gamil M et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med* 2013; 19: 747.

112. Linnemann C, van Buuren MM, Bies L et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat Med* 2015; 21: 81–85.

113. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015; 348: 69–74.

114. Sadeghi Rad H, Bazaz SR, Monkman J et al. The evolving landscape of predictive biomarkers in immuno-oncology with a focus on spatial technologies. *Clin Transl Immunology* 2020; 9: e1215.

115. Brown SD, Warren RL, Gibb EA et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res* 2014; 24: 743–750.

116. Rizvi NA, Hellmann MD, Snyder A et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; 348: 124–128.

117. Van Allen EM, Miao D, Schilling B et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015; 350: 207–211.

118. Snyder A, Makarov V, Merghoub T et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; 371: 2189–2199.

119. Cristescu R, Mogg R, Ayers M et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based immunotherapy. *Science* 2018; 362: eaar3593.

120. Samstein RM, Lee C-H, Shoushtari AN et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019; 51: 202–206.

121. Kulasinghe A, Taheri T, O’Byrne K, Hughes BG, Kenny L, Punyadeera C. Highly multiplexed digital spatial profiling of the tumor microenvironment of head and neck squamous cell carcinoma patients. *Front Oncol* 2020; 10: 607349.

122. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015; 517: 576.

123. India Project Team of the International Cancer Genome Consortium, Maitra A, Biswas NK et al. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. *Nat Commun* 2013; 4: 2873.

124. Leemans CR, Snijders PJ, Brakenhoff RH. The molecular landscape of head and neck cancer. *Nat Rev Cancer* 2018; 18: 269–282.

125. Chai AWY, Lim KP, Cheong SC. Translational genomics and recent advances in oral squamous cell carcinoma. *Semin Cancer Biol* 2020; 61: 71–83.

126. Chen SC, Chang PMH, Wang HJ, Tai SK, Chu PY, Yang MH. PD-L1 expression is associated with p16INK4A expression in non-opharyngeal head and neck squamous cell carcinoma. *Onco Lett* 2018; 15: 2259–2265.

127. Haddad RI, Seiwert TY, Chow LQM et al. Genomic determinants of response to pembrolizumab in head and neck squamous cell carcinoma (HNSCC). *J Clin Oncol* 2017; 35(15 Suppl): 6009.

128. Balermpas P, Rödel F, Krause M et al. The PD-1/PD-L1 axis and human papilloma virus in patients with head and neck cancer after adjuvant chemoradiotherapy: a multicentre study of the German Cancer Consortium Radiation Oncology Group (DKTK-ROG). *Int J Cancer* 2017; 141: 594–603.

129. Vétizou M, Pitt JM, Daillère R et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015; 350: 1079–1084.

130. Barriga V, Kuol N, Nurgali K, Apostolopoulos V. The complex interaction between the tumor micro-environment and immune checkpoints in breast cancer. *Cancers (Basel)* 2019; 11: 1205.

131. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci* 2012; 125: 5591–5596.
This manuscript describes the tumor microenvironment of head and neck cancers that is integral for understanding effective therapies.