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REVIEW ARTICLE

Current Advances in Immuno-oncology for Head and Neck Cancer

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ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer globally, originating from the epithelial surface of the upper aerodigestive tract from the lips to the larynx. It commonly presents with locally advanced disease, with a recurrence rate of around 50% despite aggressive multimodality treatment involving surgery, radiotherapy and chemotherapy or EGFR inhibition as appropriate. Improvements in understanding the underlying cancer biology and its evolution within the complex interactions of the tumor microenvironment, there is gathering interest in and evidence for the use of immunomodulating agents in the management of HNSCC. Immune checkpoint inhibitors, primarily programmed cell death protein 1 (PD-1) inhibitors to date, which inhibit the inhibitory interaction between PD-1 and its ligand PD-L1, have demonstrated durable improvements in patient outcomes in advanced/metastatic HNSCC, with both nivolumab and pembrolizumab being granted FDA approval in 2016.

There are numerous clinical trials ongoing exploring the role of checkpoint inhibitors both as single agents and in combination, administered with established modalities such as chemotherapy and radiotherapy, as well as alongside other novel immune modulators. These trials are not limited to advanced/metastatic HNSCC, but also explore neoadjuvant or adjuvant settings. As studies complete and more data become available, immunotherapy agents are likely to have expanding roles within the treatment algorithms of HNSCC, and with greater biomarker development have the potential to further improve patient outcomes via a personalized therapy approach.

Keywords: Cancer immunology, Cancer immunotherapy, Head and neck cancer, Head and neck squamous cell carcinoma, Immune checkpoint inhibitors, SCCHN

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INTRODUCTION

Head and neck cancers encompass malignancies that originate in the paranasal sinuses, nasal cavity, oral cavity, pharynx and larynx. With more than 550,000 cases diagnosed worldwide each year and around 300,000 deaths, it is the sixth leading cancer by incidence.[1]

Over 90% are squamous cell carcinoma (SCCHN), with a male predominance. Certain lifestyle and environmental risk factors, such as alcohol consumption and smoking, have long been established as risk factors for the development of SCCHN with a multiplicative risk for patients who are regularly exposed to both.[2] More recently human papillomavirus (HPV) type 16 has emerged as a driver for a significant proportion of oropharyngeal squamous cell carcinomas, with more uncertainty over its implication in other sites, and for other subtypes of the virus.[3]. This HPV driven subtype is associated with an epidemiology, local pattern of disease spread and outcome that is quite separate from non-HPV associated disease and as such it is increasingly being regarded as a distinct clinical entity.[4-6]

Despite aggressive primary multimodality treatment involving surgery, radiotherapy, chemotherapy and, where appropriate EGFR inhibition, locally advanced SCCHN has a recurrence rate of ~50%, with very poor outcomes despite subsequent therapies.[7]

IMMUNOLOGY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

In order to proliferate locally and metastasize, cancer cells must develop mechanisms that allow them to avoid detection and subsequent elimination by the host immune system. The basis of immunotherapy is the idea that the host immune system can be activated to overcome these acquired mechanisms, recognize the cancer as non-self and eliminate it (Fig. 1).

To achieve an effective antitumor immune response, T-lymphocytes must be able to both infiltrate the tumor and perform their function appropriately[8] with the frequency of tumor infiltrating lymphocytes (TILs) and their subtypes being linked to prognosis in multiple cancer pathologies.[9] In SCCHN, an increased TIL presence,[10] particularly CD3+, CD8+ and FOXP3+ sub-types,[11] is associated with a more favorable prognosis.

FOXP3+ regulatory T-cells (Tregs) suppress immune response and as such should hinder both detection and elimination of cancer cells[12] and are associated with a poor prognosis in multiple tumor types.[13] The positive association observed between infiltration with FOXP3+

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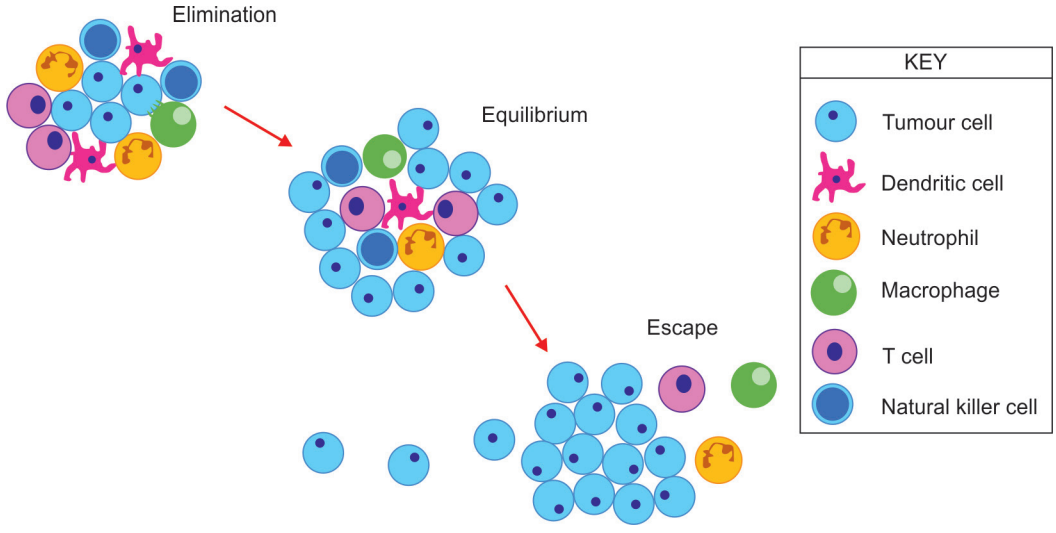

Fig. 1: Immune control and evasion: Interaction between tumor cells and the host immune system within the tumor microenvironment

cells and an improved prognosis in SCCHN seems counter-intuitive but it is also observed in colorectal cancer, epithelial ovarian cancer and lymphoma.[14-16] The balance between the different TIL clonal populations may influence the effects they have within the tumor microenvironment. Alongside differences in the tumor microenvironments between pathologies, FOXP3+ T cells exist as three phenotypically and functionally distinct subtypes,[17] which may account for this prognostic variation observed across disease sites. In colorectal cancer, it has been found that the FOXP3+lo non-suppressive T cells act in a proinflammatory manner and indicate a better prognosis in than FOXP3+hi cells.[18] Whether similar findings exist in SCCHN is an area that requires more research.

Some cancers avoid an immune response by preventing lymphocyte infiltration into the tumor and as such are often referred to as either 'immune-excluded tumors' or 'immune deserts' depending on the immune response around the tumor periphery.[19]

SCCHN does not typically fall into these categories, having one of the more immune-infiltrated cancer types,[20] suggesting that the tumors use other mechanisms to modulate the immune environment to avoid elimination by the TILs that are present.

This may be achieved through numerous mechanisms such as an increase in the immunosuppressive cytokines IL-10,[21] IL-6[22] and TGF-в,[23] overexpression of antigens causing T-cell tolerance,[24] deficiency or alterations of tumor human leukocyte antigen (HLA) class I molecules expression[25,26] and aberrant activation of the transcription factors signal transducers and activators of transcription 3 (STAT3)[27] and NF-kB.[23] Better understanding of the key mechanisms involved in the maintenance of an immunosuppressive tumor microenvironment in SCCHN may lead to more effective strategies to overcome them and hence improve disease control and patient outcomes.

Immune Checkpoint Inhibition

To avoid autoimmunity a series of checkpoints exist on the surface of immune cells, with the activation of a T-cell response being a balance of coinhibitory and costimulatory molecules and their ligands.[28] By preventing signalling from coinhibitory checkpoints, checkpoint inhibitors aim to generate an enhanced activation of the immune response to tumor cells. In SCCHN, agents that inhibit interaction between, programmed death-1 (PD-1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), or lymphocyte activation gene-3 (LAG-3) and their ligands are currently undergoing evaluation, with other newer agents in development (Fig. 2).

PD-1 and PD-L1 Checkpoint Inhibition

When PD-1 interacts with its ligand PD-L1, anticancer immunity is suppressed via decreased cytokine production, alongside induction of T lymphocyte anergy and apoptosis. As such, cancer cells can upregulate PD-L1 in order to functionally inactivate T-cell immune surveillance[29,30] and PD-L1 overexpression has been reported at between 46–100% across studies in SCCHN,[31] the wide range likely owing to a combination of variation in staining techniques and sample preservation.

Nivolumab, an IgG4 PD-1 inhibitor, demonstrated an improvement in both overall survival (OS) and progression free survival (PFS) in the phase III study CheckMate 141.

This study compared nivolumab with the physician's choice of second line single agent (docetaxel, methotrexate or cetuximab) in patients with platinum resistant, recurrent or metastatic HNSCC. A total of 361 patients were recruited and allocated to treatment with a 2:1 randomisation. Whilst response rates (RR) were low in all arms, it was higher in the nivolumab group (13.3%), with 6 complete responses (CR) and 26 partial responses (PR) reported.

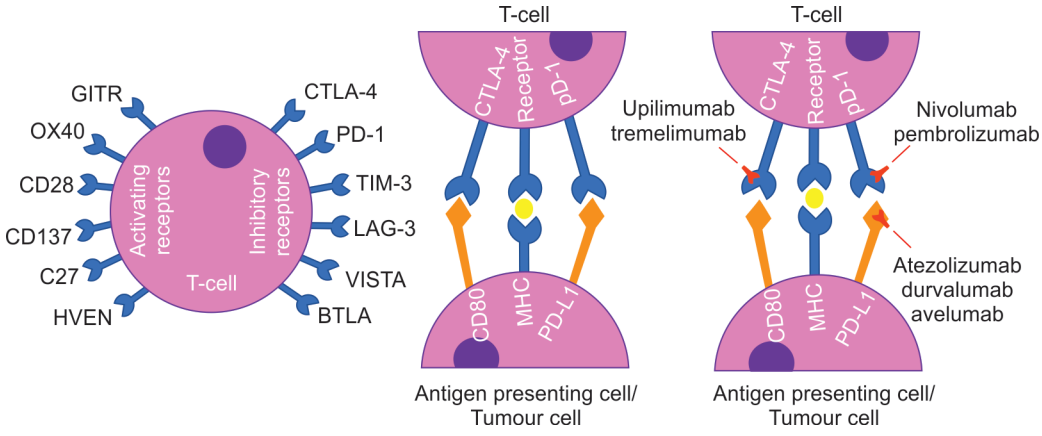

Fig. 2: Immune checkpoints: Coinhibitory and costimulatory checkpoints are potential targets for immunotherapies

In comparison, the RR in the chemotherapy arm was only 5.8% (including 1 CR). Patients who received nivolumab had a 30% lower risk of death (HR 0.70; 95% CI 0.51–0.96) with an increase in both median overall survival (OS) (7.5 months versus 5.1 months) and estimated landmark progression free survival (PFS) at 6 months (19.7% versus 9.9%). This increased benefit at later time-points gives promise to durable benefit for responding patients, as seen in other tumor types where data are more mature.[32] In addition to the higher efficacy seen, treatment with nivolumab was found to be more tolerable with a reduction in grade 3 or 4 adverse events (13.1% versus 35.1%) and a relative improvement in patient reported outcomes (PROMS) and other quality of life parameters.[33]

Patterns of response to immunotherapy agents differ from that of standard treatment, with delayed response commonly being observed and less commonly initial enlargement of tumors or the development of new lesions giving the appearance of disease progression and such patients can receive treatment beyond this 'pseudo-progression'.[32,34] The recognition of these unique response characteristics has lead to the development of the immune-related RECIST criteria.[35] The outcomes of patients who received treatment beyond progression as part of CheckMate 141 were presented at the European Society of Medical Oncology 2017 Annual Congress (ESMO 2017). Of those who progressed, 62 patients (42%) received at least one further dose of nivolumab and 15 had a subsequent reduction in target lesion size with 3 achieving a reduction of greater than 30%, with no increase in grade 3 or 4 toxicities,[36] suggesting that treatment beyond progression may be considered for patients where there appears to be some clinical benefit observed and it is felt safe to do so.

Pembrolizumab is another IgG4 PD-1 antibody, which has also been explored in the treatment of patients with recurrent or metastatic SCCHN. KEYNOTE-012 was a phase Ib study that included an expansion cohort of 132 SCCHN patients. This trial reported a RR of 18% at 9 months, which included 4 CR and 20 PR, with a median OS of 8 months and a 6-month PFS of 23%. Overall the treatment was felt to be very tolerable with grade 3 or 4 toxicity was reported in 9% of patients.[37] These data led to the accelerated approval of pembrolizumab by the FDA for the treatment of SCCHN. Early outcomes from the confirmatory phase III trial, KEYNOTE-040, were presented at ESMO 2017 and AACR 2018[38,39] although the final published manuscript is awaited. This study compared pembrolizumab with standard therapies in a similar design to CheckMate 141 study, although with a complex statistical analysis plan. It demonstrated a median OS of 8.4 months with pembrolizumab compared to 7.1 months in the control arm, with a hazard ratio of 0.80 (95% confidence interval 0.66–0.99), reaching statistically significance (p=0.016) with the updated data from the full population. It is also likely that the OS data may have been confounded by some patients within the control arm (12%) going on to receive immune checkpoint inhibitors post progression (cross-over effect).

The benefit of other agents that interrupt the interaction between PD-1 and PD-L1 in SCCHN, such as durvalumab, atezolizumab and avelumab, is currently being investigated in ongoing trials.

Double Checkpoint Inhibition

The upregulation of alternative immune checkpoints may cause resistance to anti-PD-1/PD-L1 treatment[40] and has driven the rationale for combining treatments to target more than one co-inhibitory molecule. The use of combination checkpoint inhibitors (CPI) has been successful in improving both response rates and survival in other cancer pathologies, with several combinations under ongoing investigation in SCCHN.

CTLA-4 is expressed on the surface of activated T cells, where it can prevent the co-stimulatory interaction of B7 and CD 28, subsequently down regulating T cell proliferation and IL-2 production. Inhibiting CTLA-4 results in an increase in T-Cell activation alongside maintenance of high-frequency T-cell receptor clonotypes.[41,42]

The combination of CTLA-4 and PD-L1 inhibition has resulted in higher response rates than monotherapy alone in metastatic melanoma, renal cell cancer and small cell lung cancer.[43-45] In SCCHN, this combination is being evaluated in a number of ongoing trials both in the first line and metastatic settings with results eagerly awaited.

LAG-3 is expressed on the surface of activated CD4+ and CD8+ T-cells and certain subtypes of natural killer and dendritic cells. It suppresses both activation and proliferation of T cells and assists in Treg suppressive function,[46-50] promoting a immunosuppressive immune microenvironment. BMS-986016 is an anti-LAG-3 antibody that is being explored both as monotherapy in combination in a phase I/IIa dose expansion and escalation study (NCT01968109) that involves an SCCHN cohort.

Checkpoint Inhibition and Chemo Radiotherapy

Chemo Radiotherapy (CRT) can trigger immunogenic cell death creating a cascade of endogenous molecules called "damage-associated molecular patterns" (DAMPs) which recruit and stimulate antigen presenting cells, eventually resulting in an adaptive immune response.[51] This is particularly relevant in SCCHN where a significant proportion of patients will receive (chemo/bio) radiotherapy at some point in their primary management.

In SCCHN, CRT increases the number of CD8+ T effector cells, CD4+ regulatory cells and T cells expressing PD-1, TIM3 and LAG3 within the tumor microenvironment, particularly in oropharyngeal cancers.[52] An increase in CD3+ and CD8+ TILs has also been shown to positively correlate with clinical outcome to definitive CRT in SCCHN.[53]

The addition of checkpoint blockade following platinum-based CRT has been shown to be feasible, safe and demonstrated an improvement in PFS for patients with non-small cell lung cancer, as demonstrated by the phase III PACIFIC study.[54]

Preclinical data suggest benefits may be better if the CPI are delivered concurrently with the radiotherapy.[55]

There are a number of studies underway investigating the benefits of adding checkpoint inhibition to CRT in the management of SCCHN. The preliminary results of a safety study combining pembrolizumab with cisplatin-based CRT in locally advanced SCCHN was presented at ASCO 2017. Pembrolizumab was administered 4–7 days prior to CRT, three weekly for the duration of CRT and then a further five doses following completion. In total 27 patients were enrolled, of which 74% had HPV positive oropharyngeal tumors. All subjects received their planned XRT dose, 85% received target cisplatin dose and 78% completing the planned doses of pembrolizumab. The addition of pembrolizumab was not felt to have significantly increased treatment toxicity, however three patients required treatment-discontinuation due to immune related adverse events (G2 peripheral motor neuropathy, G3 AST elevation and G1 Lhermitte-like syndrome). The study has now reopened expansion cohorts in 34 HPV positive and 23 HPV negative patients to further evaluate efficacy.[56]

Cetuximab can be administered as an alternative to cisplatin alongside RT for radical treatment of locally advanced SCCHN. It influences both natural killer cell response and dendritic cell maturation[57,58] and increases expression of inhibitory checkpoints PD-1, TIM-3 and CTLA-4 on TILs.[59,60] While a number of trials are exploring the addition of checkpoint blockade to cetuximab-RT, the combination with avelumab is of particular interest due to the propensity of both avelumab and cetuximab to activate antibody-dependent cellular cytotoxicity (ADCC).[58,61]

Other Combination Approaches

Inhibiting immune checkpoints is only one approach to promoting an anti-cancer immune environment and a number of other agents are being explored in combination with anti-PD-1 and anti-PD-L1 agents to augment their effectiveness.

STAT3 signaling has been implicated in the proliferation, invasion and survival of many cancers, including SCCHN, through upregulation of inhibitory cytokines.[62] AZD9150 is an oligonucleotide antisense molecule that prevents the production of STAT3, with preclinical evidence of antitumor activity in lymphoma and lung cancer models.[63] Preclinical studies in SCCHN models have also shown that STAT3 inhibition sensitizes to both chemotherapy and radiotherapy, particularly in nasopharyngeal cancer.[64,65] AZD9150 is being administered in combination with Durvalumab, a PD-L1 inhibtior, as part of the phase Ib/II SCORES trial for patients with advanced or metastatic SCCHN (NCT02499328). Initial results were announced at the ESMO Congress 2017. Of the 35 patients included in this report, 15 had received prior treatment with an anti-PD-L1 agent. In the CPI naïve arm, a 25% objective RR was reported, with a 45% DCR at 12 weeks and 30% of patients remaining on treatment at 25 weeks. For anti-PD-L1 pretreated patients, 1 complete response and 1 unconfirmed response were reported, with a 20% DCR at 12 weeks.

Overall the combination was felt safe and deliverable with G3/4 thrombocytopenia and increases in liver enzymes reported for 3.4% patients, and two treatment-related discontinuations (unspecified). These early data are promising, and mature results are eagerly awaited.

CXC Chemokine receptor 2 (CXCR2) is a G protein-coupled receptor for a number of cytokines and is

implicated in disease proliferation via IL-8 signaling. It is known to be frequently overexpressed in SCCHN. AZD5069 is a novel selective antagonist of CXCR2 and is being given in combination with Durvalumab as a separate arm of the SCORES trial, with results awaited.[66, 67]

Indoleamine 2,3-dioxygenase 1 (IDO1) is a catabolizing enzyme that suppresses T-cells and promotes immune suppression and has been associated with poor outcome in laryngeal squamous cell carcinoma.[68] Epacadostat is an oral inhibitor of IDO1 which is being administered concurrently with pembrolizumab was part of the phase I/II KEYNOTE-037 study for patients with recurrent/metastatic SCCHN. Preliminary results were presented at ASCO 2017 with 36 of 38 patients being efficacy-evaluable at the initial data cut off. The ORR was reported at 31% with DCR of 58%, regardless of the number of previous lines of treatment. Overall the treatment was tolerated well with fatigue (24%), nausea (11%) and decreased weight (11%) being the most commonly reported adverse events. These promising data have led to plans for a phase III study.[69]

Oncolytic viruses have been found to reduce tumor burden and stimulate antigen presentation preclinically,[70,71] and when administered concurrently, may help overcome anti-PD-1 resistance by broadening neoantigen-directed T-cell responses.[72]

Granulocyte macrophage colony-stimulating factor (GM-CSF) promotes the attraction and maturation of dendritic cells and amplifies antigen presentation.[73] One of the more advanced oncolytic viruses in development is the locally administered Talimogene Laherparepvec (T-VEC), a modified, live, attenuated herpes simplex virus type 1. T-VEC has been shown to preferentially infect cancer cells in patients with melanoma,[74] where it has gained a license after demonstrating improved DRR and mOS.[75,76] It is being administered alongside Pembrolizumab as part of the phase Ib/III KEYNOTE-137 study for patients with recurrent metastatic SCCHN (NCT02626000).

ADXS11-001 is an immunotherapeutic based on live, attenuated *listeria monocytogenes*, which secretes HPV-E7 tumor antigen as a truncated fusion protein resulting in HPV-specific T-cell generation.[77] Inhibition of PD-L1 increased ADXS11-011 activity preclinically[78] and is being administered both as a monotherapy and in conjunction with Durvalumab for the treatment of HPV positive HNSCC as part of a phase I/II study (NCT02291055).

Biomarkers

Both CheckMate-141 and KEYNOTE-12 explored the impact of HPV positivity as a predictor of response. In Checkmate-141, OS appeared to be longer with nivolumab than chemotherapy regardless of p16 status, however the benefit appeared more pronounced in patients with p16-positive tumors (mOS 9.1 months versus 4.4 months respectively), than in the p16-negative tumors (mOS 7.5 months versus 5.8 months).[33] KEYNOTE-012 reported better outcomes to pembrolizumab in the HPV-positive patients compared to those that were HPV negative (RR 32% *vs* 14%; 6 month PFS 37% vs 20% and 6-month OS 70% *vs* 56%)[37] again suggesting that the viral-associated SCCHN might gain greater benefit from checkpoint inhibition.

In the survival analysis of CheckMate-141, PD-L1 expression >1% was associated with a hazard ratio for death of 0.55 (95% CI 0.36–0.83) when treated with nivolumab compared to standard therapy. Where PD-L1 expression was <1%, this HR was increased at 0.89 (95% CI 0.54–1.45).[33] Similarly, KEYNOTE-012 described PD-L1 expression >1% or <1% and found that patients with PD-L1 positive tumors had a RR to pembrolizumab of 22% compared to 4% in negative tumors, and median OS of 303 days versus 151 days, respectively,[37] Interestingly, both studies examined PD-L1 positivity on tumor cells alone, however overexpression on immune cells has been linked to response to CPI in metastatic urotherlial cancer[79] and the combination of expression on both tumor and/or immune cells is being explored in HNSCC within the KEYNOTE-040 study.

Of the 61 patients in KEYNOTE-012 with PD-L1 positive disease, 43 had RNA expression profiling, evaluated with multigene expression signatures derived from patients with melanoma. Of these signatures, a 6-gene INF-γ was the top-performing, with significant associations to OR ($p = 0.005$) and PFS (< 0.001). On evaluation of the individual signature genes, INF-γ inducible MHC-II expression was felt to be the key biological link. Using an optimal cutoff for INF-γ, positive predictive value for response was 40% with a negative predictive value of 95%; AUC = 0.8 (95% CI 0.61–0.95). This information may assist in identifying patients who are most likely (or least likely) to gain clinical benefit from anti-PD-1 therapy,[80] either alone or in combination with PD-L1 data.

A high mutational burden or neoantigen load, is associated with response to checkpoint blockade across a number of disease sites[81,82] with mutational burden decreasing with successful anti-PD-1 treatment[83] suggesting that selection against mutant neoepitopes may be a critical mechanism of action of this class of drug. The somatic mutational load (ML) and INF-γ gene expression profile were both found to be independently predictive of response to pembrolizumab in the 73 patients within KEYNOTE-012 who were HPV and Epstein-Barr Virus (EBV) negative, with ML and INF-γ gene expression profile being significantly associated with OR ($p = 0.064$

and $p = 0.001$; AUROC 0.82 and 0.74, respectively). INF-γ gene expression profile also remained a significant predictor in HPV and EBV positive patients showing promise as a biomarker of response regardless of viral status.[84]

Several other potential biomarkers are currently under exploration including the epigenetic modification of genes associated with homologous recombination, such as RAD51 and XRCC3, which are thought to alter checkpoint expression[85] and the identification of different subtypes of SCCHN, each with a distinct microenvironment,[86] but whether these predict for OS or response to immunotherapy has yet to be established.

CONCLUSION

Immunotherapy agents have shown promising results in the treatment of HNSCC, with the 2016 FDA approval of both pembrolizumab and nivolumab offering new hope for patients with this disease. As the results of ongoing trials are made available, checkpoint inhibitors in particular may find new roles in the neoadjuvant or adjuvant settings, either as monotherapy or in conjunction with other agents. Along with a deepening appreciation of the tumor–host interaction and careful biomarker selection we are entering an era of personalized immunotherapy treatment, which should translate to improved outcomes for patients with squamous cell carcinoma of the head and neck.

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