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Neoadjuvant immunotherapy in resectable head and neck cancer: oral cavity carcinoma as a potential research model

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***Abstract*:** Squamous cell carcinoma of oral cavity (OCSCC) accounts for approximately 25% of cases of head and neck squamous cell carcinoma (HNSCC). Tobacco and alcohol consumption are the main risk factors for both cancers. Surgical resection, combined with adjuvant radiotherapy or radiochemotherapy in patients with high risk of relapse, is the key element in management in the initial stages. However, despite the availability of aggressive multidisciplinary treatments, advanced resectable OCSCC carries poor prognosis; only half of the patients are disease-free 5 years after the surgery. Immunotherapy based on the use of immune checkpoint inhibitors has been proven to be effective in a wide variety of tumours, including recurrent and metastatic HNSCC. These positive results resulted in investigations into its effectiveness in earlier stages of the disease with OCSCC emerging as an interesting research model because of the accessible location of the tumours. This article reviews the potential advantages of emerging immunotherapeutic agents [mainly monoclonal antibodies against programmed cell death-1 (*PD-1*) immune checkpoint inhibitors] as neoadjuvant treatment for OCSCC at locoregional stages as well as the ongoing clinical trials, challenges in evaluating tumour response, and possible predictive biomarkers of response with highlights regarding the role of oral microbiota as modulators of immune response. The efficacy and safety of anti-*PD-1* drugs in these patients have been proven in preliminary trials. If there is a decrease in the relapse rate and an improvement in the overall survival after surgical resection in ongoing trials, preoperative immunotherapy may be established as a treatment option for patients with early stages of the disease.

Keywords: head and neck cancer, immunotherapy, neoadjuvant, oral cavity

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Introduction

Head and neck squamous-cell carcinoma (HNSCC) is the sixth most frequent neoplasm worldwide.[1,2] In HNSCC, squamous-cell carcinoma of oral cavity (OCSCC) constitutes approximately 25% of the total cases, with tobacco and alcohol consumption being the main risk factors for OCSCC. Despite easy self-examination, diagnosis is usually established in locally advanced stages that affect the regional lymph nodes. Surgical resection, combined with adjuvant radiotherapy or radiochemotherapy in patients with high risk of relapse, is the key element of treatment.[3,4] However, despite the availability of aggressive multidisciplinary treatment, advanced resectable OCSCC carries a poor prognosis – only half of the patients are disease-free 5 years after the surgery.[5] Over the recent years, these data have not improved despite the intensification of adjuvant treatments and preoperative chemotherapy.[6,7] Neoadjuvant treatment with docetaxel, cisplatin, and 5-fluorouracil in patients with resectable OCSCC was reported to be ineffective in improving survival in comparison with an approach of surgery initially; however, a subgroup analysis has demonstrated improvements

in patients in whom bilateral cervical lymph nodes are affected.[8] The current clinical scenario warrants new approaches oriented towards the study of drugs with different mechanisms of action.[9]

Immunotherapy based on cytotoxic T-cell immune checkpoint inhibitors (ICIs) is effective in recurrent or metastatic HNSCC after progressing to platinum[10–12] and recently it has also demonstrated its effectiveness as first-line treatment in selected subgroups of patients.[13,14] These positive results have led to studies on the effectiveness of these drugs during earlier stages of the disease, with OCSCC emerging as an interesting research model because of the accessible location of the tumours and large tumour sample for studying biomarkers. Neoadjuvant immune treatment may reduce the risk of both local and distant relapse due to early initiation of systemic treatments, which are linked to a better toxicity profile than that in traditional chemotherapy,

This article reviews the potential advantages of emerging immunotherapeutic agents, mainly anti-programmed cell death-1 (anti-*PD-1*) ICI as neoadjuvant treatments for OCSCC at locoregional stages, as well as the ongoing clinical trials, challenges in evaluating tumour response, and possible predictive biomarkers of response, and highlights the role of oral microbiota as modulators of immune response.

Rationale for neoadjuvant immunotherapy in OCSCC: advantages and limitations

Advances in the field of genetics have led to investigations in the molecular pathogenesis of HNSCC and discoveries of a heterogeneous disease with different mechanisms of carcinogenesis and signalling pathways as well as gene expression profiles.[15,16] The following are the three different variants of HNSCC: (1) human papilloma virus (HPV)-positive tumours with viral transcriptional activity identified by the expression of *p16* and presence of viral DNA. The most frequent type is HPV-16, which makes up approximately 31% of head and neck tumours, with increasing incidence globally. They are more frequent in young males and are not so strongly linked to classical risk factors. They normally have a better prognosis irrespective of the type of treatment. (2) HPV-negative tumours with high copy number alterations (CNAs), which represent the most prevalent phenotype and are linked to smoking and drinking habits. Their main feature is the presence of mutations in *TP53, CDKN2A, PIK3A, FAT1*, and *NOCHT* genes. (3) HPV-negative tumours with low CNA (silent-CNA), which are more frequent in women than in men and do not carry the classical risk factors. Unlike high-CNA, the most frequent alterations in silent-CNA tumours occur in *HRAS* (Harvey rat sarcoma viral oncogene homolog) and those that result in the inactivation of caspase-8, whereas *TP53* does not usually demonstrate mutations in this type of tumour.

Despite the great complexity and diverse aetiologies, recent studies have demonstrated that the three subgroups share an inflammatory phenotype that can potentially benefit from immunotherapy.[17] HNSCC is one of the tumours with the highest immune infiltrate in the stroma. However, unlike other neoplasms, regulatory T-cells (Tregs) are the prevailing cells in both the tumour microenvironment and the peripheral blood. Tregs – induced mainly by the phosphorylation of *FOXP3* in the presence of tumour growth factor *(TGF)-beta* and retinoic acid – restrict the immune response against tumour-associated antigens, thus additionally promoting the apoptosis of the rest of the immune cells.[18] The number and activity of natural killer (NK) cells are also high in HNSCC, which highlights the capacity of detecting tumour cells that have lost their human leucocyte antigen (*HLA*) as a mechanism to escape the immune escape. Myeloid suppressor cells (MSCs) in HNSCC also play a pro-tumour role due to their involvement in the suppression of non-specific T-cells. Tumour progression promotes their induction through *TGF-beta*, *VEGF*, and *IL-6*. A drop in MSC has been linked to reduction in tumour growth and inhibition of immunosuppression of the microenvironment by the increase in the subpopulation of CD8+ cells. All these cells were also proven to be associated with tumour angiogenesis and alterations in the *JAK/STAT* (Janus kinase/signal transducers and activators of transcription) pathway.

Other specific immune features include alterations in both NK cells and maturation of dendritic cells, lower capacity of antigenic presentation, lower absolute lymphocyte count with higher number of Tregs, tumour-associated macrophages, and higher expression of immunosuppressive cytokines. Collectively, these elements promote an immunosuppressive tumour microenvironment that can be targeted by immune-based therapeutic strategies.[19,20] The high mutational burden[20,21] in HNSCC (higher in HPV-negative

Table 1. Advantages and disadvantages of neoadjuvant treatment with immunotherapy.

Advantages	Disadvantages
Surgery is the basis of treatment	Low rate of objective response with *PD-1* inhibitors in advanced disease
Large tumour sample for studying biomarkers	Absence of biomarkers for the selection of patients
Primary tumour accessible for clinical monitoring	Risk of delay in surgical treatment due to toxicity or hyperprogression
Better immune status of the patient	Alterations in wound healing
Higher tumour antigenicity	Lack of reliable tools for evaluating the response to treatment
Reduction in the risk of local or distance relapse	Absence of homogeneous criteria for evaluating the pathological response

PD-1, programmed cell death-1.

tumours, particularly, in smokers) correlates with a higher burden of target neoantigens for the immune response, which may contribute to the increase in the lymphocyte population density in the tumour microenvironment and improves the specific response of cytotoxic T-cells. Therefore, strategies for improvements in antitumour immune activity focus on promoting an effector response mediated by cytotoxic T-cells and NK cells and/or inhibiting suppressor signals provided by Tregs, tumour-associated macrophages, and suppressor myeloid cells. The effects of anti-*PD-1* immunotherapy on the inflammatory infiltrate within the tumour microenvironment and peripheral blood T-cell subpopulations in HNSCC and, especially, patients with OCSCC have not been extensively studied in prospective trials.

OCSCC is a very interesting neoplasm in the assessment of neoadjuvant immunotherapy strategies and represents a unique model for the study of the efficiency of neoadjuvant immunotherapy due to the following reasons (Table 1): (1) surgery is still the key element in the treatment of patients with local or locally advanced resectable disease;[2] (2) the primary biopsy is larger in size than the biopsies in other HNSCCs, such as those involving the lung, larynx, or pharynx, which may allow for a larger number of translational studies aimed at identifying biomarkers linked to the efficacy and resistance to different immunotherapy strategies; and (3) the whole tumour is accessible to the doctor by physical examination (i.e. the tumour of the oral cavity and locoregional nodes), which allows for closer monitoring during the preoperative treatment period. Other advantages include a better immune status of the patient due to the earlier stage of the disease; potentially greater tumour antigenicity in comparison with adjuvant treatment administered after the macroscopic removal of the neoplasm;[22,23] reduction of the risk of local or distant relapse due to the early introduction of systemic treatment;[24] and the possibility to explore the role of oral microbiota as a predictive biomarker of response and their potential therapeutic role in better responses to immunotherapy.

Nevertheless, neoadjuvant immunotherapy includes some limitations, such as low rate of objective response by *PD-1* inhibitors in advanced disease in the trials published thus far,[10–12] absence of biomarkers that could aid in appropriate selection of patients, potential delay in secondary surgical treatment due to toxicity or hyperprogression,[25,26] changes in the tumour microenvironment that may affect wound healing, lack of reliable tools to assess the efficiency of the treatment before tumour resection, and the need to obtain homogeneous and validated criteria for suitable evaluation of the pathological response.[27]

Clinical trials with neoadjuvant immunotherapy

Immunotherapy based on cytotoxic T-cell ICIs has been demonstrated to be effective in HNSCC. The administration of nivolumab for patients with recurrent or metastatic HNSCC who showed progression during the first 6 months after receiving systemic treatment with platinum-based chemotherapy, doubled the survival after 1 year (36% with nivolumab *versus* 16% with the treatment chosen by the researcher), almost tripled the overall survival after 2 years (16% *versus* 6%), and improved patients' quality of life.[10,28,29]

In this context, positive results with pembrolizumab have allowed for its approval in this scenario[11,12,30] and as first line treatment (alone or in combination with platinum plus 5-fluorouracil) in specific subgroups in recurrent or metastatic HNSCC.[13,14]

These positive results have led to investigations of the efficiency of these drugs during the earlier stages of the disease and in combination with chemo-radiotherapy in locally advanced irresectable disease.[31] A phase II trial in 27 patients with locally advanced resectable HNSCC explored the safety and efficiency of these drugs preoperatively in combination with several immuno-stimulating cytokines from healthy donors, which revealed improvements in survival after 3 years with minimum toxicity and did not involve a delay in the surgery.[32] Another study in 39 patients with OCSCC investigated the effects of intratumour administration of IL-2 and low doses of oral cyclophosphamide before the surgical resection. Two pathologically complete, two major (>50%), and four minor responses (>30% but <50%) resulted from treatment (overall response rate, 42%) and finding specific changes in composition of tumour-infiltrating mononuclear cells, with increased CD4+:CD8+ ratio, and increased tumour stroma to epithelial ratio.[33]

Several ongoing studies are assessing the activity of neoadjuvant anti-*PD-1*/*PD-L1* in different tumours such as non-small cell lung cancer, urothelial cancer, melanoma, and glioblastoma.[34–39]

A pilot study assessed the efficacy and safety of preoperative nivolumab in patients with non-small cell lung cancer (NSCLC).[40] The study included 22 patients with potentially resectable stage IB–IIIA tumours who received two doses of nivolumab during the 4 weeks before the surgery. The efficacy was assessed using the objective criteria of pathological response[31] and translational studies of the tumour microenvironment, mutational and neoantigen loads, and changes in the clonality of T-cell receptor in the blood and tumour both before and after the treatment. Nivolumab was well tolerated and there were no delays in the surgery. The tumour was resected in 21 patients, and it was irresectable in only one patient. Overall, 9/20 [45%, 95% confidence interval (CI) 24–63%] patients demonstrated a greater pathological response (defined as <10% of viable tumour cells in the tumour tissue). The pathological response was not correlated with the radiological response observed on computed tomography (CT). Over a median postoperative follow-up of 9 months, 18 (86%) patients were alive without relapse. Sequencing of the pre-treatment tumour exome demonstrated a correlation between the pathological response and mutational and potential neoantigen burden. Similarly, an immunohistochemistry study of tumour samples, before and after the treatment, revealed an increase in the infiltration of *PD-1*+CD8+ T-cells in the tumours that responded[30] both in the tumour and in the peripheral blood.

Preliminary findings of two studies that assessed neoadjuvant treatment with *PD-1* inhibitors in operable HNSCC have been published. In 2017, Ferris *et al.* published the results of the first 29 patients included in a phase I/II CheckMate 358 (NCT02488759) trial.[41] This trial assessed the safety and feasibility of nivolumab before the surgery in a cohort of neoplasms etiologically related to viral infections. The head and neck cohort included patients with a newly diagnosed squamous carcinoma of the oral cavity, pharynx, or larynx that were resectable (≥T1) and affected the ganglia (≥N1) who received two doses of 240 mg nivolumab on days 1 and 15 with scheduled surgery on day 29. The primary objective of the study was the safety (incidence of adverse effects) and delay of the scheduled surgery by >4 weeks due to toxicity. Of the 29 patients, 12 had HPV+ tumours and 17 had HPV– tumours. CT revealed a reduction in tumour size before the surgery in 11/23 (48%) assessable patients (5/10 were HPV+ and 6/13 were HPV–). Three patients experienced a reduction of >40% and a patient with HPV+ status demonstrated tumour reduction of 75%. The toxicity was mild or moderate, and only four patients demonstrated adverse effects of grade 3 or 4; however, surgery was not delayed in any of the patients.

Another phase II trial (NCT02296684) assessed 46 patients with locally advanced resectable HPV– HNSCC. They were administered a unique dose of 200 mg pembrolizumab 1–3 weeks before the surgery.[42] High-risk patients – those with affected margins and/or extracapsular extension – received cisplatin and adjuvant radiotherapy together with six additional doses of pembrolizumab. In the first 21 patients treated, the following observations were noteworthy: (1) no unexpected adverse effects, delays, or postsurgical complications; (2) no events regarding locoregional relapse or distance metastasis in the

first 10 patients with more than 1 year of postoperative follow-up; (3) affected margins/extracapsular extension rate was 38% (95% CI: 18–62%); (4) pathological tumour response was observed in 43% of patients (95% CI: 22–66%), which included tumour necrosis or presence of giant cells/histiocytes together with keratin removal in >10% of the tumour tissue; and (5) necrosis >70% of the tumour area was observed in 6/21 (29%) patients. Basal tumour samples tested positive for PD-L1 (>1% of tumour cells) in 11/19 (58%) of assessable samples and 7/8 (88%) of patients who responded, thus highlighting a correlation between *PD-L1* expression in tumour cells and the pathological response (correlation coefficient: 0.72; $p = 0.0005$).

Other investigations are now enrolling participants to study the combination of nivolumab and ipilimumab (NCT02919683 and NCT03700905), assess the increase in doses of preoperative or postoperative nivolumab (NCT03021993 and NCT03721757), evaluate neoadjuvant pembrolizumab in stage III/IVA resectable cancers (NCT03765918), and investigate preoperative durvalumab in patients with resectable OCSCC (NCT02827838) (Table 2).

In conclusion, these studies suggest that this approach is safe, does not delay nor compromise radical surgical treatment, and results in pathological response in a significant number of patients.

Future directions

Combined treatments

Despite the positive published results of clinical trials on recurrent/metastatic disease, only a small subgroup of patients will benefit from anti-*PD-1* therapies. HNSCC is one of the tumours with the largest infiltration of immune cells and features the largest infiltration by Tregs and NK cells in the tumour microenvironment.[9,18–20] Consequently, patients with these tumours could benefit from specific therapies aimed at those cells. Patients who have tumours with genetic signature highly linked with smoking feature high mutational loads but low levels of immune infiltration and interferon-gamma (*IFN-γ*) expression. These immunologically 'cold' but mutation- and neoantigen-rich tumours might benefit from the synergistic effects of combined therapy (co-stimulating agonists and anti-*PD-1*). There is a clear association between the activity of NK cells and tumour immune surveillance;[44,45] therefore, increasing the activity of NK cells might be beneficial, especially in tumours that have escaped from the adaptive immune system due to defects in the antigen processing mechanism. NK cells, despite their inability to identify an antigen, possess target cell selectivity. This mechanism is mediated by the *NKG* family of receptors and their respective ligands, which are expressed preferentially in infected cells and tumour cells.[46] Therefore, the combination of *PD-1* blockade and blockade of inhibitory receptors of killer-cell immunoglobulin-like receptors as well as *TLR* (toll like receptors) agonists or inhibitors of the 'do not eat me' signal might be an attractive strategy to explore.[44,45,47]

Approximately 50–60% of HNSCC tumours express *PD-L1*, which is induced by an increase in *IFN-γ* in the tumour microenvironment. Tumour cells and microenvironment cells develop a wide variety of mechanisms of immune evasion through diverse immune inhibitory checkpoints (e.g. *CTLA-4, PD-1/PD-L1, TIM-3, LAG3*, and *TIGIT*), which are currently being investigated as potential targets.[48,49] Antibodies that act as agonists on the members of the *TNFR* family that stimulate T-cells and NK cells (*CD137, OX40, ICOS*, and anti-*GITR*) are other potential new theraphies.[50,51] After positive pre-clinical results, MEDI6469 (agonist monoclonal antibody *OX40*) is being studied in several phase I trials in patients with recurrent or metastatic platinum-refractory HNSCC and other advanced solid neoplasms as well as its preoperative administration (NCT 02274155). Cetuximab is a chimeric IgG1 mouse/human monoclonal antibody against the epidermal growth factor receptor (*EGFR*). It is capable of inducing antibody-mediated cell cytotoxicity through the activation of NK cells and conducts the overexpression of *CD137* co-stimulatory receptor. This receptor promotes T-cell effector functions and its potential in combination with urelumab (*CD137* agonist antibody) is being currently investigated.[52,53] A phase Ib trial comparing the combination of motolimod (*TLR*-8 agonist) and cetuximab with or without nivolumab as neoadjuvant treatment in patients with locally advanced HNSCC is also currently underway (NCT02124850).[43]

The safety and activity of local immune treatments (vaccination against viral or tumour antigens, genetically modified tumours, such as T-VEC among others) linked to immunotherapy are being investigated in different tumour types,

Table 2. Ongoing or planned trials on neoadjuvant immunotherapy in head and neck squamous-cell carcinoma including oral cavity tumours.

Trial	Patient population	Neoadjuvant treatment	Adjuvant treatment	Status	Primary endpoint
NCT02488759 (CheckMate 358)[41]	Neoplasms related to viral infections (incl. HPV+ head and neck cancers)	Nivolumab →S (single arm)	Specific to cancer type	Active, not recruiting	Safety, objective response rate, rate of surgery delay
NCT02296684[42]	Any site (HPV–) platinum and anti-*PD-1* for high-risk (+margins or ECE)	Pembrolizumab × 1→S	S→ RT ± C ± pembrolizumab × 6	Ongoing	LRR and DFR at 1-year, rate of major pathologic response
NCT02919683	Oral cavity only; platinum and RT considered post-operatively	Cohort 1: nivolumab → S cohort 2: nivolumab + ipilimumab × 1–2 →S	S → ±(C)RT	Active, not recruiting	ORR to treatment
NCT03021993	Oral cavity only; stages T2–4	Nivolumab × 3–4 →S	S→ ±(C)RT	Ongoing	Pathologic response rate
NCT03721757	Oral cavity only; T1–4, N1–3 or any T3–4 N0	Nivolumab × 1 →S	Nivolumab × 1 → (C)RT → nivolumab × 6	Not yet recruiting	Disease-free survival
NCT03765918	Stage III–IVA resectable	Pembrolizumab × 2 →S	S → RT ± C + pembrolizumab × 6	Ongoing	Major pathological response, EFS
NCT02827838	Oral cavity or oropharyngeal; platinum and RT considered postoperatively	Durvalumab × 2→S	S→ ±(C)RT	Ongoing	Changes in immune biomarkers
NCT03708224	Stage III/IV resectable, HPV–	Atezolizumab × 2 →S	S→ ±(C)RT + atezolizumab × 12	Ongoing	Effect on CD3+ T-cell infiltration, R0 resection rate
NCT02274155	Stage III/IV resectable	Anti-*OX40* antibody (MEDI6469) × 1–3→ S	S→ ±(C)RT	Active, not recruiting	Safety, feasibility of definitive surgical resection
NCT02124850[43]	Any site; platinum and RT considered post-operatively	Cohort 1: motolimod + cetuximab → S Cohort 2: motolimod + cetuximab + nivolumab →S	S→ ±(C)RT	Terminated	Changes in immune biomarkers
NCT02002182	HPV+ oropharyngeal, platinum and RT considered post-operatively	ADXS 11–001 vaccine →TORS	TORS → ±(C)RT	Active, not recruiting	Change in E6/7-specific CD8 + T-cell response
NCT04080804	Stage III/IVA resectable	Nivolumab ± anti-LAG3 (relatlimab) ± ipilimumab →S	S→ ±(C)RT	Ongoing	Adverse events related to treatment

C, chemotherapy; DFR, distant failure rate; ECE, extracapsular extension of lymph nodes; EFS, event-free survival; HPV, human papillomavirus; LRR, locoregional recurrence rate; ORR, overall response rate; *PD-1*, programmed cell death-1; S, surgery; RT, radiotherapy; TORS, transoral robotic resection.

especially in severe/advanced disease, with positive results reported in patients with melanoma.[54] In locally advanced OCSCC, tumour lesion is easily approachable for the administration of intratumour treatments, which makes it a promising investigation pathway. The results of a phase II trial with ISA 101 (synthetic peptide derived from HPV-16) plus nivolumab in advanced oropharynx carcinoma p16+ reported an overall response rate (ORR) of 36%.[55,56] Furthermore, the administration of ADXS 11-001 attenuated the vaccine directed at viral antigens, before robotic transoral resection in a selected cohort of patients with HPV+ oropharynx carcinoma (NCT02002182), which is also being investigated.

Different approaches are being developed in the field of immunotherapy, including tumour-specific monoclonal antibodies, vaccination against tumour or viral antigens, immunomodulatory antibodies, oncolytic viruses, and cell therapy with adoptive cell transfer. Immunotherapy features a more favourable toxicity profile than neoadjuvant chemotherapy, which makes it another promising pathway in the combination of these drugs with alternative induction chemotherapy patterns such as platinum–taxane doublets. In this sense, the phase II clinical trial NADIM explores the viability, safety, and efficacy of a three-cycle administration of neoadjuvant nivolumab in combination with carboplatin and paclitaxel in patients with stage IIIA resectable NSCLC followed by 12 months of nivolumab postoperatively. Data of 41 patients have been published, which confirm a higher pathological response rate (<10% acceptable tumour cells) of 86.4% (complete pathological response: 71.4%) and a favourable toxicity profile and no delays in the surgery.[57]

Therapeutic response assessment criteria

To implement this new therapeutic strategy, it is essential to establish reliable tools to assess the efficacy of the treatment before tumour resection and homogeneous and validated criteria for appropriate evaluation of pathological response with the aim of dismissing a quick disease progression that could compromise the execution of a drastic surgical treatment. Due to the differences in the mechanisms of action between immunotherapy and chemotherapy, adjustments to the classic criteria of radiological and pathological response are needed.[58,59] The RECIST criteria used for the evaluation of chemotherapy response are based on the premise that therapeutic response leads to a decrease in the size of tumour. It is known that anti-*PD-1*/*PDL-1* drugs can result in responses with diverse kinetic patterns. There may be an initial growth of the lesion that is accredited to an increase in the tumour lymphocytic infiltrate through immune effector cells or to the period up to the activation of immune cells where the tumour may grow while the immune system is preparing an antitumour response. Clinical trials have revealed that pathological response does not correlate with the radiological response observed on CT. Therefore, specific criteria of the response are required (iRECIST criteria).[59]

In addition to radiological assessments, metabolic imaging studies using positron emission tomography (PET) with 2-deoxy-2-(fluorine-18)fluoro-D-glucose integrated with CT may add relevant information in assessing the response. It is accepted that metabolic changes in the tumour precede anatomic changes, which might allow for more precise early assessment of the response despite the limited ability of PET/CT in delineating tumour inflammation when the capture is low.[60–62] The complete pathological response of a tumour has been the primary objective of neoadjuvant chemotherapy trials. However, the evaluation of the pathological response in trials on immunotherapy must assess the tumour and stromal changes in the immune microenvironment. There are no specific criteria to assess the pathological response in patients treated with immunotherapy. However, there are recent criteria for NSCLC that have defined the specific histological findings of immune tumour regression with high reproducibility and low variability between pathologists.

Cottrell *et al.* assessed the relationship between the pathological response to immunotherapy and prognosis[27] in patients with NSCLC treated with preoperative *PD-1* blockade. Resection samples of 20 patients who went through complete resection were analysed before and after the treatment; the evolution of lesions was assessed using CT. Based on the findings the 'immune-related pathologic response criteria' were developed, which allow for a more accurate assessment of the prognosis and comparisons between clinical studies on neoadjuvant environment. They classified patients as complete pathological response with 0% post-treatment residual viable tumour (RVT) and major pathologic response with ⩽10% RVT.

In order to measure the immune-related pathologic response, the regression site was considered as the main feature of the immune-mediated pathologic response, which is defined specifically by proliferative fibrosis with neovascularization and evidence of immune activation and cell death. In this system, the tumour site is determined by the addition of RVT + necrosis + regression site. Therefore, assessing the response by comparing immune-mediated changes of the tumour tissue before and after immunotherapy will provide valuable information regarding the development of a treatment strategy.

Effectiveness predictive biomarkers

The main disadvantage of neoadjuvant immunotherapy is the low rate of objective response (lower than 20%) and its limited effects in delaying the progression in most patients with advanced disease (although results in earlier lines of treatment were better). Reports suggest that 70% of patients make progress during the first months of taking nivolumab and have a median time of 2 months until disease progression is observed. Similar results were obtained with other anti-*PD-1* and anti-*PD*/*L1* drugs.[10–12,63,64] Several mechanisms have been suggested for this observation, such as poor tumour immunogenicity, weak intratumoral immune cell infiltration, co-expression of inhibitory receptors, and immunosuppressive tumour microenvironment. Therefore, it is important to understand the immunological events that occur as a result of anti-PD-1 antibodies. Specifically, in HNSCC, none of them have been validated and investigations are underway.

The identification of biomarkers predictive of response can prevent the commitment of the surgical treatment due to progression. The neoadjuvant scenario allows for translational studies and monitoring of molecular changes in biological samples (peripheral blood, saliva, pre- and post-treatment tumour tissue), thus correlating the pathological response and mutational and potential neoantigen load. *PD-L1* expression on immunohistochemistry in tumour cells and immune cells of the tumour microenvironment is one of the extensively studied biomarkers.[65–67] Although data suggest that the expression of *PD-L1* correlates with more effectiveness of treatment with ICI,[10–14,63,64] this correlation is not definitive and its potential as the only biomarker for the selection of subgroups of patients is limited due to its dynamic nature, high intratumour heterogeneity, and technical aspects related to its assessments. Different companies use different antibodies and platforms with specific quantification and interpretation criteria – 22C3 and 28-8 (Dako) for pembrolizumab; SP142 and SP263 (Ventana) for atezolizumab and durvalumab, respectively, with different cut-off points on immunohistochemistry (from >1% to >50% of cells that stain for *PD-L1*) – which results in non-specific definition of positive staining with up to 25% difference depending on the antibody used. In light of the different expression of *PD-L1* in tumour cells and inflammatory cell infiltrate, two methods that can be useful are the tumour proportion score (TPS) and combined positive score (CPS).

Recently, the phase III trial KEYNOTE-048 demonstrated overall survival benefits with pembrolizumab in comparison with EXTREME regimen chemotherapy in tumours with *PD-L1* expression measured using CPS $\geqslant$1 and $\geqslant$20.[13,14] However, in the phase III KEYNOTE-0-40 trial, clinical benefit is relevant only in tumours with high expression of *PD-L1* in tumour cells (TPS: 50%). These results highlight the limited role of *PDL-1* as a unique biomarker, although CPS appears to be more predictive than TPS in HNSCC. It is also known that *PDL-1*-negative tumours also benefit from ICI treatments. Therefore, factors beyond *PD-L1* expression might contribute to response.

The HPV status[67,68] correlates with better prognosis in patients with HNSCC and appears to remain stable in patients treated with ICI. Patients with HPV+ squamous oropharynx carcinoma demonstrate a lesser 'immunosuppressor' microenvironment compared with patients with HPV– status along with greater tumour-infiltrating lymphocytes (TILs) infiltration, greater proportion of *CD8*+ T-cells, increase in *IFN-γ* levels, decrease in the *CD4*+/*CD8*+ rate, and smaller presence of Tregs. These findings are linked to a previous adaptive immune response against viral antigens that, in turn, can result in *PD-L1* expression in immune cells. The theory of better response to treatment with ICIs based on HPV status was initially confirmed by the results of subgroups analysis in phase I and II trials of pembrolizumab that revealed a response rate of 22% (*p16*+) *versus* 16% (*p16*–) in the KEYNOTE-055 trial and 32% *versus* 14%, respectively, in the KEYNOTE-012 trial. However, these results were not confirmed in other studies. In the CHECKMATE-141 trial, there were no significant differences in ORR or overall survival (OS)

between HPV+ and HPV– patients. This variability could be explained by interventions due to other coexisting factors, such as smoking, immune infiltrate, and mutational load.[69]

The composition of the inflammatory infiltrate within the tumour microenvironment has prognostic implications. However, it is still to be determined whether it can be a predictor of the response because *CD8+* T-cell infiltrate is the only subgroup that has been demonstrated to increase in response to treatment and survival of HNSCC.[67,70,71] In addition to the composition, the distribution of different immune cell subtypes may play a predictive role; a higher proportion of *CD8+* T-cells in the tumour centre *versus* the invasive margin (high 'immunoscore') is correlated with lower infiltration by Tregs and increase in *PD-L1* and *MHC-I* expressions in tumour cells. A sub-study of the CHECKMATE-141 trial analysed patients who were treated with nivolumab after progression; it reported that patients with a favourable response demonstrated a lower basal count of circulating *CD8+ PD-1+* T-cells and lower Treg count on day 43.[72]

In a recent study of 10 patients with oral cavity carcinoma, the phenotypes of *CD4+* and *CD8+* T-cells subpopulations as well as their expressions of immune mediators were assessed before and after treatment with nivolumab prior to definitive surgical resection. The results demonstrated that nivolumab resulted in a reduction in blood levels of *CD4+* T-cells but an increase in the proportion of *Foxp3+ CD4+* T-cells. An increase in the proportion of *CD8+* cells (specifically, *CD8dimCD3+* T-cells) and expressions of immune mediators *IFN-γ* and *granzyme B* were also reported.[73]

Although the available data are limited, they suggest that there is potential for the identification of a subgroup of tumours with increased sensibility to ICI treatment based on the immune cells and co-expression of inhibitory checkpoint ligands in tumour microenvironment and peripheral T-cell subpopulations. Prospective validation is necessary and oral cavity cancer is an interesting model that allows for repeated biopsies to study the dynamic changes that occurred during treatment using immunophenotyping of circulating T-cell subpopulations *versus* TILs.

In regular tissue, *PD-L1* expression is induced by *IFN-γ* as a protective mechanism against exaggerated immune response. Therefore, the assessment of *PD-L1* and *IFN-γ* can represent a way to establish the presence of TILs. The cancer genome atlas assessment revealed that between one-third and one-half of patients with HNSCC presented with an 'inflamed' phenotype based on gene expression signatures of *IFN-γ*. Apparently, 'inflamed phenotype' signatures can allow for the selection of patients who will benefit from anti-*PD-1*/*PD-L1* treatment.[16] In the populations of the KEYNOTE-012 and KEYNOTE-055 trials, six genes regulated by *IFN-γ* (*CXCL9, CXCL10, IDO1, IFN-γ, alpha chain HLA-DR*, and *STAT1*) were analysed; the results demonstrated that higher scores correlate with response to pembrolizumab with longer progression-free survival and overall survival (irrespective of HPV status).[74,75] However, additional studies are required to assess gene signatures prospectively.

The correlation between the tumour mutational burden (TMB) and the probability of response to immunotherapy has been widely researched in different tumours, including HNSCC.[76,77] Melanoma and cutaneous squamous-cell carcinoma (15–50 mut/MB) followed by smoking-related tumours (NSCLC, urothelial cancer, and HNSCC) with 5–10 mut/MB are malignant tumours with the highest TMB. Retrospective analysis of subgroups of clinical trials that assessed pembrolizumab, atezolizumab, and nivolumab in metastatic melanoma, NSCLC, urothelial cancer, and HNSCC proved not only an increased response rate, but also survival benefits in patients with high TMB.[78–80] In a retrospective analysis of 126 patients with HNSCC treated with anti-*PD-1*/*PD-L1* therapy, TMB was higher in responsive patients (21.3 *versus* 8.2 mut/MB [mutations per magabase]; $p < 0.01$) and correlated with increase in the median OS (20 months if TMB >10 mut/MB *versus* 6 months if TMB <5 mut/MB; $p = 0.01$) in HPV– tumours.[70]

PD-L1 expression influences the response to anti-*PD-1* in tumours with high TMB. However, the response may not depend on the expression of *PD-L1* in the context of the combination therapy of anti-CTLA-4 or anti-PD-1/CTLA-4. The different techniques used for tumour tissue and peripheral blood and cut-off points used to determine 'high' TMB for effective prediction of response are not well established and require further prospective validation studies.

The oral cavity is constantly exposed to environmental factors that can alter the oral microbiota.

Multiple retrospective cohort studies on HNSCC have suggested that the specific composition of the digestive tract microbiome is associated with chronic inflammatory events and higher risk of carcinoma, treatment-related toxicity, and disease recurrence. The variability in the microbiota composition in oropharyngeal and oral cavity carcinomas indicates the presence of specific microbiota according to tumour location and HPV status. The relationship between the composition and higher microbial diversity of the intestine microbiota, anticancer immune responses, and efficacy to immunotherapy is well established in melanoma and lung and kidney cancers.[67,81–83] A study on the microbiome present in the saliva of patients with HNSCC before and after treatment (including surgery, chemo-radiotherapy, and ICI) reported a link between the specific presence of certain oral bacteria, such as *Fusobacterium* and *Lactobacillus*, the inhibition of immune checkpoint signalling pathways, and the stimulation of *Wnt/Beta-catenin* oncogenic pathways.[84] However, it has not been possible to establish a correlation between the composition of oral microbiome and the efficacy of anti-*PD-1* treatment in HNSCC,[85] possibly due to the heterogeneity and small size of the study populations in the retrospective studies.

The potential use of oral microbial composition as a predictive biomarker remains to be determined. Oral cavity tumours are an ideal model to investigate whether the changes observed in the oral microbiota are influenced by tumour microenvironment and/or local and systemic treatment, the relationship between oral and intestinal microbiota, and potential therapeutic modulation of the oral microbiota to increase the response to immunotherapy.

Conclusion

Despite aggressive multidisciplinary treatment, the prognosis of advanced resectable OCSCC is poor. Advances in the field of immunotherapy during the last decade have demonstrated improvements in survival and better toxicity profile with a variety of tumours and clinical scenarios. OCSCC is a highly interesting neoplasm in the assessment of neoadjuvant immunotherapies because of its accessible location that allows for closer monitoring during the preoperative treatment period and the possibility of serial tumour, blood, and saliva samples for translational studies aimed at identifying biomarkers linked to the efficacy and resistance. Preliminary trials have proven the efficacy and safety of anti-*PD-1* drugs in these patients. If the ongoing trials prove a decrease in the relapse rate and improvements in the overall survival after surgical resection, preoperative immunotherapy will probably be established as a treatment option for patients with early stages of the disease. Despite the promising results observed, key aspects regarding the duration of treatment, combination patterns, biomarkers of response, optimal monitoring of therapeutic response, and the long-term effects are yet to be clarified.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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