Therapeutic Approaches With Immune Checkpoint Inhibitors in Head and Neck Cancers and the Role of PD-L1 as a Biomarker

Karima Oualla, MD1, Luis Castelo Branco, MD2, Lamiae Nouiyakh, MD1, Lamiae Amaadour, MD1, Zineb Benbrahim, MD1, Samia Arifi, MD1, and Nawfel Mellas, MD1

Abstract
Head and neck squamous cell carcinoma (HNSCC) is a potential curative disease at its localized stage, by the use of multimodal treatment including surgery, radiation, and chemotherapy. While the metastatic stage is considered incurable and is characterized by poor prognosis. Conventional cytotoxic chemotherapy in addition to cetuximab were the only available systemic treatment with limited efficacy and modest median overall survival barely crossing the 1 year limit. Immunotherapy with PD-1 and PD-L1 inhibitors has revolutionized the treatment of multiple cancers. Recently, Immunotherapy is being extensively explored in head and neck cancer and clinical trials have shown impressive results that allowed to immune check point inhibitors to be the new standard of care. In this article we tried to explain the rationale and mechanisms of targeting the immune system in head and neck carcinoma and to report the results from the phase III clinical trials that put the immunotherapy as a new standard of care for head and neck cancer.

Keywords
head and neck, squamous cell carcinoma, PD-L1, immune checkpoint inhibitors

Introduction
Worldwide, head and neck cancer accounts for more than 650,000 cases and 330,000 deaths annually.1 In the United States, head and neck cancer accounts for 3 percent of malignancies, with approximately 53,000 Americans developing head and neck cancer annually and 10,800 dying from the disease.2

The main conventional implicated risk factors are consumption of tobacco and alcohol. However, the incidence of smoking related HNSCC is decreasing over years and the Human papillomavirus (HPV) is becoming a causative agent in about 25% of all HNSCC especially the HPV 16 and HPV 18.2

The relative prevalence of these risk factors contributes to the variations in the observed distribution of head and neck cancer in different areas of the world.

The outcomes of patients with HNSCC have been considerably improved with the multimodal treatment. However the prognosis of metastatic patients remain poor.

Recently, the better understanding of the immune system led to development of novel immunotherapies, including anti-PD-L1 blockers, that had shown promising results with improvement of survival rates in the advanced stage.

Immunogenicity of Head and Neck Cancers
Head and neck squamous cell carcinoma (HNSCC) is a highly immunosuppressive cancer characterized by high immune infiltration.
Cytotoxic T-lymphocytes, characterized by the expression of CD8, play a crucial role in targeting and destroying cancer cells through binding to MHC class I molecules. The presence of CD8+ lymphocytes in the tumor microenvironment has been associated with favorable prognosis.\(^3\)

Additionally, Human papillomavirus positive (HPV+) HNSCC has one of the higher levels of infiltrating regulatory T-cells (Tregs) that has been associated with better prognosis.\(^3\)

The Tregs may be assessed by immunohistochemical analysis for FoxP3, which is the most specific Treg marker. They play a major role in maintaining immunological tolerance and are considered to be suppressors of the anti-tumor immune response.

The presence of Tregs in the micro-environment of the tumor is predictive of unfavorable outcome but with variable prognostic value among different types of cancer. For HNSCC, previous studies suggested that high Treg counts are associated with better prognosis.\(^4\)

Additionally, HNSCC genomes showed high instability with relatively high tumor mutation burden (TMB). The Cancer Genome Atlas (TCGA) has generated a comprehensive landscape of genomic alterations by analyzing molecularly 279 HNSCCs. It was found that HPV+ cancers are characterized by helicase domain mutations of the oncogene PIK3CA, loss of TNF receptor-associated factor 3 (TRAF3), and amplification of the cell cycle gene E2F1.\(^5\)

While smoking-related HNSCCs showed loss of TP53 mutations and CDKN2A with frequent alterations including a novel amplification of 11q22.

In the distinct subgroup of laryngeal cancers, there were frequent novel loss of function alterations of the chromatin modifier NSD1, Wnt pathway genes AJUBA and FAT1, and activation of oxidative stress factor NF2L2.

Regarding oral cavity cancers associated with favorable evolution, they were found to harbor activating mutations of HRAS or PIK3CA, in addition to inactivating mutations of CASP8, NOTCH1 and wild-type TP53.\(^5\)

Various suppressive mechanisms are involved including alterations of tumor human leukocyte antigen (HLA) class I molecules expression associated with overexpression of antigens causing T-cell tolerance, and high level of immunosuppressive cytokines such IL-10, IL-6 and TGF-β. Another mechanism is the aberrant activation of the transcription factors Signal Transducers and Activators of Transcription 3 (STAT3) in addition to NF-kB, which are linked to IL-6 and TGF-β signaling. HNSCC is also characterized by the presence of immunomodulatory agents such as Cytotoxic T-Lymphocyte-associated antigen 4 (CTLA-4), Programmed death-1 (PD-1) and their ligands which has allowed the development of new therapeutic strategies.\(^3\)

It was demonstrated that metastatic sites have different genomic alterations from the primary site. Therefore, circulating tumor cells (CTCs) may have a crucial role to identify patients at-risk of developing metastases. It was also shown that PD-L1 is expressed in HNSCC tumors and CTCs and may contribute to the tumors ability to evade the immune system. Therefore, CTCs may provide the possibility to analyze the metastatic seeds in circulation and identify patients that are likely to benefit from PD-L1 blockade.\(^6\)

Immune-checkpoint inhibitors (ICI) are now the standard of care in head and neck squamous cell carcinoma (HNSCC). However, it’s being reported around 60% of primary resistance to anti-PD-1/PD-L1 in these tumors.\(^7\) Several mechanisms might be implicated such as expression of other inhibitory receptors, activation of immunosuppressive pathways within the tumor microenvironment and poor immunogenicity of the tumor with low intratumoral immune cell infiltration.\(^8\)^\(^-\)^\(^10\)

Therefore, the concept of combination of ICI with other classes of ICI, chemotheraphy, radiotheraphy or targeted therapies has emerged to overcome this resistance in addition to the investigation on potential predictive biomarkers such as PDL1 immune infiltration, tumor mutational burden (TMB) and immune-gene expression profiling.\(^11\)

**Phase III Clinical Trials Leading to New Standards of Care**

In CheckMate-141 phase III study that compared nivolumab to the investigator’s choice (IC) after failure of platinum-based therapy,\(^12\) nivolumab showed better OS of 7.5 months versus 5.1 months and hazard ratio (HR) = 0.68 (95% CI 0.54-0.86) (Table 1). Nivolumab demonstrated OS benefit across patients with tumor PD-L1 expression ≥1% (HR [95% CI] = 0.55 [0.39-0.78]) and < 1% (HR [95% CI] = 0.73 [0.49-1.09]), and regardless of tumor HPV status.\(^12\) Therefore, the effect of nivolumab on OS was more pronounced in PD-L1 positive (≥1%) patients but there was no correlation in this study where PD-L1 expression was only determined in tumor cells, although the thresholds used were different (>1%, 5% and 10%). Updated results concluded that patients benefited from nivolumab regardless of PD-L1 expression.

In KEYNOTE-040 phase III clinical trial, in second line after platinum therapy, pembrolizumab compared to chemotherapy, showed marginal improvement of median OS (8.4 versus 7.1 months, [HR] 0.81 95% CI 0.66-0.99, P = .0204). In patients with tumors expressing PD-L1, the benefit was significantly higher with pembrolizumab specifically in patients with combined tumor and immune cell PD-L1-expression CPS ≥1%. Median OS was 8.7 months with pembrolizumab versus 7.1 months with standard treatments (HR 0.75; 95% CI 0.59-0.95, P = .0078), and also in patients with PD-L1-expression ≥50% of their cancer cells, median OS was 11.6 versus 7.9 months (HR 0.54; 95% CI 0.35-0.82, P = .0017).\(^13\)

These findings support the use of pembrolizumab in this population, especially among those with high PD-L1 expression.

In first line setting, the phase III KEYNOTE-048 study that randomized patients to platinum-based chemotherapy and cetuximab versus pembrolizumab alone; or the combination of pembrolizumab and platinum-based chemotherapy.\(^14\) In the comparison of pembrolizumab alone to standard treatment, results showed that OS was significantly improved with pembrolizumab for the subgroups of patients with...
PD-L1 CPS ≥1 and PD-L1 CPS ≥20. In the CPS ≥1 subgroup, the median OS was 12.3 months for the pembrolizumab arm and 10.3 months for the standard treatment (HR = 0.78; 95% CI = 0.64-0.96; P = 0.0086). For the CPS ≥20 subgroup, the median OS was 14.9 months with pembrolizumab and 10.7 months with standard treatment (HR = 0.61; 95% CI = 0.45-0.83; P = 0.0007).

There was no significant difference in OS between the 2 arms for the overall population.\(^\text{14}\)

In the second comparison of the combination of pembrolizumab and platinum-based chemotherapy versus standard treatment in all comers regardless of PD-L1 status, OS was improved with the combination in overall population (13.0 months versus 10.7 months, HR 0.77, P = 0.0034).

Results were similar in the CPS ≥20 subgroup (HR = 0.69; 95% CI = 0.51-0.94) and CPS ≥1 subgroup (HR = 0.71; 95% CI = 0.57-0.88).\(^\text{9}\) Therefore, the addition of chemotherapy to pembrolizumab has an additional effect in PDL-1 negative HNSCCs by improving significantly survival in this subgroup of patients where the monotherapy with pembrolizumab didn’t show significant benefit.

Regarding safety, grade 3 or worse all-cause adverse events occurred in (55%) of treated patients in the pembrolizumab alone group, 85% in the pembrolizumab with chemotherapy group, and 83% in the cetuximab with chemotherapy group. Adverse events leading to death were noted in 8% of participants in the pembrolizumab monotherapy group, 12% in the pembrolizumab with chemotherapy group, and 10% in the cetuximab with chemotherapy group.\(^\text{14}\)

After these findings, Pembrolizumab was approved for use as first line treatment in combination with platinum and fluorouracil (FU) for all patients and as a single agent for patients whose tumors express PD L1 ([CPS] ≥1). These results also established PD-L1 CPS as a valid marker for head and neck cancer that should be measured to select patients for pembrolizumab alone, or combined with chemotherapy.

Higher PD-L1 expression is associated with more benefit but more analyses are needed to determine the right cut-off and potential new biomarkers especially in patients who have tumors with low or absent PD-L1 expression such as human papillomavirus (HPV) status, tumor immune infiltration or TMB.

### Learned Lessons and Future Research

KEYNOTE-048 showed improvement of OS with pembrolizumab monotherapy in first line R/M HNSCC when PD-L1 expression ≥1% and ≥20% by CPS. While combination of pembrolizumab and chemotherapy showed benefit regardless of PDL-1 expression.

However, in KEYNOTE-040, the correlation with clinical outcome was also strongly positive when using PD-L1 expression in tumor cells only (TPS ≥50%).

In contrast, no correlation in the nivolumab CHECKMATE-141 study where PD-L1 expression was exclusively determined in tumor cells. No firm conclusion can be made, although CPS seems to be more predictive than TPS in HNSCC.

To date, no validated predictive biomarkers that are applicable uniformly to all HNSCC patients, other biomarkers are under investigation: HPV status, tumor immune infiltration, gut microbiota and TMB. More research is needed to validate these new promising prognostic and predictive biomarker in this disease.

Future directions should focus on next generation sequencing and identification of more refined biomarkers for better selection of patients who can benefit from different immune check inhibitors.

### Authors’ Note

All authors contributed in writing and approving the final manuscript. Our study did not require an ethical board approval because it did not contain human or animal trials.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD
Karima Oualla https://orcid.org/0000-0002-7677-6492

References
1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6):394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-164.
3. Forster MD, Devlin MJ. Immune checkpoint inhibition in head and neck cancer. Front Oncol. 2018;8:310.
4. Shang B, Liu Y, Jiang SJ, Liu Y. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep. 2015;5:15179.
5. The Cancer Genome Atlas Network, Genome sequencing centre: Broad Institute, Lawrence M, et al. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517(7536):576-582. doi:10.1038/nature14129
6. Kulasinghe A, Perry C, Kenny L, et al. PD-L1 expressing circulating tumour cells in head and neck cancers. BMC Cancer. 2017; 17(1):333. doi:10.1186/s12885-017-3316-3
7. Kok VC. Current understanding of the mechanisms underlying immune evasion from PD-1/PD-L1 immune checkpoint blockade in head and neck cancer. Front Oncol. 2020;10:268. doi:10.3389/fonc.2020.00268
8. O’Donnell JS, Long GV, Scolyer RA, et al. Resistance to PD1/PD-L1 checkpoint inhibition. Cancer Treat Rev. 2017;52:71-81.
9. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016;8(328):328rv4.
10. Schalper KA, Kaftan E, Herbst RS. Predictive biomarkers for PD-1 axis therapies: the hidden treasure or a call for research. Clin Cancer Res. 2016;22(9):2102-2104.
11. Oliva M, Spreat hico A, Taberna M. Immune biomarkers of response to immune-checkpoint inhibitors in head and neck squamous cell carcinoma. Ann Oncol. 2019;30(1):57-67.
12. Ferris RL, Blumenschein G, Fayette J, et al. Nivolumab vs investigator’s choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. Oral Oncol. 2018; 81:45-51.
13. Soulieres D, Cohen E, Le Tourneau C, et al. Updated survival results of the KEYNOTE-040 study of pembrolizumab vs standard-of-care chemotherapy for recurrent or metastatic head and neck squamous cell carcinoma. Abstract. Cancer Res. 2018; 78(13):CT115.
14. Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet. 2019;394(10212):1915-1928. doi:10.1016/S0140-6736(19)32591-7