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Review

Immunotherapy in recurrent/metastatic head and neck squamous cell carcinoma: PD-L1 and beyond

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Summary
Head and neck squamous cell carcinoma (HNSCC) is a prominent global health concern because of its high incidence, aggressive clinical behavior, and scarce therapeutic options. The management of these neoplasms in the recurrent/metastatic setting has been revolutionized following the results of key clinical trials, leading to the advent of immunotherapeutic agents targeting the PD-1/PD-L1 axis. Despite the exciting results obtained with the new drugs, immunotherapy is helpful only in a sizable minority of patients, and there is a pressing need to identify reliable predictive biomarkers for patient selection. The immunohistochemical assessment of PD-L1 expression was initially identified as a powerful and easily accessible predictive tool, and gained its place as the current standard for patient selection, but it has clear limitations. The imperfect predictive power of PD-L1 has resulted in a strong effort to discover additional clinical, pathological and molecular biomarkers such as tumor HPV status, mutational burden, microsatellite instability, and much more. In addition, the tumor microenvironment has been extensively studied searching for promising new biomarkers as potential avenues for refining patient selection and improvement of treatment outcomes. As we gain deeper understanding of the complex interplay between tumor biology, immune system, and tumor microenvironment, we are rapidly realizing that the perfect biomarker, the magic bullet, probably doesn't exist. On the other hand, with the introduction of new drugs on the horizon, integration of multiple variables in the context of combined predictive scores is shaping up to be our best weapon in this strife to treat each patient with the best possible drug.

Key words: head and neck squamous cell carcinoma, immunotherapy, biomarkers, PD-L1

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Background

Head and neck squamous cell carcinoma (HNSCC) is a significant global health concern, representing the sixth most common cancer worldwide with almost 900,000 new cases and 450,000 deaths annually [1]. It comprises a heterogeneous spectrum of diseases originating from the oral cavity, oropharynx, nasal cavity, hypopharynx, and larynx.
HNSCC is characterized by aggressive behavior, high rates of recurrence, and limited treatment options, especially in the recurrent and metastatic (R/M) settings [2]. Indeed, despite the multimodality treatments available, disease recurrence and/or metastasis are frequently associated with a poor prognosis, with survival averaging less than one year [3-5]. Furthermore, most HNSCCs are diagnosed at advanced stage, with loco-regional lymph node involvement, and approximately 10% of

patients have distant metastases at initial presentation [6]. Before the advent of immunotherapy, first-line treatment options included combination regimens of cytotoxic agents in combination with cetuximab, a chimeric human anti-epidermal growth factor receptor monoclonal antibody [7,8]. On the other hand, taxanes and methotrexate were the most widely used chemotherapeutic agents in platinum-refractory disease, but none of these drugs showed a clear benefit in terms of overall survival (OS) [9]. The advent of immune checkpoint inhibitors (ICIs) has remarkably changed the management of R/M HNSCC [10].

Normally, immune checkpoints enable the immune system to respond to infections and malignancies and to protect normal tissues from damage. However, this machinery can be hijacked by neoplasms to induce immune tolerance [11]. The complexity of this escape strategy is far to be fully understood and can pivot around the several receptors and ligands involved, including the programmed death receptors (e.g. PD-1), their ligands (PD-L1 and PD-L2), and all the costimulatory and inhibitory associated proteins (e.g. CD40L, CTLA-4, LAG-3, and TIM-3) (Fig. 1) [12]. When a programmed death receptor and its ligand interact, the effector T cells carrying the receptor become unable to eliminate tumor cells, resulting in immune escape by the tumor. On this basis, ICI therapy aims to prevent the interaction between the programmed death receptor (PD-1) on the surface of T cells and its ligand PD-L1, expressed by the tumor cell [12].

In the last years, the discovery of ICIs has revolutionized oncology, and the field of HNSCC was involved in a series of clinical trials to assess a possible role for immunotherapy in this neoplasm [13]. Two ICI agents, in particular the PD-1 inhibitors nivolumab and pembrolizumab, have been approved by FDA in 2016 for R/M HNSCC following the results of the CheckMate 141 and KEYNOTE 040 trials, respectively [14,15]. These landmark trials demonstrated significant and durable clinical benefits with immunotherapy, in terms of both response rates and overall survival, for a subset of HNSCC patients who had failed prior platinum-based chemotherapy [16,17].

A few years later, in 2019, after the result of the KEYNOTE 048 trial [18] the FDA granted approval for PD-1 inhibition as first-line treatment for patients with metastatic or unresectable, recurrent HNSCC, approving pembrolizumab in combination with platinum and fluorouracil for all patients with HNSCC and pembrolizumab as a single agent for patients with HNSCC

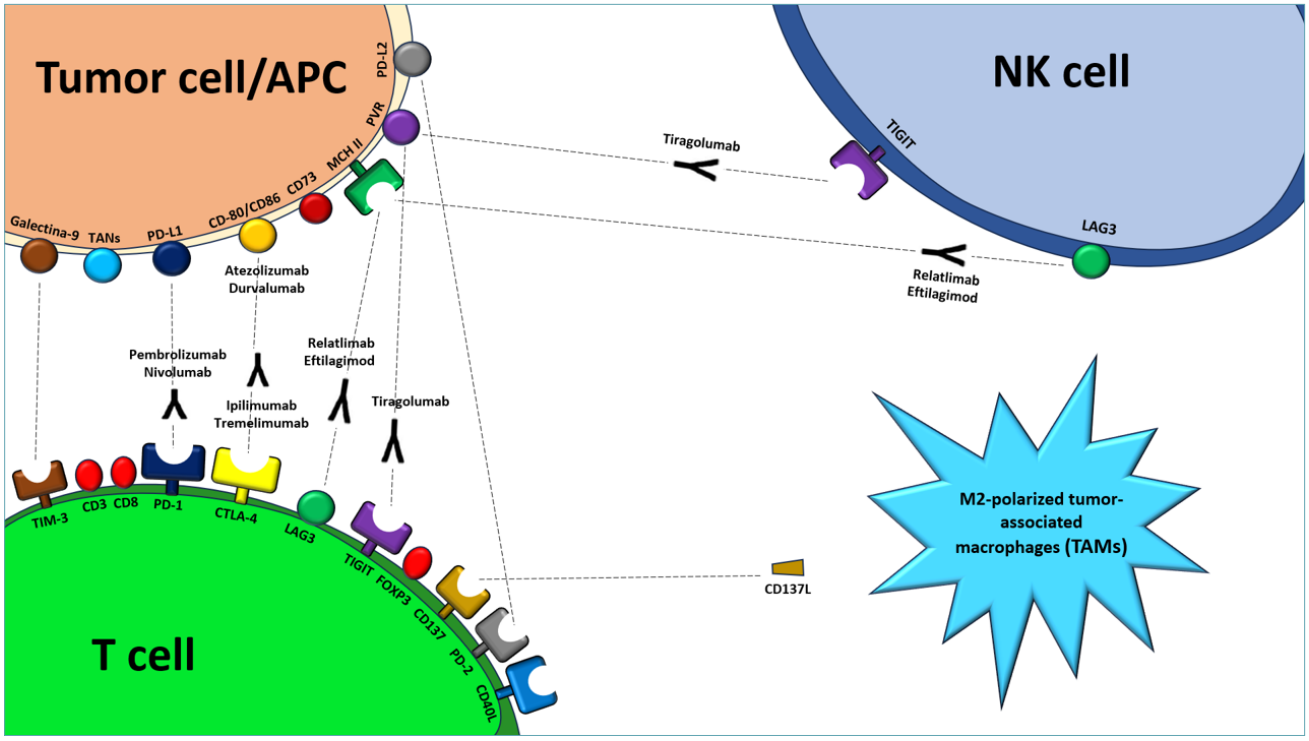

Figure 1. The interplay between tumor cells and the immune microenvironment is a complex balance regulated by a plethora of receptors, ligands and co-stimulatory/inhibitory molecules. Several of these interactions can be affected by existing drugs, mostly monoclonal antibodies, and many others are undergoing research to assess their potential as therapeutic targets.

whose tumors express a PD-L1 combined positive score (CPS) ≥1. The expression of PD-L1 on tumor cells and infiltrating immune cells is currently assessed by immunohistochemistry on formalin-fixed paraffin-embedded (FFPE) tissue to identify patients eligible for ICI therapy. The CPS is calculated as the number of PD-L1 positive invasive cancer cells, lymphocytes, and macrophages, divided by the number of viable tumor cells and multiplied by 100. Unfortunately, this predictor is not infallible and may not indicate a long-term response. In fact, only a marginal subset of patients with advanced HNSCC derives meaningful clinical benefit from the new agents (overall response rate not exceeding 35%) [19,20]. Lastly, the onset of serious immune-related adverse events is not an unlikely occurrence, and mandates caution in the administration of these treatments [21]. Consequently, a tremendous need emerged to identify reliable and practical predictive biomarkers to optimize patient selection and to guide the development of more cost-effective immunotherapeutic strategies for HNSCC.

This review aims to provide a comprehensive overview of the currently available and promising predictive biomarkers in the landscape of immunotherapy in R/M HNSCC.

Biomarkers in HNSCC

PD-L1: LIGHTS AND SHADOWS

Advances in immunology and oncology have expanded our knowledge on the topic of immune checkpoints and their role in solid neoplasms, including HNSCC. Immune checkpoints are essential for regulating the immune response and maintaining self-tolerance, but they can also be hijacked by tumor cells to evade immunosurveillance [22]. Several immune checkpoint proteins have been identified as relevant therapeutic targets, and the first wave of immune checkpoint inhibitors to be developed and clinically tested in HNSCC targets the PD-1/PD-L1 axis [10]. By expressing PD-L1, tumor cells interact with the inhibitory receptor PD-1 on effector T cells, neutralizing their activity and dampening the antitumor immune response.

Several scoring systems evaluating tumoral and immunological cellular compartments were developed for quantifying PD-L1 expression in different kinds of malignancies: among these: (i) tumor proportion score (TPS) estimates the percentage of viable neoplastic cells showing partial or complete membrane staining relative among all viable tumor cells; (ii) the immune cell score (IC) refers to the area occupied by PD-L1 positive immune cells (lymphocytes, dendritic cells, macrophages, and granulocytes) as a percentage of the whole tumor area and; (iii) CPS is the ratio of the number of all PD-L1–positive cells (tumor cells, lymphocytes, macrophages) to the number of all viable tumor cells [13].

The many clinical trials performed to test immune checkpoint inhibitors in HNSCC have used different immunohistochemical assays and different thresholds to define PD-L1 positivity, leading to a notable lack of standardization across the field and eventually to the approval of companion diagnostics for the administration of specific drugs [13]. This inconsistency is evident in the development of anti-PD-1/PD-L1 agents investigated to date in R/M HNSCC, including pembrolizumab, nivolumab, atezolizumab, durvalumab and avelumab, thus impairing cross-study comparisons and undermining the value of PD-L1 as a biomarker [23].

In general, tumor PD-L1 expression is associated with improved efficacy with anti-PD-1/PD-L1 therapy in R/M HNSCC, with its predictive value being enriched by the consideration of PD-L1 expression on both tumor cells and tumor-infiltrating immune cells (CPS) [21]. In particular, post hoc analysis of data from the KEYNOTE-040 trial showed CPS and TPS to have equivalent performances at a cutoff of 50, but CPS is more sensitive than TPS at a lower cutoff of ≥1 [24]. Therefore, CPS emerged as the best scoring method and is currently recommended, with evidence pointing at the thresholds of CPS ≥20 and ≥1 as clinically significant, with improved overall response rate, overall survival and progression-free survival in this population when treated with ICIs [18,19].

The predictive value of PD-L1 expression scored as CPS ≥1 is unfortunately far from perfect, with patients testing negative for PD-L1 still occasionally responding to treatment and patients testing positive sometimes displaying only poor and temporary response.

The conflicting observations regarding PD-L1 as a predictive biomarker of tumor response likely reflects several issues, both IHC-test specific and tumor-biology-related.

Concerning the issue of the several immunohistochemical assays available for the evaluation of PD-L1, in the US pembrolizumab was approved by the FDA exclusively for patients with CPS ≥ 1 assessed with the 22C3 PharmDx assay, while in Europe EMA stated that pembrolizumab could be used as first-line treatment for R/M HNSCC in patients with CPS ≥ 1 as assessed using any validated antibody and IHC platform.

This was received positively by the European pathological departments, as many of them had in use assays different than 22C3 PharmDx [25]. Of course, the problem of the concordance of different assays and

platforms in the HNSCC setting was rapidly raised. The first studies addressing this issue reported considerable differences among the assays [26], while the later ones gave reassuring results with high agreement, also demonstrating good inter-observer reliability among pathologists [27,28]. The issue of the inter- and intra-observer reliability has often been considered a critical flaw of PD-L1 testing, but several studies have confirmed that concordance is very high among trained pathologists [29].

Currently, no recommendation exists on whether PD-L1 should be preferably tested on the primary tumor, lymph node metastasis, or distant metastasis when these options are simultaneously available. Studies have demonstrated fair concordance between these sites, with discordant cases usually characterized by higher expression in lymph node metastases [30].

Another critical issue concerns the concordance between biopsies and resection specimens, and even between different blocks of the same resection, but most studies have shown that there is significant reproducibility in both these scenarios [31-33].

A significant problem is also represented by the concordance of PD-L1 expression in tissue from primary disease at initial diagnosis and recurrent disease or metastatic localization, as it can be very difficult to obtain new material in many cases at progression. Concordance studies in this specific setting have yielded contrasting results, with discordance in up to 36% of cases using the only threshold of CPS ≥1 [34,35].

Probably underlying this discordance and also creating a significant problem on its own, is the fact that PD-L1 expression has been shown to decrease when tested on slides from the same block in a matter of months to a few years, with reduced expression involving both tumor and immune cells [36].

In the end, the choice of what exactly should be tested is up for debate and is a decision that should be taken jointly by oncologists and pathologists. Of course, when dealing with borderline cases and particularly small samples, logic dictates caution, and a properly fixed block from the most recent resection specimen is probably to be preferred, if available.

Another important controversy regarding PD-L1 testing in HNSCC concerns the reliability of fine needle aspiration-derived cell blocks as source material. This matter is of no small importance, considering that this can sometimes be the only material that is available or easily obtained. Several studies have addressed the issue, and most evidence points to cytology as underestimating CPS scores, with resulting low negative predictive value and very high positive predictive value [37,38]. Consequently, a positive CPS should be regarded as reliable, while a negative test should prompt further investigation if feasible.

Tumor mutational burden and microsatellite instability

Tumor molecular burden (TMB) can be grossly defined as the total number of mutations present in a tumor [39]. Calculation of the TMB used to be performed through whole exome sequencing, but has since developed to rely on extensive gene panels analyzed through next-generation sequencing [39]. Tumors with higher TMB harbor more neoantigens and are thought to be more immunogenic [40]. Several studies have also shown that, in various solid neoplasms, TMB-high status is associated with improved response to ICIs [41]. Consequently, FDA has granted accelerated approval to the administration of pembrolizumab in patients with metastatic disease found to be TMB-high (≥10 mutations/Mb) by an FDA-approved assay and having no other satisfying treatment option [42].

Regarding HNSCC, the most recent meta-analyses confirm that patients with TMB-high tumors treated with pembrolizumab had a significantly improved overall response rate (OR = 2.62; 95% CI 1.74–3.94; $p < 0.0001$) and a survival advantage (HR = 0.53; 95% CI 0.39–0.71; $p < 0.0001$) compared with patients with TMB-low tumors [43] These results are largely independent of PD-L1 expression [41].

While TMB accounts for a plethora of different kinds of genetic alterations, the type of mutation can also be particularly significant. In fact, the quality of the neoantigens has been postulated to be more important than their quantity, and in the case of HNSCC frameshift mutations have been associated with improved response to ICIs [44].

A recent advance in the field of TMB is its characterization from blood samples using circulating tumor DNA, a reliable, non-invasive technique that has several advantages, including the possibility of repeated sampling during therapy and the possibility of testing patients for which no solid tissue sample is available [40].

Limitations of TMB as a predictive biomarker certainly include the cost of the assays and the fact that the predictive power is currently low, with around 5% of patients with low TMB positively responding to ICIs and > 50% of patients with high TMB not responding [40].

In 2023, the American Society of Clinical Oncology (ASCO) published guidelines for immunotherapy and biomarker testing in R/M HNSCC stating that TMB testing may be performed in patients with recurrent

or metastatic HNSCC when CPS is not available or in patients with rare tumors, and that TMB ≥ 10/Mb should be interpreted as high, correlating with a clinical benefit to PD-1 inhibitors [45].

Currently, no recommendation exists to test TMB in all R/M HNSCC [21] but according to these results, TMB is expected to play an important role in the future.

Microsatellite instability (MSI) is a molecular condition caused by impairment of the DNA mismatch repair system and characterized by genetic alterations in the length of microsatellites, which are short, repetitive DNA sequences scattered throughout the genome. Studies across many different cancer types have suggested that tumors with high MSI (MSI-H)/mismatch repair deficient are associated with higher TMB and display higher sensitivity to ICIs, a consequence of the large proportion of mutant neoantigens that characterize these neoplasms [46,47]. According to these results, in 2017 FDA approved ICI treatment (pembrolizumab) for patients with deficient mismatch repair or MSI-H tumors regardless of histology.

However, the proportion of MSI-H HNSCC is very low (around 1-3%) and so, even though sporadic reports of complete and lasting response to ICIs in these cases exist, there is currently no translational role for MSI in this field, and the current consensus documents recommend against standard MSI testing [21,48,49].

Human Papilloma Virus

In the last decades, the role of Human Papilloma Virus (HPV) as a risk factor for HNSCC, especially in the oropharynx, has become increasingly acknowledged, to the point that HPV+ tumors are now regarded as biologically and clinically distinct from HPV- tumors. Furthermore, with reduction in smoking habits, HPV infection is now considered as the most important risk factor for oropharyngeal HNSCC in the developed world, underlying 45-90% of these cases and around 26% of all HNSCCs [50,51].

HPV+ tumors tend to affect younger patients, male, Caucasian and non-smokers, and often present with large, cystic cervical lymph node metastases. HPV status has important positive prognostic value, as HPV+ tumors are highly responsive to standard therapies [51].

Both HPV+ and HPV- HNSCCs are highly immune-infiltrated neoplasms, but HPV+ HNSCC typically has the highest density of tumor infiltrating lymphocytes (TILs). Patients with HPV+ HNSCC show improved outcomes with PD-1/PD-L1 axis blockade compared to those with HPV– tumors [52,53].

Interestingly, the most recent evidence from large meta-analyses across many cancer types from several different organs, has shown that viral-associated neoplasms (HPV, HBV, HCV) generally show a better response to ICIs, probably due to their increased immunogenicity [51,53].

Nevertheless, there is currently no specific recommendation to test HPV status in R/M HNSCC before starting immunotherapy, as this information is not likely to change therapeutic planning [21].

Tumor Immune Microenvironment

Tumor response to ICIs is known to be not only up to the biology of the neoplastic cells, but also of the surrounding microenvironment, with its specific immunological milieu consisting of the complex interplay of cell populations and molecular signaling pathways.

TILs are known to be direct effectors of antitumor immunity and can be predictors of prognosis in several solid neoplasms, but their role as predictors of response to immunotherapy is still being determined [54-56].

Research in this field is hindered by several issues related to the heterogeneity of available studies, including the exact method of TILs scoring, with some researchers assessing TILs in H&E slides and others using a plethora of possible immunohistochemical molecules/markers (CD3, CD8, FOXP3, etc.). The recently published guidelines of the International Immuno-oncology Biomarker Working Group (IIBWG) formed an essential step towards a standardized assessment method and implementation of TILs in pathology reporting, but they are not yet considered mature for introduction in the clinical routine of HNSCC reporting [55,57].

HNSCCs are known to have a specific tumor microenvironment (TIM), on average being one of the most immune-infiltrated among the solid neoplasms (especially true for HPV+ tumors), with high ratio of Treg/CD8+ T cells and large numbers of CD56dim NK cells [58]. Using gene expression analysis, the TIM of HPV+ HNSCCs was found to have higher expression of genes encoding PD-1, CTLA-4, and TIM3, among others, a possible piece of evidence that the immune infiltrate of these tumors could be largely exhausted [59].

In the setting of HNSCC, higher numbers of CD3+ and CD8+ T cells have generally been linked to improved clinical outcomes [60]. However, results have been heterogeneous when stratified for tumor anatomic subsite and HPV status. For instance, an association between high CD8+ T cells and tumor recurrence was found in oral squamous cell carcinomas [61].

Interpretation of the number of CD4+ T cells comes

with several inherent problems, as many different subsets of these lymphocytes exist, with wildly different immunological roles. Even levels of FoxP3+ Treg lymphocytes, historically considered to have a role in tumoral immune escape, were found to have a much more controversial role across several solid neoplasms, including HNSCC, where some studies have found a positive effect on survival [55,62].

When it comes to prediction of response to immunotherapy, there is already evidence from a large meta-analysis across many cancer types that high CD8+ T cells can predict treatment outcomes in patients with ICIs across different cancers, both in monotherapy and in combination with other therapies [63]. This meta-analysis included HNSCC in the form of only one study, using non-standard ICI regimen, and its specific value in the field is therefore up for debate.

Efforts focusing specifically on HNSCC have given conflicting results, with one study showing positive prediction of anti PD-1 response by CD8+ T cells [44], and another finding no correlation between single subsets of TILs and response [64].

It is important to highlight that TILs are not the only cells playing a role in the TIM. In fact, the presence of myeloid-derived suppressor cells (MDSCs), M2-polarized tumor-associated macrophages (TAMs), and N2 tumor-associated neutrophils (TANs) has been associated with attenuated response to ICI therapy [65,66].

While TILs may not yet be ready to be used as biomarkers, numerous immune-related molecular biomarkers are being investigated in HNSCC, and many seem to hold great promise [13]. Here we will only review some of these.

PD-L2 is a second possible ligand for PD-1. It has been observed that PD-L2 expression is an independent predictor of response to ICI in HNSCC. Furthermore, positivity to both PD-L1 and PD-L2 entails a better response than what is seen with PD-L1 positivity alone [67].

Interferons of type I and II are increasingly recognized as fundamental for the interaction between the immune system and tumor. IFN-γ is considered to be a strong inducer of PD-L1 expression in cancer cells, but its direct effect on response to ICIs is very complicated to predict and probably dependent on several other dynamics [68].

CD73, a protein involved in the extracellular adenosine-generating pathway, is known for its immunosuppressive role in solid neoplasms, and has been linked to reduced response to immunotherapy [69,70].

Indoleamine 2,3-dioxygenase (IDO) is an enzyme produced in inflammatory states that plays a role in limiting harmful inflammation by promoting immunosuppression. IDO has been shown to play a role in the strategy that various tumors, including HNSCC, utilize to escape the immune system [71]. Higher IDO expression carries negative prognostic value in HNSCC and other carcinomas and has already been linked to ICI resistance in non-small cell lung cancer [72]. IDO is shaping up to become a potential therapeutic target in and of itself, but will possibly also have a role as a biomarker for ICI response [68,73].

Exciting advancements are also coming from the characterization of TIM by gene expression profiling. Analysis of hundreds of genes across different cancer types, including HNSCC, has led to the discovery of specific signatures associated with worse clinical outcomes in patients treated with ICIs [14]. Many of the top-ranked genes were directly linked to IFN-γ signaling. Composite scores depending on the expression of these genes were formulated, allowing the identification of populations with overall response rates as high as 40% [74]. These important studies promoted the search for other gene expression profile signatures linked to ICI response, leading to the recent finding of the exceptional positive predictive abilities of a profile linked to overexpression of IFN-I related genes [75].

Liquid profiling

Considerable efforts have been made in oncology to harvest as much information as possible from liquid biopsies, a technique that is now considered ready to move from the bench to the bedside [76]. The term liquid profiling can be used to define the in-depth characterization of the biological information gathered from a liquid biopsy.

The advantages of the liquid biopsy are manyfold: it is easily performed, it circumvents the need for a solid tissue sample, it allows repeated testing over time and is representative of the overall tumor burden across the body and not only of the selected site [77]. The liquid biopsy holds promise to advance our ability to monitor and predict treatment response, detect early relapses and check for minimal residual disease [77]. Introduction of liquid profiling in clinical practice is now a matter of standardization of the pre-analytical and analytical phases, and of approval of certified panels and biomarkers [77].

In the field of HNSCC many possible biomarkers are being investigated, including circulating tumor and immune cells, circulating nucleic acids, tumor-derived vesicles and metabolomic markers [78].

Time will be required to understand which of these biomarkers will predict response to ICIs. Interestingly, PD-L1 can be found in peripheral blood in a soluble form, inside vesicles and on circulating tumor cells,

opening the possibility to test this already established marker on a different type of materials [79]. High levels of blood PD-L1 have been correlated with poor prognosis in patients with HNSCC [80].
Among circulating immune cells, there is evidence that the levels of the $CD3^{+}CD137^{+}$ lymphocyte population, already known to play a role in the antitumor response in several solid neoplasms, may positively predict response to ICIs when tested before the initiation of immune therapy in patients with R/M HNSCC [81].

Other

Smoking

Beyond its role as an important risk factor and carcinogen, smoking is known to influence the biology of solid neoplasms in several ways. In particular, smoking is a known cause of DNA damage, and smoke-related cancers tend to have higher overall mutational loads, leading to the formation of more immunogenic neoantigens [47,82]. These effects could possibly lead to improved immune activity against the neoplasm, but seem to be canceled by the severe and multifaceted immunosuppressive activity that smoking also possesses [76]. The effects of smoking on response to immunotherapy have yet to be elucidated, but smoker HNSCC patients were found to have poorer clinical outcome when treated with ICIs than non-smokers [15,82]. Whether smoking is an independent factor or not is still up for debate in this specific context.

Microbiome

The term oral microbiome defines the complex community of microorganisms that populates the oral cavity. This microbial community consists of bacteria, fungi, and viruses that colonize various surfaces within the oral cavity and beyond. The oral microbiome is incredibly diverse, with hundreds of different species present in a healthy individual [83]. These microorganisms play a crucial role in maintaining oral health through a complex interplay with the host immune system. In fact, the microbiome shapes the local immune system and likely plays an important role in the biological history of neoplasms arising here, across all steps going from carcinogenesis to treatment response [83].
The role of the microbiome has been extensively studied in colorectal cancer, where several different genera of microbes (*Akkermansia*, *Fecalibacterium*, *Bifidobacterium*, etc.) have shown association with response to ICI therapy [2,84,85]. Also interesting is the finding that fecal microbiota transplantation from patients who responded to ICIs into germ-free or antibiotic-treated mice improved the antitumor effects of PD-1 blockade, while microbiota from non-responders failed to do so [86].
Active research is also ongoing in the specific field of HNSCC, which has a direct interplay with its specific microbiome, especially in the oral cavity. No significant associations were detected between oral bacterial diversity and clinical response to nivolumab in the CheckMate141 population [87]. On the other hand, another study demonstrated that antibiotic treatment within one month before the initiation of immunotherapy for the treatment of R/M HNSCC was significantly associated with decreased survival [88].
Ongoing studies are focusing on the possible role of the oral microbiome in the management of HNSCC, and whether its characterization will be of use in the selection of patients who are fit for immunotherapy.

Conclusions

Accurate prediction of ICI response is still far from being reached and will probably never depend on a single magic bullet. On the contrary, any advancement in this field will likely rely on an improved understanding of the complex interplay between tumor cells, immune cells, and the tumor microenvironment and, judging from the current trends, it will probably integrate multiple heterogeneous variables into composite predictive scores. Among these variables there will probably be a role for TMB, expression of PD-L1 and related molecules, and for many other factors that were discussed herein. The future increases in our ability to characterize the specific tumor's signature and the individuality of the patient are likely to play a pivotal role.
In order to integrate these different variables, the organization of large-scale trials, with rigidly standardized and reproducible methodology, is going to be of key importance.

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Conflicts of interest statement

The authors have no conflict of interest relevant to the present work.

Author contributions

AA: investigation, project administration, visualization, writing – original draft, writing – review and editing; AB: project administration, supervision, writing – review and editing; ML: investigation, writing; EC: in-

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