An update on head and neck squamous cell carcinoma in respect to classification and systemic therapy. Extended review

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Head and neck squamous cell carcinoma (HNSCC) is among the 10 most common cancers and demonstrates high mortality rates. Well known for its relation to smoking and alcohol consumption, in within the past 10 years a high number of cases, especially of the oropharynx could be shown to be based on an infection of human papilloma virus, high risk subtypes, mostly type 16 and 18. Often it is connected to younger age at onset, better outcome and better response to radiochemotherapy.

Next to well established radiochemotherapy schemes, including targeted therapy as cetuximab, in the recent 2 years a complete new therapeutic approach manifested, called checkpoint inhibition. The drugs of this class block a binding of a membranous ligand to a surface receptor on T-cells. Without blocking, this binding is physiological in antigen-presenting cells or many other normal tissue cells (e.g. heart muscle, trophoblast) inhibiting activation of the cytotoxic T-cells. Research revealed an identical way of tumor cells escaping cell death caused by patient’s own immune system. By blocking this inhibition, in several carcinoma entities, a very effective disease control was achieved. So far, 2 pairs of binding are approved for treatment: CD80-CTLA4 and PD1-PDL1. In CD80-CTLA4, it is an inhibitor to CTLA4 (e.g. Ipilimumab), in PD1-PDL1, for both, the ligand an receptor are several inhibitors on the market (e.g. Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab). So far, there could not be found a highly predictive marker for the grade of efficiency of these inhibitors. Next to very recent and complex markers, tumor mutational burden, the immunohistochemical staining for the ligand, PD-L1 could be established in a few number of carcinoma, incuding adenocarcinoma of the lung. Next to different ways of interpretation of the staining, also different staining procedures used in the trials are hindering an easy establishment of this marker in pathology laboratories. The staining procedures used in trials are not comparable with common immunohistochemistry due to very high costs of reagents (test-kits sold by the involved companies, so called companion diagnostic). To overcome these drawbacks, different studies were performed comparing the different antibody clones of immunohistochemical stains used in the official trials and antibodies of other companies. These harmonization studies brought to light that most antibodies stain equally, even the antibodies available from other companies thus making this stain more effordable and possible for introduction in the marker-portfolio of labs of pathology.

In HNSCC there is a better response to checkpoint inhibitors in cases of high PD-L1 expression, but also in negative cases, an effect could be seen. The actual approval are exclusively for patients in second line without PD-L1 testing. Upcoming approvals for first line treatment by checkpoint inhibitors are likely to include immunohistochemical testing for PD-L1.

Key words: head and neck squamous cell carcinoma, radiochemotherapy, checkpoint inhibition, Ipilimumab, Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab


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Squamous cell carcinoma of the head and neck derive from different sides, including oral cavity, oropharynx, nasopharynx, hypopharynx and larynx. This group of carcinomas, head and neck squamous cell carcinoma (HNSCC), show a wide range of incidences among the world, being more prevalent in some parts of South America and Asia, due to consumption of alcohol, betel quid or cigarette smoking and, rising in recent years, infection by high-risk human papilloma virus (HPV) types, mostly HPV type 16 and 18 [1, 2]. The way of interacting of HPV high risk types and the host genome is based on expression of viral gene products, E6 and E7, leading to an uncontrolled cell proliferation via inactivation of p53 and rb (retinoblastoma-gene product) [3].

HNSCCs are linked to high morbidity and mortality rates (up to 50%) due to the spectrum of affected patients and high recurrence rates. While the incidence of HPV related HNSCC rise, non-HPV related HNSCC are decreasing [4, 5]. In general, the age-standardization frequency of head and neck cancer worldwide is 8.1 per 100,000. In 2008, 324,834 related deaths [6]. Classical site for HPV related squamous cell carcinoma (SCC) is the oropharynx, as palatal or lingual tonsils, but lately, the number of HPV positively tested cases of sinonasal SCC and laryngeal SCC are rising or diagnosed in a significant number as well [7, 8]. The 5-year survival rate is more favorable in HPV positive cases than in HPV negative cases, due to less recurrence rates and better response to radiochemotherapy [8, 9]. One explanation of better prognosis in HPV positive SCC is the intrinsic immune response to the tumor, reflected by infiltrating T-cells [10].

The classical surrogate marker for HPV positive cases is the immunohistochemical stain for p16 or the product of the gene CDKN2A [11]. In oropharyngeal squamous cell carcinoma, in some extent depending on the country or continent, p16 marks HPV high-risk positive cases up to 100 %, but down to 80 % [12, 13], due to the prevalence of infections by subtype HPV type 16. Other types, including type 18 does not seem to be related to stable p16 expression. By publications using meta-analysis, it could be shown that p16 status is not a complete independent marker for outcome, but the combination of HPV testing by DNA extraction and PCR based detection: HPV+/p16+ cases...
had the best prognosis, HPV−/p16+ intermediate and HPV+/p16- and HPV−/p16− the worst [9].

By screening HNSCC for HPV relation, p16 is the recommended marker, especially in oropharyngeal SCCs with a cut off value of 70% stained tumor area [11]. There are no recommendations concerning the used antibody clone. As it is well established as being strongly expressed in almost 100% of SCC of the cervix uteri [14], it is up to the pathological laboratory to establish a specifically staining antibody using for example SCC of cervical SCC as a positive control.

**Diagnosis**

Diagnostic approaches include always a biopsy taken of the tumor side by endoscopy. Histologically, a squamous cell carcinoma can be challenging in case of low differentiation or basaloid differentiation and depending on the surrounding inflammatory infiltration. HPV positive squamous cell carcinomas show generally a distinct pattern of basaloid cell types and no typical squamous stratification or basaloid differentiation and depending on the cell carcinoma can be challenging in case of low differentiation of tumor cells without keratinization or differentiated epithelium [15]. As shown in figure 1, a typical keratinizing squamous cell carcinoma of the tongue, p16 negative, is shown in 1A and 1B. In comparison, a basaloid squamous cell carcinoma origin in the tonsil, p16 positive, is shown in 1C and 1D.

**New approaches for therapy**

Within the guidelines in western countries (for example NCCN, USA), as for most carcinomas, complete surgical resection comes with best prognosis. In unresectable cases or unresectable recurrence, systemic therapy as a combined chemoradiotherapy, using classical chemotherapeutics, for example cisplatin and radiation or targeted therapeutics, as cetuximab and radiation [16]. In non-naso-pharyngeal SCC in cases of recurrence after platinum containing therapy or progression under platinum containing therapy, a relatively new therapeutic agent, Pembrolizumab (MSD), was approved by the FDA and EMA [16]. Pembrolizumab (MSD) counts to the new group of so called checkpoint-inhibitors, representing a class of therapeutics interacting with the communication of tumor cells and cytotoxic T-cells [17]. In general, checkpoint inhibitors are blocking the ligand binding of the tumor cell on the receptor on the cytotoxic T-cell in order to activate the T-cells to attack tumor cells. In many different tumor entities, trials had been executed with different types of checkpoint inhibitors, mainly blocking interactions of PD-1/PD-L1 and CD80/CTLA4. By several authors and speakers, checkpoint inhibitors are called “the fourth modality” next to the well established treatment options: surgery, radiation and chemotherapy [18].

One of the first tumor entities in which checkpoint inhibitors were tested in trials was the malignant melanoma of the skin. Here, CTLA4 inhibitor Ipilimumab (BMS) didn’t reveal high response rates (5–15%), but still better than prior regimes. With PD-1 inhibitors like Nivolumab, up to 30–45% stable response rates were achievable [18].

For HNSCC, in case of CTLA4 several inhibitors are under investigation and mostly in combined therapies with another drug, none of the studies revealed a predicting biomarker as a immunohistochemical staining [19]. In case of PD-1 and PD-L1 inhibitors, many different drugs were developed (a selection is listed below) and set up in numerous trials from phase I to phase III. From beginning on, immunohistochemical expression of PD-L1 in tumor cells or so called “immune cells” in inflammatory infiltrates within the tumor (mainly histiocytic cell types) were chosen as a major depending variable in the analysis of efficacy of the drug [19]. The number of PD-1 positive lymphocytes within or adjacent to the tumor was discussed in the beginning but not followed [10]. In the last years, an additional predictive biomarker for the positive response to checkpoint inhibitors revealed the tumor mutational burden, TMB. The TMB had been tested in HNSCC and showed to be much higher in non-HPV related carcinomas [20]. In theory, TMB high tumors should be related to a high internal immune response due to a higher number of neo antigens on the cell surface. Other factors are discussed to play a role as microbiome or inflammatory signatures [21].

As for Pembrolizumab (MSD), equally for Nivolumab (BMS), it inhibits the receptor on the T-cell (see figure 2). Other inhibiting drugs are blocking the ligand as there are Atezolizumab (Roche), Durvalumab (AstraZeneca) and Avelumab (Merck). With these 5 drugs, the interaction
of the receptor programmed-cell death-1 (PD-1) and programmed cell death-ligand-1 (PD-L1) is meant to be suppressed [22]. As depicted in figure 2, PD-L1 is not only expressed on tumor cells, but also on antigen-presenting cells, as dendritic cells. This kind of treatment is not meant to be curative but to control the disease in order to reduce tumor burden or to receive stable disease. Side-effects of these drugs are in general less severe, as inflammation of lungs, colon and liver, but mostly well treatable [22].

So far, of the five mentioned actual drugs in use or in close future use, are primarily tested in second line and after successful first line application. Nivolumab and Pembrolizumab had been under investigation in Phase III trials in HNSCC: Nivolumab in CheckMate studies (No 141) and Pembrolizumab in Keynote studies (No 012, 048, 055). Atezolizumab and Durvalumab had also been investigated on HNSCC in phase I and II studies [23, 24]. In the trial of Atezolizumab, there could not be shown any connection of the response to HPV status (p16 positivity) or PD-L1 immunohistochemical expression. In Durvalumab, only tumors with a PD-L1 expression of at least 25% of tumor cells were included. Here p16, resp. HPV positive patients showed a better outcome [24].

In actual studies, the single drug as second line or first line is no longer much under investigation, but combination with other drugs/therapies or an established treatment followed by a checkpoint inhibitor (see table 2).

In the diverse trials of the four main drugs, different staining protocols and data assessment for the immunohistochemistry of PD-L1 expression were carried out (see table 1). Staining was always counted positive as a membranous staining, independently from the amount of staining and % stained portion of the membrane. The staining procedures were different in means of used staining machines and staining reagents, playing a minor role. The main role comes down to the used primary antibodies. As shown in figure 3, PD-L1 staining is mostly seen and counted on the membrane. Cytoplasmic stain is generally not mentioned in the trials. As demonstrated in figure 4 and revealed in harmonization studies of different groups, the antibody clones: 28.8, 22C3 and SP263 are staining more equally with differences in intensity. The clone SP142 stained significantly less tumor cells but highlight more immune cells [25, 26].

Even if many trials resulted in an approval of one of the drugs in different entities without necessary immunohistochemical testing for PD-L1, it still remains a useful biomarker. In many entities, higher response rates or progression free survival time could be described in tumors showing high expression of PD-L1 in tumor cells. But, as in approved treatments without mandatory testing, also low-expressing or not expressing tumors revealed good enough response rates. In special situations, an immunohistological testing is useful to determine if the treatment with checkpoint inhibitors might work better: malignant melanoma and single treatment with Nivolumab due to higher side effects of Ipilimumab in combination therapy; or in patients who had to pay for the drug themselves.

In general, there was no correlation mentioned concerning PD-L1 status and p16/HPV [19]. But HPV posit-

![Cell interaction of checkpoint ligand/receptors: between antigen-presenting cells (APC) and T-cells via PD-1 and PD-L1 and PD-L2 in order to inhibit cytotoxic effects on APC. Tumor cells escape cytotoxic effects by inhibiting T-cells the same way, here demonstrated CD80 and CTLA4 and PD-1 and PD-L1. By blocking either one of them, the immune system can be reactivated by the ‘inhibition of the inhibition’, calling these drugs checkpoint inhibitors](image)

![Fig. 2. Cell interaction of checkpoint ligand/receptors: between antigen-presenting cells (APC) and T-cells via PD-1 and PD-L1 and PD-L2 in order to inhibit cytotoxic effects on APC. Tumor cells escape cytotoxic effects by inhibiting T-cells the same way, here demonstrated CD80 and CTLA4 and PD-1 and PD-L1. By blocking either one of them, the immune system can be reactivated by the ‘inhibition of the inhibition’, calling these drugs checkpoint inhibitors](image)

Table 1. Main four checkpoint-inhibitors of PD-1 and PD-L1 and their detection systems/methods used in trials

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nivolumab (BMS)</th>
<th>Pembrolizumab (BMS)</th>
<th>Atezolizumab (Roche)</th>
<th>Durvalumab (AstraZeneca)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody clone</td>
<td>28.8</td>
<td>22C3</td>
<td>SP142</td>
<td>SP263</td>
</tr>
<tr>
<td>Company</td>
<td>Agilent/DAKO</td>
<td>Agilent/DAKO</td>
<td>Ventana/Roche</td>
<td>Ventana/Roche</td>
</tr>
<tr>
<td>Compartment</td>
<td>TC</td>
<td>TC and TC/IC: CPS</td>
<td>TC and TC</td>
<td>TC</td>
</tr>
<tr>
<td>Cut offs, in %</td>
<td>≥1, ≥5, ≥10</td>
<td>≥1, ≥50</td>
<td>TC: ≥1, ≥10, ≥50</td>
<td>IC: ≥1, ≥5, ≥10</td>
</tr>
</tbody>
</table>
The only actual need for an immunohistochemical grading of PD-L1 is in the application of Pembrolizumab in first or second line in NSCLC (at least 1% in second line and at least 50% in first line treatment). In the close future, Pembrolizumab might be approved for first line treatment in HNSCC in higher expressing tumors of a so called “combined-positive-score (CPS)” for PD-L1. The CPS counts positive staining of tumor cells and adjacent inflammatory cells divided by vital tumor cells and need to be greater than 1.

**Fig. 3. Different main types of staining of PD-L1 in HNSCC (T – tumor cells, IC – “immune cells”): A – homogenous, strong membranous stain of exclusively tumor cells (T); B – a case of heterogeneous stain within the tumor infiltrates (T) (left strong stain, right almost no stain); C – a case of slightly heterogeneous stain within the tumor (T) but also positivity of tumor adjacent inflammatory cells (IC), so called “immune cells” mainly stained are histiocytic cells as macrophages; D – exclusively stained tumor adjacent “immune cells” (IC) – tumor cells are negative (T). All photographs at 200, immunohistochemistry by Agilent/DAKO monoclonal mouse, 28.8; B – Agilent/DAKO monoclonal rabbit, 22C3; C – Ventana/Roche monoclonal rabbit, SP142; D – Ventana/Roche monoclonal rabbit, SP263 (Ventana, Tucson, AZ, USA). All stains performed with Agilent/DAKO Autostainer and Flex system (Agilent, Waldbronn, Germany)

**Fig. 4.** As demonstrated also in different studies, the three stains in A (clone 28.8), B (clone 22C3) and D (clone SP263) stain differently, but the same amount of tumor cells (staining counted irrespectively the strength). The stain in C does stain much less – in studies significantly less – tumor cells, but highlights positive immune cells with a dot-like staining pattern. The four different types of antibodies/test kits used in the trials: A – Agilent/DAKO monoclonal mouse, 28.8; B – Agilent/DAKO monoclonal rabbit, 22C3; C – Ventana/Roche monoclonal rabbit, SP142; D – Ventana/Roche monoclonal rabbit, SP263 (Ventana, Tucson, AZ, USA). All stains performed with Agilent/DAKO Autostainer and Flex system (Agilent, Waldbronn, Germany)

**Table 2. Actual studies on combination of checkpoint inhibitors and other treatments**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Short description</th>
<th>Name</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-CTLA4</td>
<td>Ipiilimumab (BMS)</td>
<td>Combination of Cetuximab, Radiation and Ipiilimumab in first line PULA HNSCC</td>
<td>—</td>
<td>[27]</td>
</tr>
<tr>
<td>Tremelimunab (AstraZeneca)</td>
<td>Ipiilimumab plus Nivolubum</td>
<td>CheckMate 651&amp;714</td>
<td>[28, 29]</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Tremelimunab plus Durvalumab</td>
<td>Kestrel</td>
<td>[30]</td>
<td></td>
</tr>
<tr>
<td>anti-PD-1</td>
<td>Pembrolizumab</td>
<td>Combination with Chemo and Radiation and Pembrolizumab</td>
<td>—</td>
<td>[31]</td>
</tr>
<tr>
<td>Nivolubum</td>
<td>Ipiilimumab plus Nivolubum</td>
<td>CheckMate 651&amp;714</td>
<td>[28, 29]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nivolubum plus Chemotherapy</td>
<td>Checkmate 227</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of Chemo plus Nivolubum followed by surgery (similar to neoadjuvant)</td>
<td>Optima2</td>
<td>[33]</td>
<td></td>
</tr>
<tr>
<td>anti-PD-L1</td>
<td>Durvalumab</td>
<td>Durvalumab plus Tremelimunab</td>
<td>Kestrel</td>
<td>[30]</td>
</tr>
</tbody>
</table>
With these special scoring systems and the need of accuracy, next to the different assays used, there was a big need for pathologists to simplify this field and to build up a common base. Harmonization studies of different working groups, the antibody clones: 28.8, 22C3 and SP263 are staining more equally with differences in intensity. The clone SP142 stained significantly less tumor cells but highlight more equally with differences in intensity. Therefore, Agilent/Dako and MSD organized training work-shops for pathologists.

Due to high costs for the testing kits for the described antibodies (e.g. reagents only DAKO/Agilent: ~3150€/50 tests), also other commercially available antibodies developed for research use were tested. Here, a significant number of well working antibodies could be found [25] and (table 3). Thus, several alternative antibodies are available with much less costs for the pathologist and patient.

Conclusion

Squamous cell carcinomas of the head and neck are since recent years a field of plasticity and new approaches: after research discovered and established the connection of HPV infection and a subpopulation of HNSCC, the upcoming drug group of checkpoint inhibitors is about to revolutionize the field of treatment which was dominated for decades by radiochemotherapy.

Table 3. Antibodies to test for PD-L1 immunohistochemistry (adjusted from S.Kintsler et al. [34])

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Agilent</th>
<th>Agilent</th>
<th>Ventana</th>
<th>Ventana</th>
<th>Cell Signalling</th>
<th>Abcam</th>
<th>Zytomed</th>
<th>Quartett</th>
<th>Merck</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>mMo</td>
<td>mRab</td>
<td>mRab</td>
<td>mRab</td>
<td>mRab</td>
<td>mMo</td>
<td>mRab</td>
<td>mRab</td>
<td>polyRab</td>
</tr>
<tr>
<td>Clone</td>
<td>28.8</td>
<td>22C3</td>
<td>SP142</td>
<td>SP263</td>
<td>E1L3N</td>
<td>28.8</td>
<td>Cal10</td>
<td>QR1</td>
<td></td>
</tr>
<tr>
<td>Efficacy concordant</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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M. Bolotин: article writing, reviewing of publications of the article’s theme; obtaining data for analysis; analysis of the obtained data;
T. Braunschweig: developing the research design, article writing, reviewing of publications of the article’s theme; obtaining data for analysis; analysis of the obtained data.

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M. Болотин: написание текста статьи, обзор публикаций по теме статьи, получение данных для анализа, анализ полученных данных;
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