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Study of the PD-L1 expression, T-cells density and immunoscore in paired baseline tumor biopsies and surgical specimens in squamous cell carcinoma of the oral cavity

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ABSTRACT

Objectives: Primary objective was to evaluate the correlation between immune marker expression in baseline tumor biopsies and their respective surgical specimens in squamous cell carcinoma of the oral cavity (OCSCC). Secondary objective was to assess the impact of these markers on overall (OS) and disease-free survival (DFS).
Materials and methods: Patients with a histological diagnosis of oral squamous cell carcinoma treated surgically between 2012 and 2020 were included in this retrospective, translational monocentric study. The expression of PD-L1, T-cells markers and an OCSCC-adapted immunoscore were evaluated by multiplex immunohistochemistry.
Results: One hundred and four patients (mean: 58 years) were included. Seventy patients had paired samples available. Poor correlation was highlighted for PD-L1-positive surface expression ($r = 0.29$) and combined positive score (CPS). For CPS ≥ 20 and CPS ≥ 1, correlation coefficient r was 0.24 and 0.46 respectively. T-cells density showed also poor correlation with a r of 0.57 and 0.31 for CD3 and CD8 T-cells, respectively. Univariate survival analyses showed significant better OS and DFS ($P < 0.05$) for patients with stage III-IV OCSCC with a high compared to a low immunoscore, based on surgical samples only.
Conclusion: Our study showed poor correlation in PD-L1 expression, CPS, T-cells density and immunoscore between baseline tumor biopsies and surgical resection specimens. In addition, the immunoscore may emerge as a potential prognostic factor in advanced squamous cell carcinoma of the oral cavity. If surgical specimens are available, they may be of interest for clinical practice decision.

Introduction

Head and neck squamous cell carcinoma (HNSCC) affect more than 650,000 people annually worldwide. Surgery or radiotherapy is recommended for localized disease American Joint Commission on Cancer (AJCC) stages I and II. The standard of care for locally advanced disease (AJCC stages III and IV) is multimodal and based on surgery followed by post-operative (chemo)radiotherapy or primary (chemo)-radiation, depending on the site of the disease and the patient's medical condition. Despite this heavy multimodal local therapy, less than 60 % of patients with locoregionally advanced HNSCC remain disease-free at three years [1].

Immune checkpoint inhibitors, such as anti-programmed death (PD)-1 antibodies, increase overall survival in patients with recurrent and/or metastatic (R/M) HNSCC [2]. For PD-ligand (L) 1 positive tumor, the Food and Drug Administration (FDA) has approved pembrolizumab for use as a single agent in first-line in recurrent and/or metastatic (R/M) HNSCC, or in combination with chemotherapy (platinum/5-

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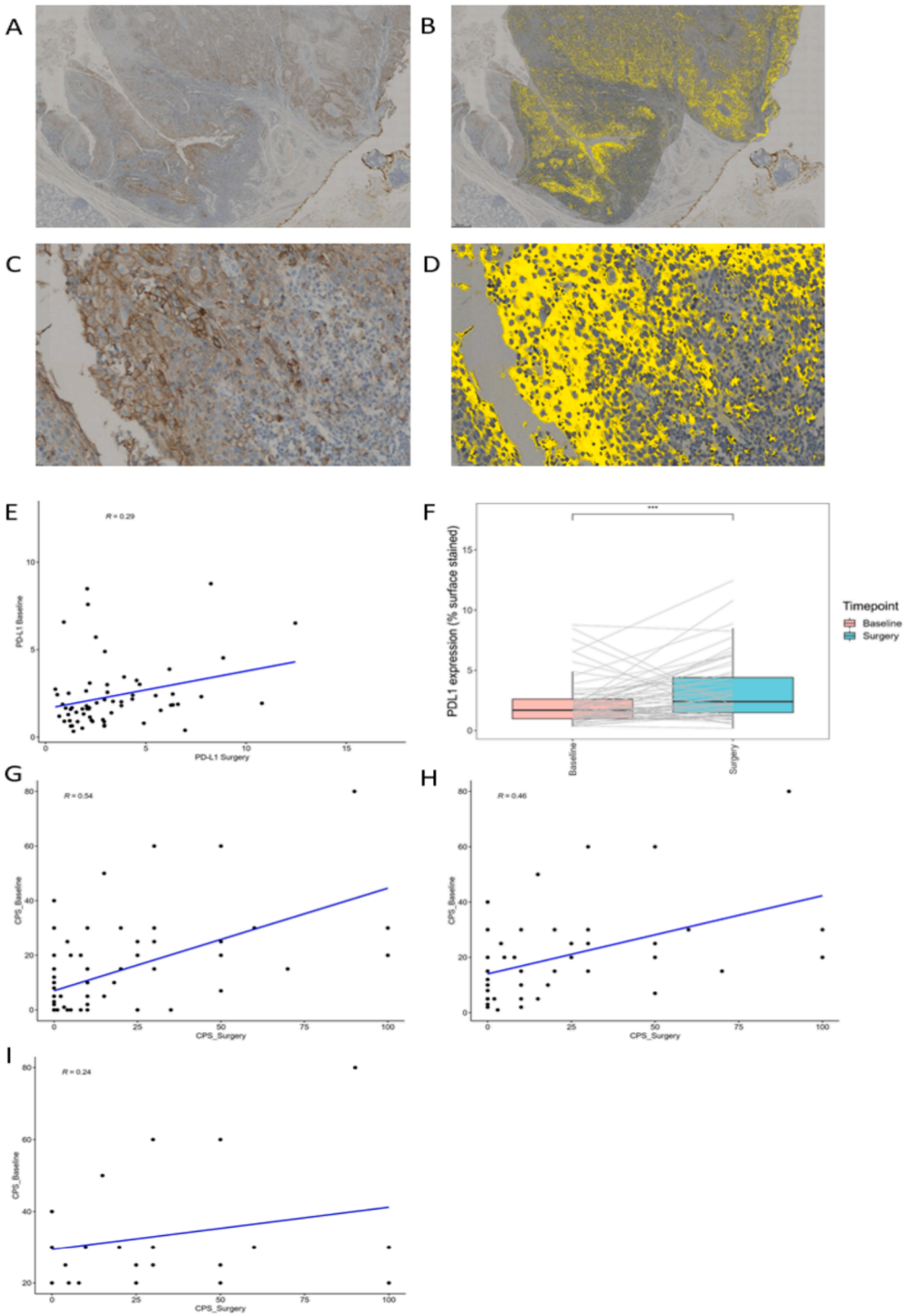

Fig. 1. PD-L1 immunohistochemitry analysis. A & C. PD-L1 staining of surgical specimens with 22C3 antibody. **B & D.** PD-L1 staining detection using the Halo software. The percentage of the surface stained by PD-L1 is automatically detected. **E.** Pearson correlation coefficient of PD-L1 expression between tumor biopsy (y-axis) and surgical specimens (x-axis). **F.** Boxplots comparing PD-L1 surface expression in baseline tumor biopsies and surgical sections. A bilateral paired Wilcoxon rank sum test was performed. **G, H & I**. Pearson correlation coefficient of paired samples in all CPS (G) and patients with baseline CPS $\geq$ 1 (H) and baseline CPS $\geq$ 20 (I) between tumor biopsy (y-axis) and surgical specimens (x-axis). ***: $P < 0.001$.

fluorouracil), regardless of PD-L1 status [1–2]. However, The European Medicines Agency (EMA) does not permit the use of pembrolizumab in patients whose tumors do not express PD-L1. Therefore, in Europe the first-line therapy in R/M HNSCC for PD-L1 negative R/M HNSCC is platin-based chemotherapy plus cetuximab (EXTREME regimen) [3]. Nivolumab, another anti-PD1 antibody, can also be used for patients with immunotherapy-naive R/M HNSCC that progress after platinum therapy [1].

However, immunotherapies are expensive, can have severe adverse events [2], and not all patients respond to treatment, including patients with PD-L1 positive and hot tumors. Indeed, only a small percentage of patients experience long-term benefits, and the objective response rate is still low around 10–18 % [2]. This emphasizes the necessity for better predictive biomarkers. Intratumor heterogeneity may explain why some

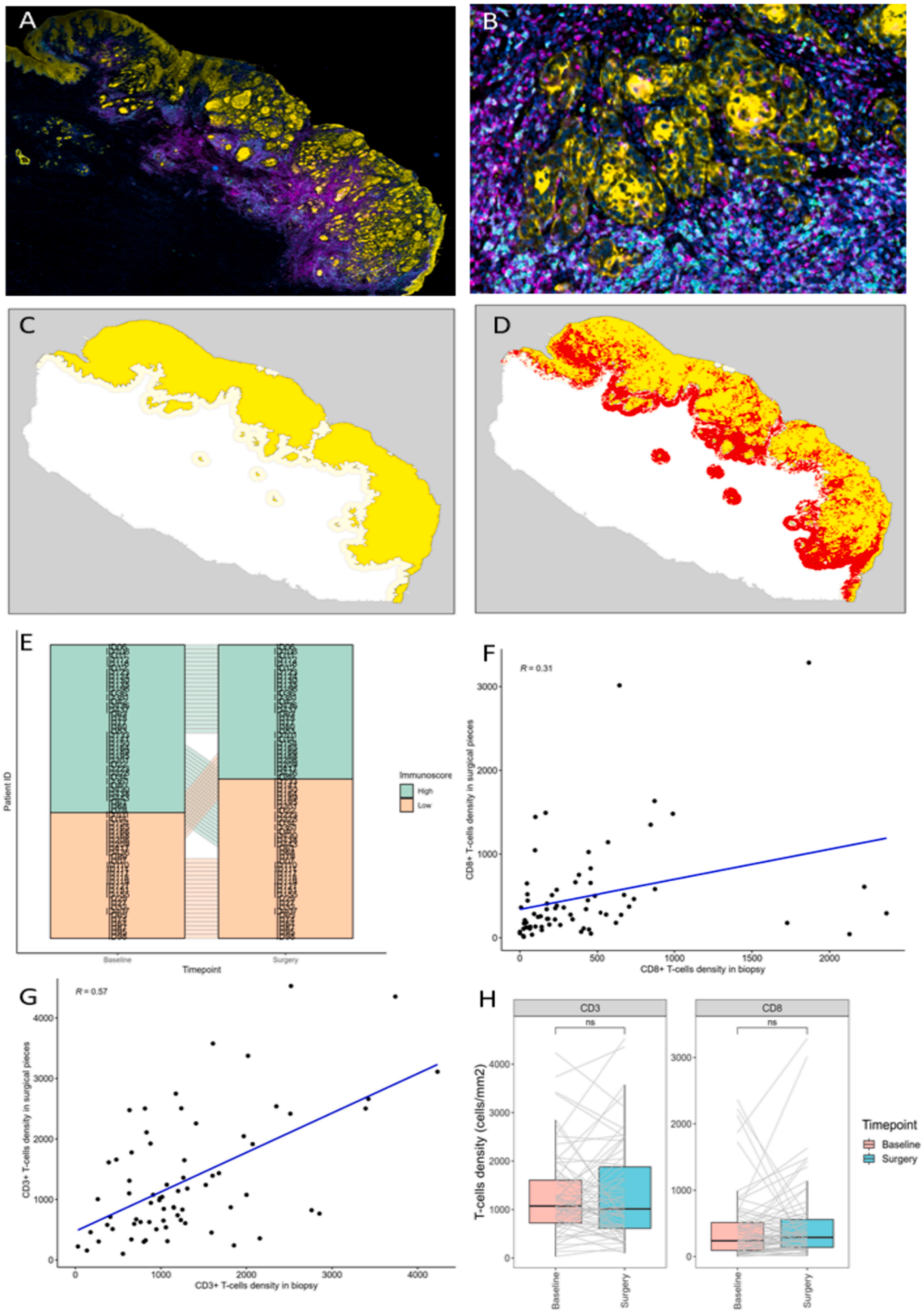

Fig. 2. Multiplex immunohistochemistry analysis on CD3 + and CD8 + T-cells. A & B. Multiplex immunohistochemistry of surgical specimens involving three markers (Pancytokeratin in yellow, CD3 in purple, and CD8 in green). **C**. Reconstruction of the surgical specimen using bioinformatics. The tumor center is shown in yellow, the invasive margin in light yellow, on an arbitrary threshold of 500 μm around the tumor center. **D.** CD8 + T-cells are represented by red dots. **E.** Alluvial plot showing variations in immunoscore calculated on biopsies (left column) and surgical sections (right column). **F & G**. Pearson correlation coefficient (r) of CD8 (Fig 2F) and CD3 (Fig 2G) between tumor biopsy (x-axis) and surgical specimens (y-axis). **H**. Boxplots comparing CD3 and CD8 densities in tumor biopsies and surgical sections (center + invasive margin). A bilateral paired Wilcoxon rank sum test was performed. ns: not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Patient characteristics.

Characteristics	N = 104
Sex	
men	66 (63.5 %)
women	38 (36.5 %)
Tobacco	
yes	69 (66.3 %)
No	35 (33.7 %)
Alcohol	
yes	49 (47.1 %)
no	55 (52.9 %)
cT	
T1	29 (27.9 %)
T2	35 (33.6 %)
T3	16 (15.4 %)
T4	24 (23.1 %)
cN	
N0	71 (68.3 %)
N1	8 (7.7 %)
N2a	1 (1 %)
N2b	16 (15.4 %)
N2c	7 (6.7 %)
N3	1 (1 %)
AJCC Stade	
I–II	50 (48.1 %)
III–IV	54 (51.9 %)
Grade	
Well differentiated	37 (35.6 %)
Moderately differentiated	53 (51.0 %)
Poorly differentiated	12 (11.5 %)
Adjuvant radio(chemo)therapy	
Yes	55 (53.4%)
No	48 (46.6 %)

patients with cold and/or PD-L1 negative tumor biopsy respond to immunotherapy, and conversely, why hot and/or PD-L1 expressing tumor biopsy do not always respond to immunotherapy. In clinical practice, PD-L1 expression is based on immunohistochemical analysis of a single biopsy taken during oncology work-up, which only allow analysis of a small and superficial part of the HNSCC that may not be representative of the entire tumor. Tumor heterogeneity has therefore a direct impact on clinicians' treatment decisions. Indeed, authors have demonstrated that PD-L1 expression can be variable in paired samples [4], and can be focally and heterogeneously expressed within tumors [5].

The objective of this study was to evaluate the correlation of the expression of immune markers like PD-L1, T-cells markers, and a (biopsy-adapted) HNSCC-adapted immunoscore in paired samples of operated oral squamous cell carcinoma, by comparing baseline tumor biopsies with their respective surgical specimens. Secondary objective was to assess if survival was associated with the expression of these markers.

Patients and material and methods

This monocentric retrospective study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice standard and the Declaration of Helsinki. The study protocol was approved by our local ethics committee in the Cliniques universitaires Saint-Luc, Brussels.

Inclusion and exclusion criteria

Patients eligible for the trial have a primary histologically proven squamous cell carcinoma of the oral cavity. Patients have to be selected for curative surgery without neoadjuvant therapy and have been fully treated in our institution between 2012 and 2022. No treatments were allowed between the tumor biopsy and the surgery.

Collection of the data

Study data were collected retrospectively and managed using REDCap electronic data capture tools hosted at Cliniques universitaires St-Luc [6–7]. No sample size estimation was made prior to data collection. Indeed, given the monocentric and retrospective nature of the study, all patients meeting the inclusion criteria were included in order to increase the statistical value of the analyses.

Tissue samples

Two formalin-fixed paraffin-embedded (FFPE) samples were taken from the Biobank of our institution; (i) the biopsy realized during the diagnostic endoscopy ("Baseline" sample), and (ii) section of operated tumors ("Surgery" sample).

Immunohistochemistry (IHC)

FFPE tumor biopsies and surgical resection specimens were cut into 5 μm-thick sections. IHC simplex was done for PD-L1 (22C3 pharmDx) (Fig 1A-1C).

For multiplex IHC, the sections were stained in multiplex immunohistofluorescence for CD3 (dilution 1:100, Abcam ab16669), CD8 (dilution 1:100, Dako M710301) and Pancytokeratin (dilution 1:50, Dako M3515). The multiplex IHC staining procedure was performed as described by Huyghe et al [8]. All the stained slides were digitized with the Zeiss AxioScan Z.1 slide scanner at 20X magnification (Fig 2A-2B).

IHC image analysis

For PD-L1, the percentage of marker expression was calculated using Halo® 3.3 software (Indica labs) (Fig 1B-1D). The thresholds for 3,3′-Diaminobenzidine (DAB) detection on tumor slides were set based on the DAB signal intensity of control slides. High and low PD-L1 surface expression were set according to the cut-off value of PD-L1 surface expression defined at the median of the cohort (50 % of patients with high PD-L1 expression and 50 % of patients with low PD-L1 expression). Combined Positive Score (CPS) was defined by a pathologist (PB).

For multiplex IHC images, the staining detection thresholds were carried out on HALO® 3.3 (Indica Labs) software and the collected raw data were processed on R in order to calculate density variations (cells/mm^2). High and low T-cells density were set according to the cutoff value of CD3 or CD8 density defined at the median of the cohort (50 % of patients with high CD3 or CD8 density and 50 % of patients with low CD3 or CD8 expression). Spatial analysis was done using the G-cross function from the R spatstat package [9], by calculating the area under the curve (AUC) of the G-cross function of T-cells for a radius of 0 to 20 μm around tumor cells, corrected with the border "rs" parameter [10]. A high AUC indicates a high probability for a tumor cell to be surrounded by lymphocytes over a radius of 0 to 20 μm around the tumor cell. High and low T-cells proximity toward tumors cells were set according to the cutoff value of the AUC of the G-cross function for CD3 or CD8 defined at the median of the cohort (50 % of patients with high CD3 or CD8 AUC and 50 % of patients with low CD3 or CD8 AUC).

The biopsy-adapted immunoscore was based on CD3 and CD8 densities according to El Sissy et al. [11]. But the biopsy-adapted immunoscores were divided only between "high" and "low", depending on whether the mean T-cells density percentile ((percentile density CD3 + percentile density CD8)/2) was above or below 50, respectively. The immunoscore on surgical sections was calculated according to the technique described by Galon et al [12–13]. The surgical specimen was first bioinformatically reconstructed as described by Huyghe et al [8] (Fig 2C-2D). Then the immunoscore was based on the density of CD3 and CD8 T-lymphocytes in the tumor center and the invasive margin. The tumor center and the invasive margin were constructed bioinformatically, with the invasive margin around the tumor center on an

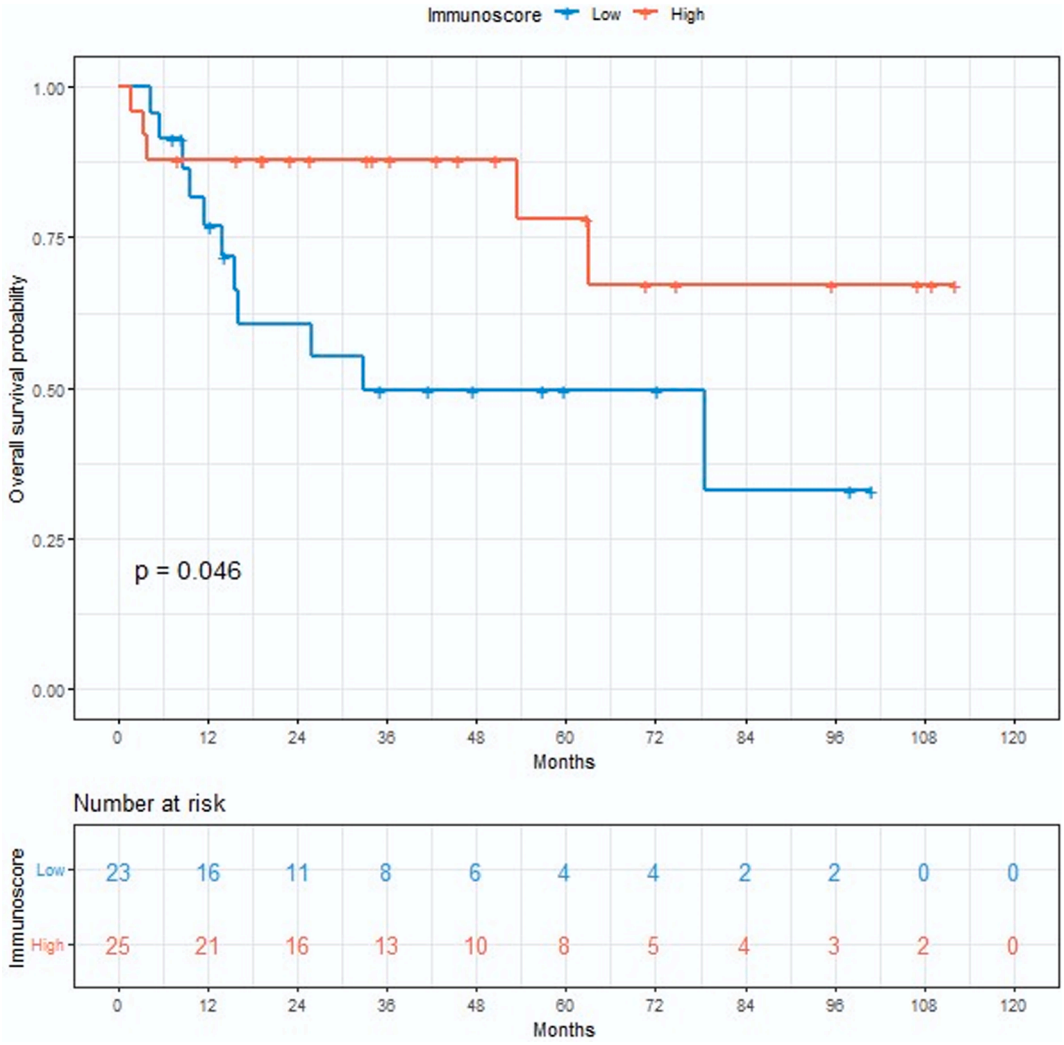

Fig. 3. Probability of overall survival in patients with advanced oral squamous cell carcinoma of the oral cavity. A. Overall survival probability based on the immunoscore. **B.** Overall survival probability based on the proximity between CD3 T-cells towards tumor cells estimated by the area under the curve (AUC) of the G-cross function in surgical specimens. Logrank test was applied to test the null hypothesis that there is no difference between the populations for overall survival.

arbitrary 500 μm basis. The immunoscore was calculated by averaging the CD3 and CD8 densities percentiles ((percentile density CD3_margin + percentile density CD3_center + percentile density CD8_margin + percentile density CD8_center)/4). If the average percentile was below or above 50, the immunoscore was considered low or high, respectively.

Statistical analysis

A coefficient of correlation Pearson R was used to calculate the association between quantitative variables used as markers in paired samples (PD-L1, CD3/CD8 densities, CD3/CD8 AUC of the G-cross function). For the secondary endpoints, overall survival (OS) and disease-free survival (DFS) were calculated. OS was defined as the time between the end of the treatment and date of death from any cause. DFS was defined as the time from the end of the treatment until disease recurrence or death from any cause [14]. OS and DFS were calculated using the Kaplan-Meier method and logrank test to assess the statistical differences between group in survivals in univariate analysis. Cox proportional hazards regression analysis was used for multivariate analyses. To compare quantitative data, a Shapiro-Wilk test was performed to determine whether the data were parametric or not. Depending on the results, a Whitney-Wilcoxon rank-sum test (for non-parametric data), or a Student's *t*-test (for parametric data), was performed to analyze the data. A chi-square test was performed to assess the correlation between immunoscore status and recurrence. P-values were indicated as "*P*" in the text. A p-value below 0.05 indicates a statistically significant result. All statistical analyses and plots were performed on R v4.1.0.

Results

Patients

One hundred and four patients (66 men and 38 women) with histologically proven squamous cell carcinoma of the oral cavity were included in the study, with an average age of 58 years (min–max: 26–87 years) and a median follow-up of 36.5 months (min–max: 1–129). Sixty-nine (66.3 %) were smokers and 49 patients (47.1 %) had a history of alcohol overuse. The other characteristics of the patients included are available in Table 1.

Correlation analysis on paired tumor samples

In our cohort, 70 patients had paired samples available. For PD-L1-positive surface expression (%) analysis between baseline biopsies and

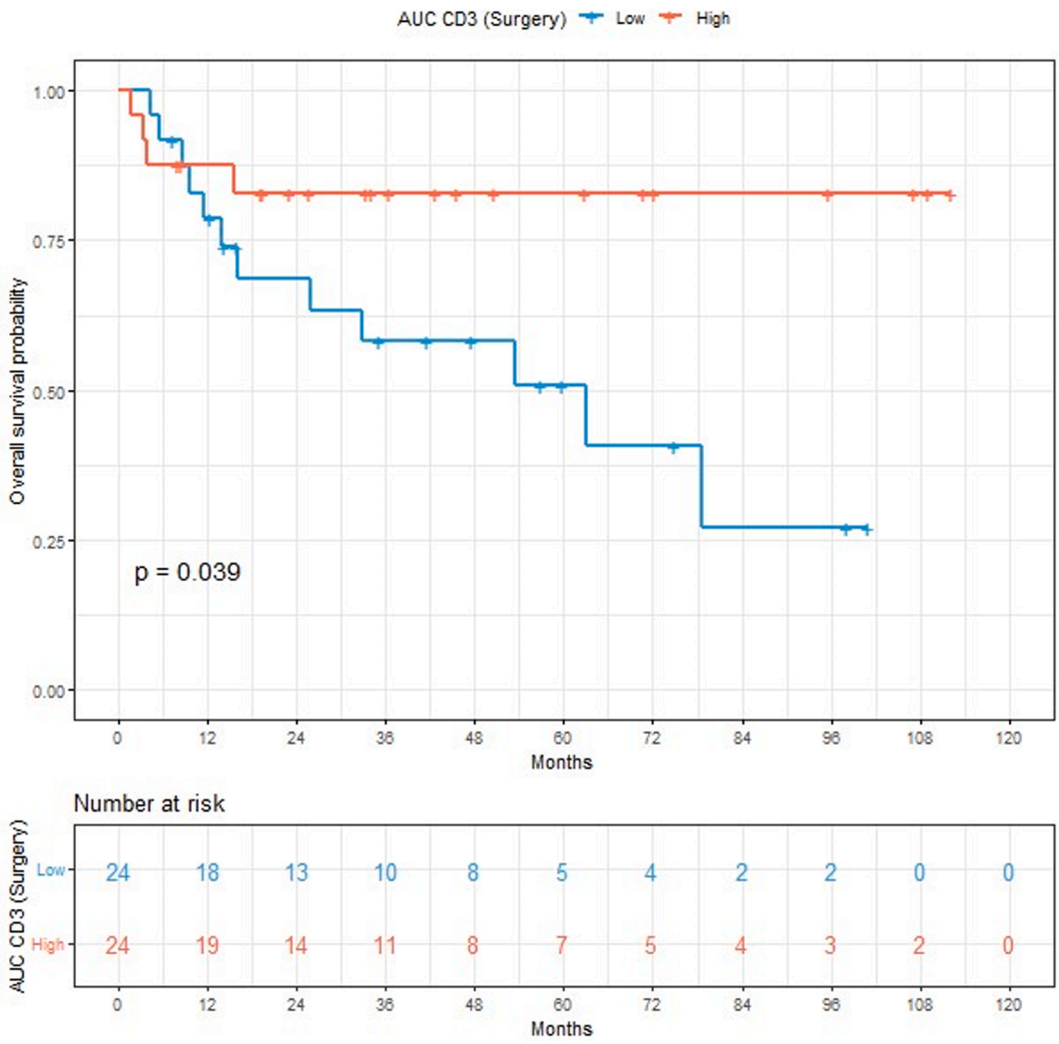

Fig. 3. (*continued*).

surgical sections, a weak Pearson correlation coefficient (r) of 0.29 was demonstrated (Fig 1E). Moreover, surgical sections showed significantly higher surface expression of PD-L1 than baseline tumor biopsies ($P < 0.001$) (Fig 1F). Furthermore, a weak Pearson correlation coefficient was found between paired samples when looking at the CPS on all paired samples (n = 97; r = 0.54), as well as when filtering samples by keeping only patients with baseline CPS $\geq$ 1 (n = 50; r = 0.46) and CPS $\geq$ 20 (n = 22; r = 0.24) (Fig 1G-1H-1I).

For CD3 and CD8 T-cells densities, the Pearson correlation coefficient (r) between biopsies and surgical samples (invasive margin + center of the tumor) was at 0.57 and 0.31, respectively (Figure 2F-2G) with no significantly different density between biopsies and surgical samples (Fig 2H). In addition, CD3 and CD8 distances from tumor cells, calculated by the AUC of the G-cross function, showed a Pearson correlation coefficient (r) of 0.59 and 0.33 respectively with no significant difference in the AUC between paired samples (Supplementary figures A-C). Variations between the immunoscore and the biopsy-adapted immunoscore in paired samples were observed in 42.8 % of the cases (Figure 2E). Sixteen percent of patients had an increased immunoscore compared with the biopsy-adapted immunoscore, and 27 % had a decreased immunoscore compared with the biopsy-adapted immunoscore. In addition, no correlation was highlighted between the biopsy-adapted immunoscore or the immunoscore and the relapses for all stages ($P = 0.11$ and $P = 1$, respectively) and for late stages only ($P = 0.40$ and $P = 0.92$, respectively).

Survival analysis

In univariate analysis, OS and DFS were calculated on several clinical variables (age, smoking status, alcohol consumption, American Joint Committee on Cancer (AJCC) stage, tumor grade) and IHC markers in biopsies and surgical specimens (PD-L1 surface expression, CD3/CD8 densities, CD3/CD8 AUC of the G-cross function towards tumor cells and the (biopsy-adapted) immunoscore). When considering the entire population, smoking is associated with worse OS (hazard ratio (HR) = 3.3; $P = 0.01$; 95 %CI 1.3–8.4) and DFS (HR = 3.0; $P = 0.0096$; 95 %CI 1.3–7.3), while high tumor grade significantly decreased DFS (For all grade $P = 0.034$; for poorly differentiated: HR = 1.11; $P = 0.82$; 95 %CI 0.45–2.75; for well differentiated: HR = 0.36; $P = 0.018$; 95 %CI 0.15–0.84).

A subgroup analysis showed that in patients with advanced stage oral cancer (AJCC stage III-IV; n = 54), OS was lower in univariate analyses for smokers (HR = 9.8; $P = 0.0067$; 95 %CI 1.3–74), high tumor grade (For all grade $P = 0.046$; for poorly differentiated: HR = 5.7; $P = 0.06$; 95 %CI 0.93–34; for moderately differentiated: HR = 5.3; $P = 0.03$; 95 % CI 1.17–24), "low" immunoscore patients (HR = 2.8; $P = 0.046$; 95 %CI 0.97–8.1) (Fig 3A) and lower proximity between tumor cells and CD3 + cells (HR = 3.1; $P = 0.039$; 95 %CI 1–9.7) (Fig 3B). Three and five-year OS rates for high immunoscore patients was 88.0 % and 78.2 %, compared to 49.7 % and 49.7 % for low immunoscore patients, respectively.

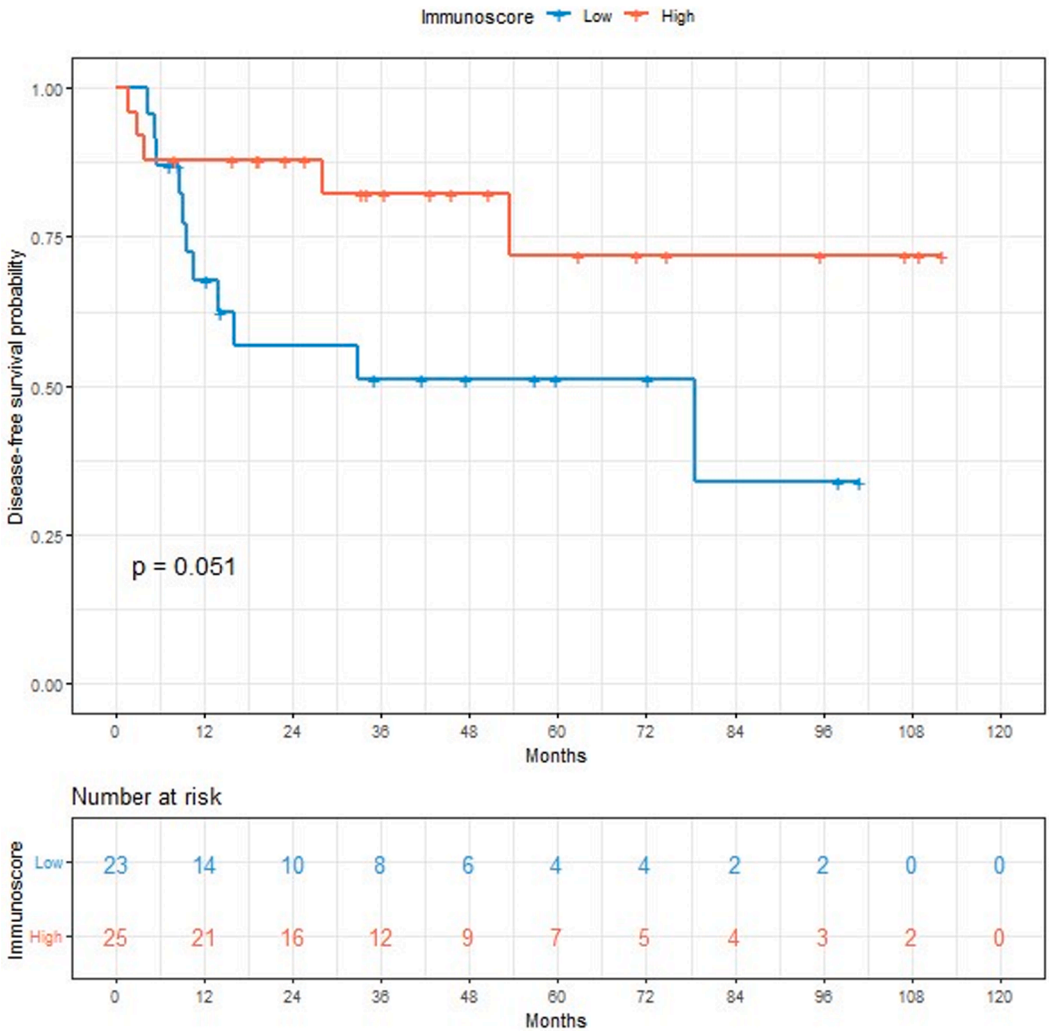

Fig. 4. Probability of disease-free survival in patients with advanced squamous cell carcinoma of the oral cavity. A. Disease-free survival probability based on the immunoscore. **B**. Disease-free survival probability based on the proximity between CD3 T-cells towards tumor cells estimated by the area under the curve (AUC) of the G-cross function in surgical specimens. Logrank test was applied to test the null hypothesis that there is no difference between the populations for disease-free survival.

Three and five-year OS rates for tumors with high proximity between CD3 toward tumor cells in surgical specimens was 82.9 % and 82.9 %, compared to 58.1 % and 50.8 % for tumors with low proximity between CD3 and tumor cells, respectively. Moreover, DFS was significantly decreased for smokers (HR = 9.8; $P = 0.0065$; 95 %CI 1.3–74), "low" immunoscore patients (HR = 2.8; $P = 0.051$; 95 %CI 0.95–8) (Fig 4A) and low proximity between CD3 and tumor cells calculated in surgical specimens (HR = 3.1; $P = 0.039$; 95 % CI 1–9.6) (Fig 4B). Three and five-year DFS rates for high immunoscore was 82.1 % and 71.9 %, compared to 51.1 % and 51.1 % for low immunoscore, respectively (Fig 3A). Three and five-year DFS rates for tumors with a high proximity between CD3 and tumor cells calculated in surgical specimens was 82.9 % and 82.9 %, compared to 53.6 % and 46.0 % for low proximity between CD3 and tumor cells, respectively. However, multivariate analyses showed no significant impact on OS and DFS for any of these variables (data not shown).

By analyzing patients with advanced oral squamous cell carcinoma who received adjuvant radio(chemo)therapy (n = 42), OS was significantly decreased for smokers (HR = 7.8; $P = 0.019$; 95 %CI 1–60), "low" immunoscore patients (HR = 3.9; $P = 0.03$; 95 %CI 1–14), low proximity between CD3 (HR = 6; $P = 0.0087$; 95 %CI 1.3–27) and CD8 (HR = 5; $P = 0.022$; 95 %CI 1.1–23) T-cells toward tumor cells calculated in surgical specimens. DFS was significantly decreased for smokers (HR = 7.8; $P = 0.02$; 95 %CI 1–60), "low" immunoscore patients (HR = 3.8; $P = 0.033$; 95 %CI 1–14), low proximity between CD3 (HR = 5.9; $P = 0.0089$; 95 %CI 1.3–27) and CD8 (HR = 5; $P = 0.021$; 95 %CI 1.1–23) T-cells toward tumor cells calculated in surgical specimens. However, multivariate analyses showed no significant impact on OS and DFS for any of these variables (data not shown).

Biopsy-adapted immunoscore and other markers measured on tumor biopsies (CD3/CD8 T-cells density and proximity towards tumor cells, PD-L1 surface expression and CPS) have shown no association with patient survival (Table 2).

Discussion

In this study, we compared the baseline biopsy realized during the oncological work-up and surgical sections from operated oral squamous cell carcinoma. PD-L1 surface expression and CPS showed poor correlation between baseline biopsies and surgical specimens, the latter having a significant higher surface expression. Rasmussen et al. [4] assessed the PD-L1 expression in six random core biopsies in HNSCC surgical specimen to evaluate the concordance between multiple core biopsies. With 1 % and 50 % cut-off values, 52 % and 55 % of the

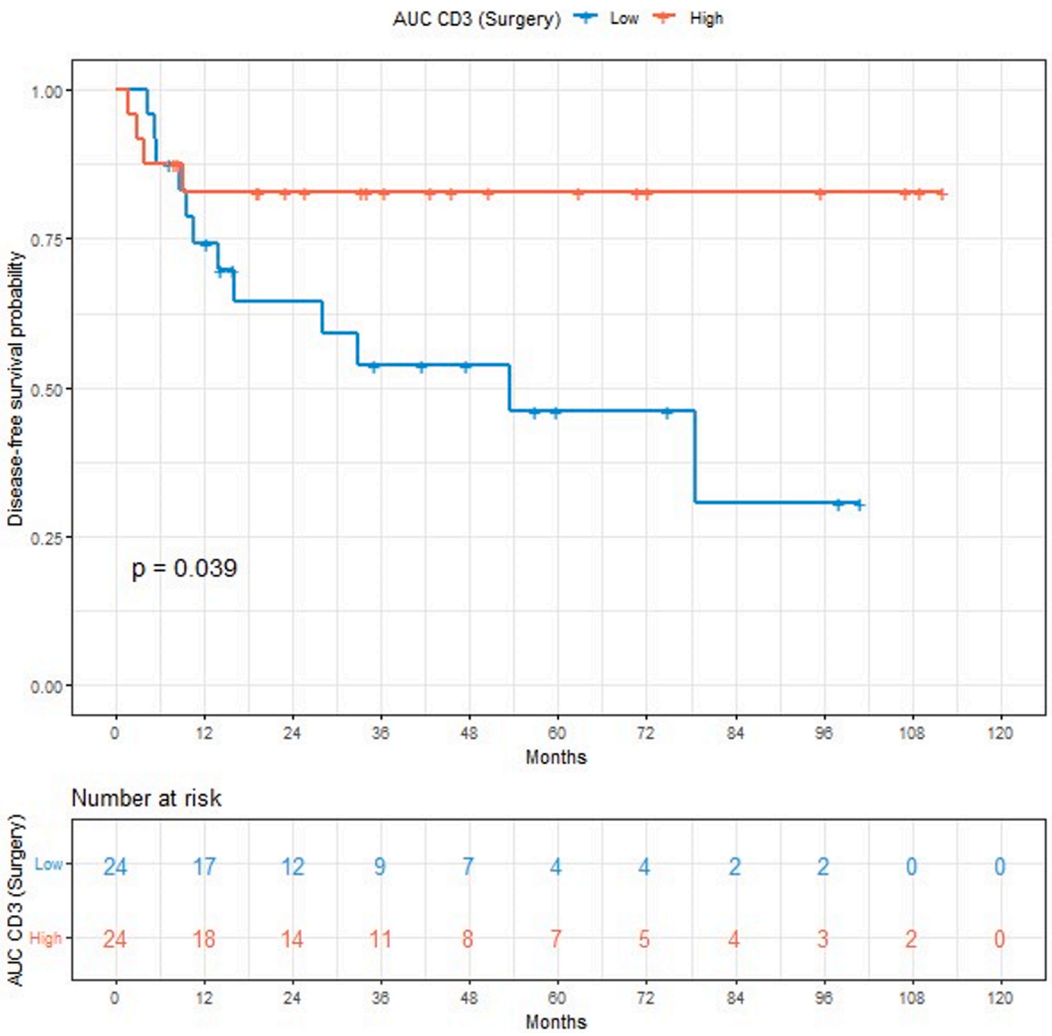

Fig. 4. (*continued*).

specimens were concordant for CPS, respectively. The negative predictive value of a single negative core biopsy was 0 % (1 % cut-off), and 62.8 % (50 % cut-off) for CPS. It should be noted that the antibody used in the study performed by Rasmussen et al. is not the antibody used in clinical practice (22C3 PharmaDx). Disparities may exist between PD-L1 expression results depending on the antibody and the protocol applied. Indeed, Crosta et al. [15] compared the CPS using five different PD-L1 antibodies and protocols, compared to the gold standard in 40 HNSCC samples. The sensitivity of the individual's protocols ranged from 79 % to 96 %, the specificity from 50 % to 100 %, and the error rates varied from 7 % to 21 %. Our study and others [4] show the importance of tumor heterogeneity on PD-L1 expression in HNSCC, which can influence the CPS and therefore oncologist's choice of treatment, illustrating the challenges of using PD-L1 in a single small biopsy as a predictive biomarker for immunotherapy. In view of these results, we suggest (i) to perform multiple biopsies in the workup of R/M HNSCC eligible for anti-PD-(L)1 immune checkpoint inhibitor, to minimize the risk of false negatives related to tumor heterogeneity, or (ii) to analyze PD-L1 expression in surgical resection sections when available.

The immunoscore calculated on biopsies and surgical sections in our cohort, based on methodologies previously described [8,13], contained only two categories: "high" versus "low", compared with the three categories "high", "intermediate" and "low" described in colorectal cancers [11,13,16]. We decided to divide our population into two categories only, given the small number of patients included in our retrospective monocentric cohort. In addition, our methodology includes the recognition of the tumor center and the invasive margin using multiplex immunohistochemistry and bioinformatics-based section reconstruction. In our opinion, this automated approach provides a reproducible and non-observer dependent method of analysis. In our cohort, we observed poor correlation between T-cells densities and spatial distribution in paired samples. In addition, we observed a 42.8 % discrepancy between the biopsy-adapted immunoscore and the immunoscore based on surgical sections in paired samples. Univariate survival analyses showed better OS and DFS for high immunoscore and high proximity of CD3 toward tumor cells in surgical specimens in advanced oral squamous cell carcinoma, in accordance with data found in international validation of the immunoscore in colon cancers [16]. In HNSCC, the immunoscore developed by Zhang et al. [17] demonstrated a prognostic value in OS and DFS. Moreover, our results showed association of a high immunoscore and high T-cells proximity toward tumor cells in surgical specimens with better OS and DFS for patients with advanced oral squamous cell carcinoma treated with adjuvant therapy. Zhang et al. also showed that patients with advanced hypopharyngeal cancer who received radiotherapy and chemotherapy before or after curative surgery showed better survival in immunoscore "high" patients compared to immunoscore "low" patients.

In patients with stage III colon cancer described in the international immunoscore study [18], chemotherapy was significantly associated with survival in the high-immunoscore patients and not in the low-immunoscore patients. Immunoscore may be of interest to select patients which may benefit most from chemoradiotherapy, or patients with

Table 2
Survival analyses.

Univariate analyses (logrank test)	All patients (n = 104)		Patients with advanced AJCC stage III-IV (n = 54)		Patients with advanced AJCC stage III-IV and adjuvant therapy (n = 42)	
	OS	DFS	OS	DFS	OS	DFS
Clinical variables						
Smoking status	**0.01**	**0.0096**	**0.0067**	**0.0065**	**0.019**	**0.02**
Alcohol	0.41	0.49	0.86	0.88	0.56	0.58
Tumor grade	0.078	**0.034**	**0.046**	0.053	0.16	0.18
AJCC stage	0.45	0.89				
Age	0.65	0.40	0.42	0.43	0.84	0.85
Adjuvant therapy			0.57	0.57		
IHC variables						
Tumor biopsies						
PD-L1 expression	0.99	0.77	0.61	0.62	0.54	0.87
CPS ≥ 1	0.93	0.70	0.70	0.79	0.71	0.87
CPS ≥ 20	0.44	0.52	0.92	0.87	0.92	0.96
CD3 density	0.76	0.53	0.83	0.70	0.72	0.58
CD8 density	0.45	0.95	0.14	0.15	0.25	0.28
CD3 AUC	0.26	0.17	0.72	0.69	0.76	0.82
CD8 AUC	0.98	0.53	0.14	0.16	0.22	0.28
Biopsy-adapted immunoscore	0.95	0.49	0.54	0.61	0.55	0.60
Surgical specimens						
PD-L1 expression	0.93	0.85	0.93	0.97	0.81	0.60
CPS ≥ 1	0.82	0.95	1	0.92	0.94	0.82
CPS ≥ 20	0.68	0.54	0.87	0.84	0.81	0.81
CD3 density	0.79	0.98	0.30	0.33	0.065	0.09
CD8 density	0.24	0.42	0.27	0.30	0.15	0.19
CD3 AUC	0.15	0.23	**0.039**	**0.039**	**0.0087**	**0.0089**
CD8 AUC	0.29	0.40	0.057	0.053	**0.022**	**0.021**
Immunoscore	0.25	0.35	**0.046**	**0.051**	**0.03**	**0.033**

AJCC: American joint committee on cancer; AUC: area under the curve of the G-cross function; CPS: combined positive score; DFS: disease-free survival; IHC: immunohistochemistry; OS: overall survival PD-L1: programmed-death ligand 1. All p-values were calculated using a logrank test. For T-cells densities, AUC and PD-L1 expression, patients were classified between "high" and "low" based on the median expression of the variable in the cohort. A high AUC indicates a high probability for a tumor cell to be surrounded by lymphocytes (CD3 and CD8 T-cells for "CD3 AUC" and "CD8 AUC" respectively) over a radius of 0 to 20 μm around the tumor cell.

low immunoscore may require treatment intensification, to be investigated in a study protocol. However, our multivariate analyses showed negative results, probably related to the low number of patients included in this study.

In our study, all IHC markers investigated on tumor biopsies showed no significant association with survival. The biopsy-adapted immunoscore did not show a prognostic value in survival analyses; as patient with neo-adjuvant chemotherapy were excluded, it was not possible to evaluate response to treatment according to the biopsy-adapted immunoscore, as it was done in rectal cancers. However, our study has several limitations. These include its retrospective nature and the limited number of patients included in this monocentric study. Extending recruitment to several centers would increase sample size and add statistical strength to the analyses.

Conclusion

To the best of our knowledge, this is the first study comparing PD-L1 expression, CPS, T-cells markers and immunoscores in paired samples in primary squamous cell carcinoma of the oral cavity eligible for surgical treatment. Our study showed poor correlation and significant differences in PD-L1 expression between baseline tumor biopsies and surgical resection specimens, questioning the current methodology used in clinical practice to assess patient eligibility for anti-PD-(L)1 immune checkpoint inhibitor. Furthermore, our results showed poor correlation between T-cells densities and disparities between the immunoscore measured on biopsies and surgical sections. The immunoscore and T-cells proximity toward tumor cells measured on surgical sections may emerge as potential prognostic factors in advanced oral cavity squamous cell carcinoma. In order to have an impact on the management of patients with squamous cell carcinoma of the oral cavity, the results of this study need to be validated in a multicenter larger cohort.

CRediT authorship contribution statement

Simon Beyaert: Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aléna Borys:** Project administration, Methodology, Investigation, Formal analysis, Data curation. **Pamela Baldin:** Validation, Investigation. **Hajar Dahou:** Methodology, Investigation. **Michèle Magremanne:** Writing – review & editing. **Pierre Mahy:** Writing – review & editing. **William Renwart:** Writing – review & editing. **Jean-Pascal Machiels:** Writing – original draft, Visualization, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Sandra Schmitz:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JPM reports grants from Boehringer-Ingelheim, Pfizer, Roche, AstraZeneca, Bayer, Innate, Merck Serono, Boehringer, BMS, Novartis, Janssen, Incyte, Cue Biopharma, ALX oncology, iTEOS, eTheRNA, NEKTAR, F-star, Seagen, Genmab, Astellas, CureVac, MSD, Gilead, and Sanofi outside the submitted work. No disclosures were reported by the other authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2024.106869.

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