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Programmed death ligand-1 expression as immunotherapeutic target in sinonasal cancer

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Abstract

Background: Sinonasal cancer carries a poor prognosis, especially in recurrent stages, and it is a disease with very limited treatment options.

Methods: The expression of programmed death ligand-1 (PD-L1) as a marker for immunotherapy was evaluated in 53 sinonasal squamous cell carcinoma (SCC) and 126 intestinal-type adenocarcinoma (ITAC) samples. Results were correlated to clinicopathological characteristics and follow-up data.

Results: Membranous PD-L1 staining of tumor cells was observed in 34% (18/53) of the sinonasal SCC samples and in 17% (22/126) of the ITAC samples. The PD-L1 positivity on infiltrating immune cells occurred in 45% (24/53) of the sinonasal SCC samples and in 33% (41/126) of the ITAC samples. Expression of PD-L1 showed no correlation to clinicopathological parameters and was not an independent risk factor for survival.

Conclusion: The PD-L1 positivity does not seem to have prognostic value. However, a proportion of patients with sinonasal SCC and ITAC may benefit from therapy with immune checkpoint inhibitors that recently have been approved for clinical application in head and neck cancer.

KEYWORDS
adenocarcinoma, immunotherapy, programmed death ligand-1 (PD-L1), sinonasal cancer, squamous cell carcinoma

1 | INTRODUCTION

Tumors in the sinonasal cavities represent 5% of all head and neck cancers with an incidence of approximately 0.5 of 100 000 inhabitants.[1,2] Squamous cell carcinoma (SCC) and intestinal-type adenocarcinoma (ITAC) represent approximately 70%-80% of sinonasal cancer, the remainder include a miscellany of neuroendocrine carcinomas, neuroectodermal neoplasms, salivary gland tumors, and undifferentiated carcinomas. Sinonasal SCC and ITAC have been etiologically related to occupational exposures, including wood and leather dust, nickel, and chromium.[3] There are also indications that human papillomavirus (HPV) and Epstein-Barr virus play a role in the development of a subset of sinonasal

cancer.[4,5] Diagnosis of these tumors is often late due to unspecific symptoms similar to inflammatory processes.

Despite improvements in the field of surgery and radiotherapy, the prognosis is still poor with a 5-year overall survival of 30%-50%.[1,2,6,7] Typically, all sinonasal tumors frequently develop local recurrences (50%-80%), the main cause of death. Lymph node or distant metastasis occur in 10%-20% of cases.[1,2,6,7] For locally advanced sinonasal tumors, treatment usually is surgery combined with radiotherapy, and, in some cases, a multimodal approach, including chemotherapy.[1,8] However, there is a clear need for new therapeutic options.

Molecular genetic studies may guide the application of modern anticancer drugs that target specific signaling pathways. Immunotherapy may be an option for treatment of sinonasal cancer. Specific monoclonal antibodies can inhibit the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) immune checkpoint pathway and enhance the antitumor activity of the immune system. In clinical trials with head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC), two tumors with histological similarity to sinonasal SCC and ITAC, immunotherapy has shown significant response rates.[9–14] In 2016, the Food and Drug Administration (FDA) approved the use of pembrolizumab and nivolumab for recurrent and metastatic HNSCC. Although this is still subject to further investigation, membranous PD-L1 protein expression on tumors and infiltrating immune cells is a means to predict clinical efficacy of immunotherapy. The purpose of this study was to evaluate the prevalence of PD-L1 expression in a series of 179 patients with sinonasal SCC and ITAC.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical variables

Primary tumor samples were obtained from previously untreated patients, 53 with sinonasal SCC who were seen between 1989 and 2009 and 126 with ITAC seen between 1978 and 2014 at the Department of Otolaryngology at the Hospital Universitario Central de Asturias (Oviedo, Spain). Written informed consent for the collection, storage, and analysis of specimens was obtained from all patients. The study had received prior approval from our institutional ethical committee. Details on the clinical features are presented in Table 1. The disease stage is according to the TNM system for tumor classification.[15] The ITAC subtypes were histologically evaluated by an experienced pathologist (B.V.) according to the World Health Organization histological classification.[2] Eleven were papillary type or papillary tubular cylinder cell I (PTCC-I), 79 colonic (PTCC-II), 9 solid (PTCC-III) and 27 mucinous type. Cases showing more than 1 type were classified according to the worst histology. A history of wood dust exposure was recorded for 106 of 121 patients (88%) with ITAC. Craniofacial resection was performed in the majority of cases; 26 cases of ITAC and 2 cases of sinonasal SCC were treated with endoscopy surgery. Neck dissection was performed in 2 patients with ITAC and 5 patients with sinonasal SCC. After radical surgery, 42 of 53 patients (79%) with sinonasal SCC and 75 of 121 patients (62%) with ITAC received radiotherapy with 60 to 70 Gy using 3D image-based treatment planning. The median follow-up was 18 months (range 1-312 months) for the patients with sinonasal SCC and 30 months (range 1-460 months) for the patients with ITAC.

2.2 | Immunohistochemistry

Tissue microarray blocks were prepared from formalin-fixed paraffin-embedded tumor tissues using the Beecher Tissue Microarrayer (Beecher Instruments, Silver Spring, MD). In total, 7 tissue microarray blocks were constructed, containing three 1-mm cores from different areas of 179 sinonasal tumors. Each block included normal sinonasal mucosa samples as internal controls. Three micrometer sections were stained with hematoxylin-eosin and reviewed by 1 pathologist to determine whether the samples contained a good representation of the original tumor blocks. Immunohistochemistry was performed on an automatic staining workstation (Dako Autostainer Plus; DakoCytomation) using a 1:100 dilution of the monoclonal rabbit anti-PD-L1 clone E1L3N (Cell Signaling Technology, Cambridge, UK) and antigen retrieval by EnVision FLEX + Mouse (LINKER), high pH (DakoCytomation) during 20 minutes. The slides were evaluated in a double-blind manner by 3 observers (C.R., B.V., and M.A. H.) and discrepancies between observers were solved afterward by looking together using a multihead microscope. Tumors were scored in 5 classes: 0%; 1%-5%; 5%-25%; 25%-50%, and 50%-100% membranous staining and then dichotomized into negative (5% or lower) or positive (5%-100%). The PD-L1 staining of immune cells was evaluated in the same manner. The 5% cutoff was chosen in accordance with most studies in the field, including clinical immunotherapy trials.[9,10,13,16,17]

2.3 | Statistical analysis

Correlations between the immunohistochemistry results and clinicopathological variables were analyzed by SPSS version 15.0 software for Windows (SPSS, Chicago, IL), using the Pearson chi-square and Fischer Exact tests. Kaplan-Meier analysis was performed for estimation of overall and disease-free survival, comparing distributions through the Mantel-Cox log-rank test. Multivariate Cox regression analysis was performed for factors possibly related to survival. Values of $P < .05$ were considered significant.

TABLE 1 Patient and tumor characteristics

	Sinonasal SCC: no. of patients (%)				ITAC: no. of patients (%)			
	53 total	18 PD-L1-positive	35 PD-L1-negative	*P* value	126 total	22 PD-L1-positive	104 PD-L1-negative	*P* value
Sex								
Female	16 (30)	8 (50)	8 (50)	.125	2 (2)	1 (50)	1 (50)	.320
Male	37 (70)	10 (27)	27 (73)		124 (98)	21 (17)	103 (83)	
Tumor site								
Maxillary sinus	42 (79)	14 (33)	28 (67)	.999	0 (0)	0 (0)	0 (0)	NA
Ethmoid sinus	11 (22)	4 (36)	7 (64)		126 (100)	22 (17)	104 (83)	
Disease stage								
I	0 (0)	0 (0)	0 (0)	.550	25 (20)	3 (12)	22 (88)	.819
II	4 (7)	0 (0)	4 (100)		14 (11)	3 (21)	11 (79)	
III	18 (34)	6 (33)	12 (67)		43 (34)	7 (16)	36 (84)	
IVa	22 (42)	10 (45)	12 (55)		16 (13)	4 (25)	12 (75)	
IVb	9 (17)	2 (22)	7 (78)		23 (18)	5 (22)	18 (78)	
Missing	0 (0)	0 (0)	0 (0)		5 (4)	0 (0)	5 (100)	
Histological differentiation								
Well	17 (32)	5 (29)	12 (71)	.655	NA	NA	NA	NA
Moderate	11 (21)	5 (45)	6 (55)		NA	NA	NA	
Poor	25 (47)	8 (32)	17 (68)		NA	NA	NA	
Histological subtype								
Papillary (PTCC-I)	NA	NA	NA	NA	11 (9)	1 (9)	10 (91)	.153
Colonic (PTCC-II)	NA	NA	NA		79 (63)	13 (16)	66 (84)	
Solid (PTCC-III)	NA	NA	NA		9 (7)	4 (44)	5 (56)	
Mucinous	NA	NA	NA		27 (21)	4 (15)	23 (85)	
Recurrence								
No	9 (17)	2 (22)	7 (78)	.701	61 (48)	13 (21)	48 (79)	.481
Yes	44 (83)	16 (36)	28 (64)		60 (48)	9 (15)	51 (85)	
Missing	0 (0)	0 (0)	0 (0)		5 (4)	0 (0)	5 (100)	
Metastasis								
No	48 (91)	16 (33)	32 (67)	.999	109 (87)	21 (19)	88 (81)	.693
Yes	5 (9)	2 (22)	3 (78)		12 (9)	1 (8)	11 (92)	
Missing	0 (0)	0 (0)	0 (0)		5 (4)	0 (0)	5 (100)	
Patient status								
Alive	5 (10)	1 (20)	4 (80)	.348	51 (40)	10 (20)	41 (80)	.925
Died of disease	41 (77)	16 (39)	25 (61)		54 (43)	9 (17)	45 (83)	
Died of other causes	7 (13)	1 (14)	6 (86)		16 (13)	3 (19)	13 (81)	
Missing	0 (0)	0 (0)	0 (0)		5 (4)	0 (0)	5 (100)	

Abbreviations: ITAC, intestinal-type adenocarcinoma; NA, not applicable; PD-L1, programmed death ligand-1; PTCC, papillary tubular cylinder cell; SCC, squamous cell carcinoma.

3 | RESULTS

3.1 | Follow-up

During the course of follow-up of the 53 patients with sinonasal SCC, 44 developed local recurrences (83%), 5 of whom also developed distant metastases (9%). At the time of the current report, a total of 9 patients remained disease free (17%; Table 1). The overall survival, disease-specific survival, and disease-free survival in relation to disease stage and localization is presented in Figure 1. The main causes of death were local recurrences and distant metastases. Seven patients died during the postoperative period or because of intercurrent causes. Survival was not significantly related to clinical variables as disease stage, histological grade, or localization.

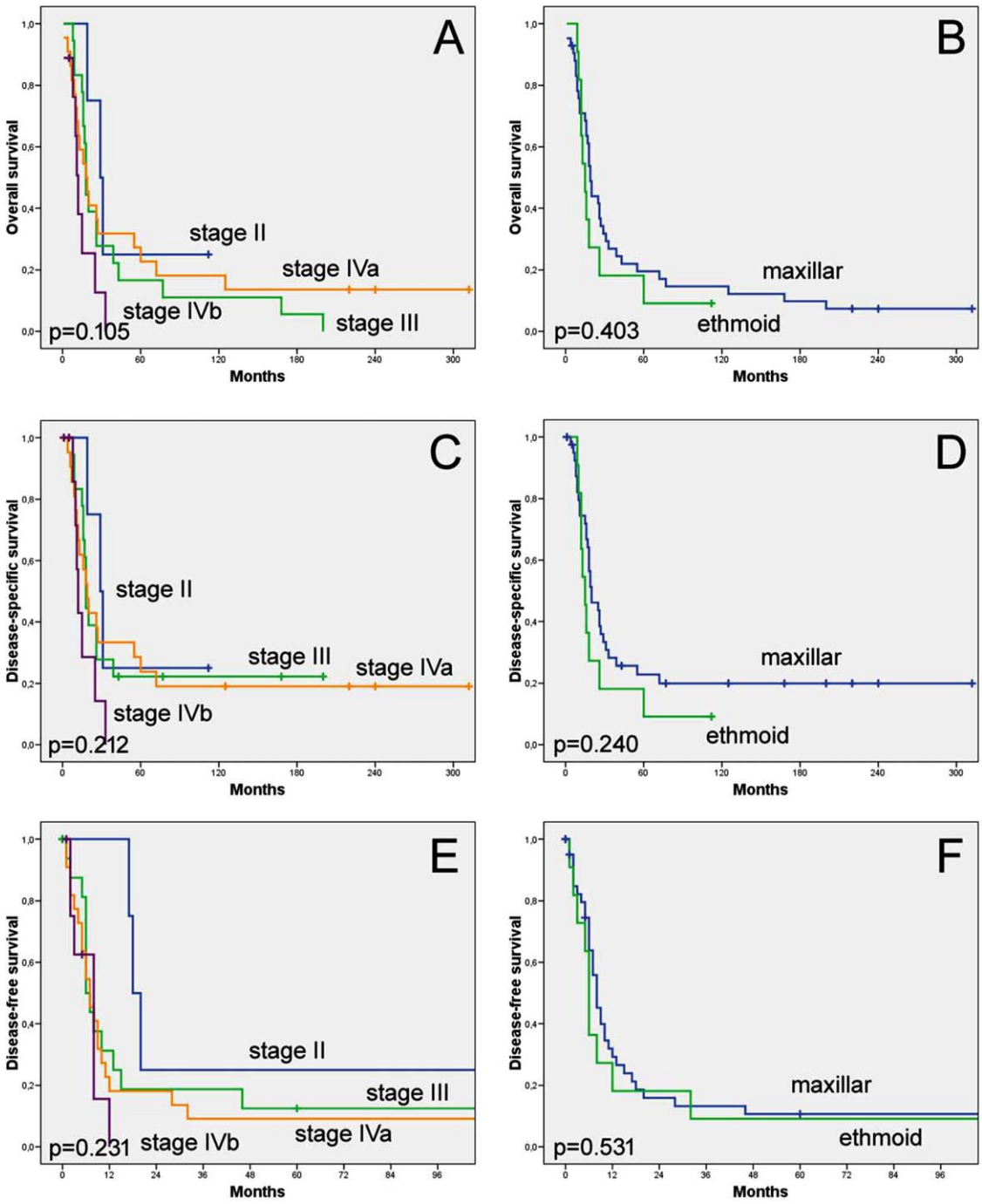

FIGURE 1 Kaplan-Meier survival analysis of 53 patients with sinonasal squamous cell carcinoma. Overall survival according to disease stage A, and tumor localization B. Disease-specific survival according to disease stage C, and tumor localization D. Disease-free survival according to disease stage E, and tumor localization F [Color figure can be viewed at wileyonlinelibrary.com]

Follow-up data was available for 121 of the 126 patients with ITAC. Sixty patients developed local recurrence (48%), 12 patients developed distant metastases (9%; Table 1), and 54 patients (43%) remained disease free. The overall survival, disease-specific survival, and disease-free survival rates in relation to disease stage and histological subtype are given in Figure 2. The main cause of death was local recurrence and intracranial invasion. Survival was significantly related to disease stage (log-rank 40.879; $P = .001$), to intracranial invasion (log-rank 27.037; $P = .001$), and to histological subtype (log-rank 12.206; $P = .007$) but not to the type of surgery (log-rank 0.198; $P = .656$).

3.2 | Programmed death ligand-1 and correlation with clinicopathological data

Membranous PD-L1 staining in $> 5\%$ of tumor cells was observed in 34% cases (18/53) of sinonasal SCC and in 17%

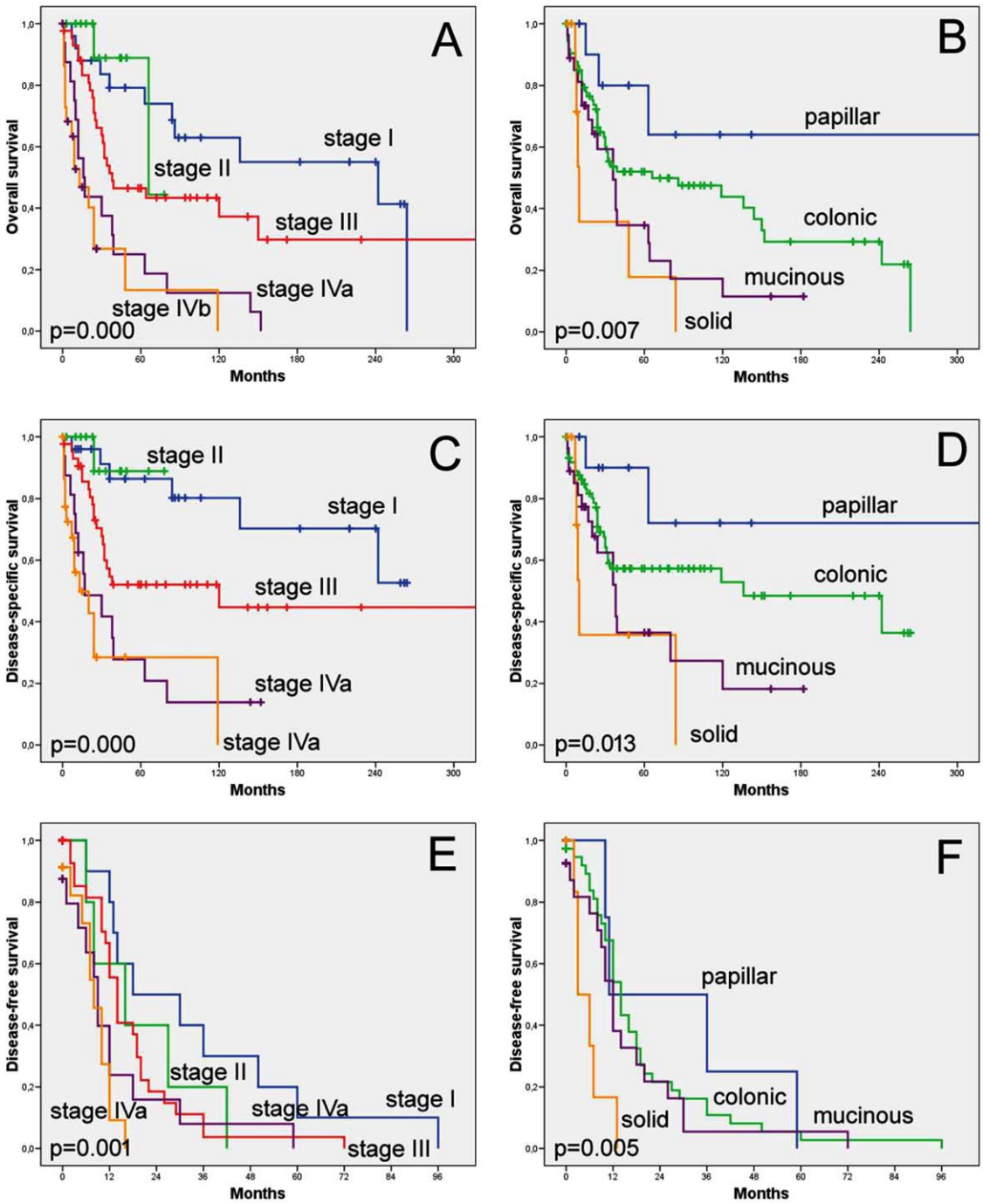

FIGURE 2 Kaplan–Meier survival analysis of 121 patients with intestinal-type adenocarcinoma. Overall survival according to disease stage A, and histological subtype B. Disease-specific survival according to disease stage C, and histological subtype D. Disease-free survival according to disease stage E, and histological subtype F [Color figure can be viewed at wileyonlinelibrary.com]

cases (22/126) ot ITAC (see Figure 3). Expression in > 50% of tumor cells occurred in 78% cases (14/18) of sinonasal SCC and in 23% cases (5/22) of ITAC with PD-L1 positivity. The staining pattern was diffuse in sinonasal SCC, whereas ITAC more frequently demonstrated focal expression. Nuclear expression of PD-L1 was observed in 11 of 179 tumors, all of which were papillary or colonic-type ITAC (see Figure 3). In neither sinonasal SCC nor ITAC, positive PD-L1 staining correlated with clinicopathological parameters, including localization, histological grade, disease stage, or the development of recurrences or metastases (Table 1). Solid-type ITAC showed a significantly ($P = .049$) higher PD-L1 positivity than papillary, colonic, and mucinous subtypes: 44% (4/9) versus 15% (18/117). In addition, the patients with ITACs with exposure to wood dust had less PD-L1 positivity than those without, 16% (17/106) versus 33% (5/15); however, this difference was not statistically significant.

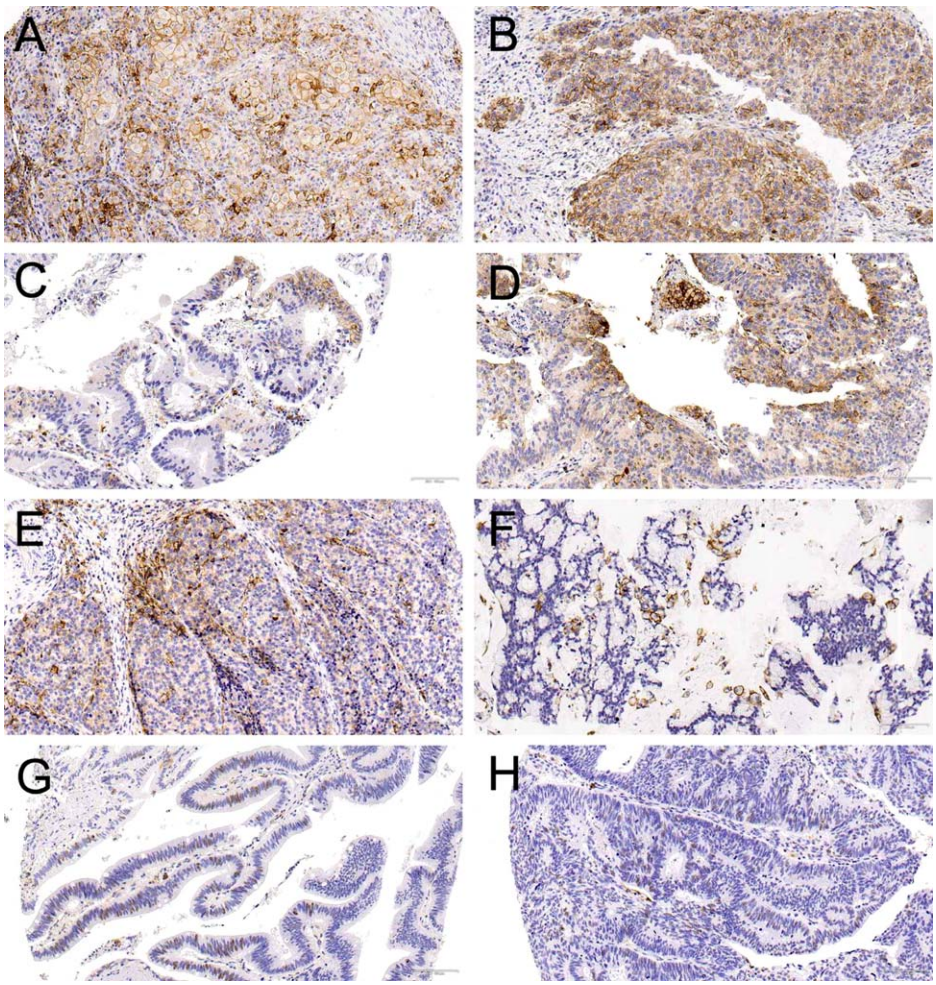

FIGURE 3 Representative membranous immunostaining of programmed death ligand-1 (PD-L1) in a well-differentiated sinonasal squamous cell carcinoma (SCC) A, a poorly differentiated sinonasal SCC B, a papillary-type intestinal-type adenocarcinoma (ITAC) C, a colonic type ITAC D, a solid type ITAC E, and a mucinous-type ITAC F. Representative nuclear immunostaining of PD-L1 in a papillary-type ITAC G, and a colonic-type ITAC H. Original magnification all images: 15× [Color figure can be viewed at wileyonlinelibrary.com]

The PD-L1 staining in > 5% of tumor-infiltrating immune cells were observed in 45% cases (24/53) of sinonasal SCC and in 33% cases (41/126) of ITAC. In both tumors, this was significantly correlated ($P = .001$) with PD-L1 expression on the tumor cells. The presence of PD-L1-positive immune cells showed no correlation to clinicopathological parameters, disease stage, localization, histological grade, or the development of recurrences or metastases (Table 1). Mucinous-type ITAC showed a significantly ($P = .010$) lower PD-L1 positivity on immune cells than papillary, colonic, and solid subtypes: 11% (3/27) versus 38% (38/99).

3.3 | Programmed death ligand-1 and correlation with follow-up data

Neither sinonasal SCC nor ITAC demonstrated a significant difference in overall or disease-specific survival according to PD-L1 positivity on the tumors (see Figure 4) or infiltrating immune cells. However, disease-free survival was significantly worse in cases with PD-L1 staining on the tumor cells (see Figure 4). The 1-year disease-free survival was 43% in PD-L1-negative cases versus 6% in PD-L1-positive sinonasal SCC cases, and 61% in PD-L1-negative cases versus 42% in PD-L1-positive ITAC cases (log-rank 5.872; $P = .015$ for sinonasal SCC and log-rank 4.592; $P = .032$ for ITAC; see Figure 4). Using multivariate analysis of disease-free survival in relation to PD-L1 status and radiotherapy, we found that PD-L1 maintained its significance in sinonasal SCC ($P = .014$) and showed a strong tendency in ITAC ($P = .052$). Multivariate Cox regression analysis of PD-L1 status, disease stage, and localization in sinonasal SCC showed that PD-L1 did not remain statistically significant with disease-free survival, although the tendency is still there. In ITAC, multivariate analysis of PD-L1 status, disease stage, and histological subtype showed that only stage maintained its statistical significance with survival (Table 2).

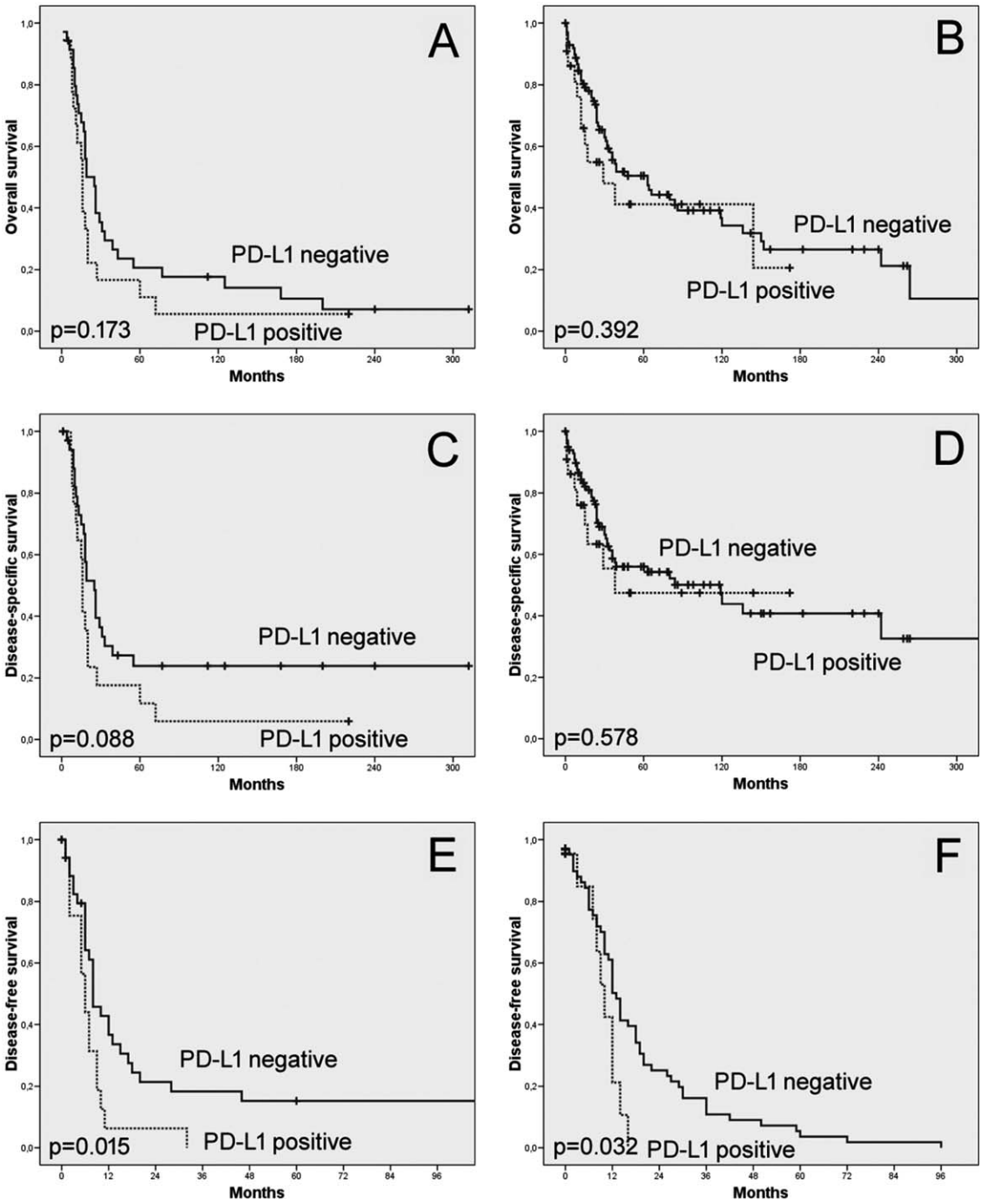

FIGURE 4 Kaplan-Meier survival analysis. Overall survival according to programmed death ligand-1 (PD-L1) immunostaining of 53 patients with sinonasal squamous cell carcinoma (SCC) A, and 121 patients with intestinal-type adenocarcinoma (ITAC) B. Disease-specific survival according to PD-L1 immunostaining of 53 patients with sinonasal SCC C, and 121 patients with ITAC D. Disease-free survival according to PD-L1 immunostaining of 53 patients with sinonasal SCC E, and 121 patients with ITAC F

4 | DISCUSSION

Patients with sinonasal cancer have a poor prognosis, especially in recurrent stages, and it is a disease with very limited treatment options. In this study, the expression of PD-L1 as a marker for immunotherapy was evaluated in 179 cases of sinonasal tumors. In addition, we investigated its possible role as a prognostic factor. Results showed that 34% cases of sinonasal SCC and 17% cases of ITAC expressing membranous PD-L1 in $>5\%$ of tumor cells. Expression in $>50\%$ of tumor cells was frequent in sinonasal SCC (14/53; 26%) in contrast to ITAC (4/126; 3%).

To the best of our knowledge, this is the first report on PD-L1 concerning sinonasal cancer, therefore, there are no other data for comparison. However, tumors with similar histology, such as HNSCC and NSCLC have been shown to

TABLE 2 Multivariate Cox regression survival analysis of 53 patients with sinonasal squamous cell carcinoma according to disease stage, localization, and programmed death ligand-1 status, and of 126 patients with intestinal-type adenocarcinoma according to disease stage, histological subtype, and programmed death ligand-1 status

	Overall survival		Disease-specific survival		Disease-free survival	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Sinonasal SCC						
Stage	1.276 (0.870-1.872)	.212	1.318 (0.875-1.984)	.186	1.357 (0.923-1.994)	.120
Localization	1.329 (0.651-2.712)	.435	1.478 (0.716-3.048)	.291	1.127 (0.546-2.327)	.746
PD-L1	1.355 (0.739-2.486)	.326	1.513 (0.798-2.872)	.205	1.834 (0.955-3.521)	.068
ITAC						
Stage	1.683 (1.370-2.068)	.001	1.837 (1.447-2.332)	.001	1.458 (1.169-1.817)	.001
Histological subtype	1.271 (0.998-1.620)	.052	1.276 (0.970-1.677)	.081	1.107 (0.876-1.399)	.394
PD-L1	1.135 (0.606-2.129)	.692	1.039 (0.505-2.136)	.918	1.706 (0.831-3.505)	.146

Abbreviations: CI, confidence interval; ITAC, intestinal-type adenocarcinoma; PD-L1, programmed death ligand-1; SCC, squamous cell carcinoma.

express PD-L1 in the same range of frequency. In HNSCC, the incidence varies between 19% and 45%, whereas HPV-positive tumors seem to have higher PD-L1 positivity.[16–19] In NSCLC, including both SCC and adenocarcinoma, frequencies of 14%-50% have been reported, with SCC generally showing higher positivity.[20–23] Among cases of adenocarcinoma-type NSCLC, those with solid histology showed the highest PD-L1 positivity, which is in agreement with the findings in ITAC in this article.[20] A limitation in our study may be that only 1 pathologist (B.V.) performed the ITAC subtyping, however, we are confident in the evaluation. The variability between the studies may be explained by geographic, technical, (sampling, choice of antibody, flow cytometry, and quantitative polymerase chain reaction), and interpretation (positivity on cell membrane/cytoplasm, cutoff points) differences.

A surprising result was the finding of nuclear expression of PD-L1 exclusively observed in papillary/colonic-type ITAC, either as the sole type of positivity (in 7 of 11 cases) or in combination with membranous staining (in 4 of 11 cases; see Figure 3). Nuclear PD-L1 has been reported in circulating prostate and colorectal adenocarcinoma cells.[24] In addition, nuclear expression of B7-H3, a protein of the same family as PD-L1, has been detected by immunohistochemistry in 30% of 277 cases of colorectal adenocarcinomas.[25] In both studies, nuclear staining was associated with poor prognosis. Being a transmembrane protein, it is not clear what could be the biological role of nuclear trafficking of PD-L1, although it has been described for epidermal growth factor receptor and other membrane proteins.[26]

By expressing PD-L1, tumor cells create an immunosuppressive microenvironment and so may avoid cytolysis by activated T cells. This may explain the relation to poor prognosis demonstrated in most reports. In this study, both sinonasal SCC and ITAC with >5% PD-L1 expression had a significantly worse disease-free survival (see Figure 4), although, in multivariate analysis with clinicopathological parameters, it lost statistical significance (Table 2). We found no significant correlation with overall or disease-specific survival. Worse survival in relation to PD-L1 expression has been found in HNSCC, including oral, laryngeal, pharyngeal, oropharyngeal, and nasopharyngeal subsites,[17–19,27] as well as in SCC and adenocarcinoma NSCLC.[20,21,28] On the other hand, some studies reported PD-L1 to have no prognostic value in NSCLC,[23] or to be associated with better clinical outcome in HNSCC[29,30] or NSCLC.[31]

Although this is still subject to further investigation, PD-L1 protein expression on tumor cells is a means to predict clinical efficacy of immunotherapy with specific monoclonal antibodies that target the PD-1/PD-L1 immune checkpoint pathway and enhance the antitumor activity of the immune system. Indeed, most clinical trials on HNSCC and NSCLC have shown a stronger response to inhibitors of PD-1 (pembrolizumab and nivolumab) and PD-L1 (atezolizumab and durvalumab) in patients positive for PD-L1 expression,[10,11,13,32] although there are also studies that reported no relation to PD-L1 expression.[9,12,14] The PD-L1 protein expression alone may not be sufficient to predict responsiveness to immunotherapy. It has been shown that tumors with a high mutational load and exposure to mutagenic compounds respond better, whereas tumors with B-type Raf or Janus kinase 1/2 mutations or loss of phosphatase and tensin homolog expression respond worse to immune blockade therapy.[33] Recently, Ayers et al[34] proposed an assay based on the expression profile of 6 or 18 genes, including interferon-gamma as a clinical predictor of response. Finally, evaluation of the density of CD8 T cell infiltrate as well as the proximity of PD-1 expressing immune cells to PD-L1 expressing tumor cells can enhance the predictive value of PD-L1 staining alone.[35]

In August and October 2016, the FDA approved the use of pembrolizumab and nivolumab for recurrent or metastatic HNSCC, based on a phase 1b and phase III clinical trial, respectively.[9,10] Chow et al[10] reported a 6-month progression-free survival of 23% in 132 patients treated with pembrolizumab. The PD-L1-positive cases (defined as $>1\%$, either on the tumor cell only or on the tumor and immune cells) showed a significant better response, 22% versus 4% in PD-L1-negative patients. Moreover, the response was more pronounced in HPV-associated cases (assessed by p16 immunohistochemistry). Ferris et al[9] compared patients treated with nivolumab to standard single-agent therapy. The 6-month progression-free survival rates were 20% and 10%, respectively. No relation was found between the response and PD-L1 or HPV (assessed by p16 immunohistochemistry) status. Importantly, both studies demonstrated that immunotherapy was well tolerated.

In conclusion, a number of clinical trials with different immunotherapeutic agents are ongoing, in patients with HNSCC whose disease has progressed during or after chemotherapy, but also in a first-line setting.[36,37] Pembrolizumab and nivolumab have already received FDA approval for clinical application in recurrent or metastatic HNSCC and may also be considered for treatment of recurrent sinonasal SCC. Immunotherapy may also be effective in ITAC, as has been shown in lung adenocarcinoma. Patients with sinonasal SCC or ITAC have very limited treatment options available, especially those with recurrent disease (in the present study, 83% and 56% of cases, respectively). Therefore, although PD-L1 positivity does not seem to have prognostic value, a proportion of cases showed PD-L1 expression and might benefit from immunotherapy.

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