Received: 20 January 2019 | Revised: 7 June 2019 | Accepted: 12 June 2019

DOI: 10.1002/hed.25857

ORIGINAL ARTICLE

WILEY

Prognostic markers in salivary gland cancer and their impact on survival

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Funding information
Greater Poland Cancer Center, Grant/Award Number: 15/2015(107)

Abstract

Background: The role of molecular markers in salivary gland carcinoma (SGC) is not well understood. We evaluated molecular marker expression and their prognostic value.

Methods: Immunohistochemical analysis of 124 tumor specimens was performed to determine expression of androgen (AR), estrogen (ER), and progesterone (PR) receptors and epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), programmed death ligand 1 receptor (PD-L1), and PD-L1 in tumor-infiltrating mononuclear cell (TIMC). Survival outcomes (disease-free survival [DFS] and overall survival [OS]), pT and N classification, margin status, and treatment failure were assessed.

Results: Most patients (78; 62.9%) had early-stage SGC. AR positivity and EGFR positivity were detected in 21.0% and 78.6%, respectively, of tumors. AR positivity and PD-L1 negativity were associated with locally advanced disease. PD-L1-negativity was associated with higher recurrence (38.5% vs 0%; $P < .001$) and worse DFS. OS and DFS were worse in patients with AR+ or HER2+ disease.

Conclusions: Several molecular markers—AR and HER2 positivity and PD-L1 negativity—were associated with worse clinical outcomes. Prospective, multi-institutional trials are needed to determine the prognostic value of these markers.

KEYWORDS
androgen, EGFR, HER2, PD-L1, salivary gland cancer

1 | INTRODUCTION

Malignant salivary gland carcinoma (SGC) accounts for approximately 5% of all head and neck cancers and there are more than 20 different pathological subtypes according to the World Health Organization.[1] Due to the highly heterogeneous nature of SGC, treatment selection can be challenging and an individualized approach is required in most cases.[2] Surgery followed by adjuvant radiotherapy is the mainstay of treatment, although oncological outcomes are often less than satisfactory.[3,4] In recurrent or metastatic disease, successful sequential treatment is limited by the poor efficacy of standard chemotherapy protocols.[2,5]

In recent years, a growing body of evidence[6] has demonstrated the value of molecular targeted therapy in numerous cancers. The success of this therapeutic approach relies on the presence and expression of certain proteins, which can then be targeted by therapeutic agents that selectively bind to the receptors on those targets. Conceivably, molecular targeted therapy could also be applied to SGC, but to date the data to support this therapeutic approach in SGC are scant, largely to the rarity of this tumor type.[7] For this reason, more research is needed to

identify the molecular receptors present in these tumors. A more comprehensive understanding of the potential molecular targets in this tumor type could improve diagnosis and facilitate the development of more effective therapies to improve outcomes.

In this context, the aim of the present study was to investigate the expression of a range of different molecular markers in patients with SGC and to evaluate the prognostic value of these markers.

2 | PATIENTS AND METHODS

2.1 | Patients

This retrospective study was based on tumor specimens obtained from 124 patients diagnosed and treated for malignant SGC. All patients underwent curative-intent surgery from 2007 to 2017 at our institution (a tertiary care hospital). All patients treated at our institution for primary SGC during the study period were included. Patients with recurrent disease or a second primary tumor were excluded from the study. Written informed consent was obtained from each patient prior to treatment. Study approval was obtained from the Research Ethics Board at Poznan University of Medical Sciences.

The following patient-related variables were recorded: sex, age at presentation, TNM stage, tumor histology and grade, surgical margin status, and adjuvant treatment. Recurrent disease was classified as local, regional, distant, or combination.

When indicated by the institutional multidisciplinary team, patients were qualified for adjuvant treatment. The standard radiotherapy protocol was 60-66 Gy (2.0 Gy/fraction) administered daily from Monday to Friday for 6-7 weeks. Patients who met the following criteria were considered candidates for adjuvant radiotherapy: stage pT3/4 tumor, close surgical margins (1-5 mm), positive nodes, and evidence of perineural/vascular invasion.

All patients with N0 neck disease were seen to a multidisciplinary tumor board, which evaluated a wide range of factors before making the treatment decision. The following variables were considered in deciding whether to propose elective nodal dissection (END; level I + III): the presence of locally advanced disease, high tumor grade, high-risk histological type (ie, salivary duct carcinoma, adenocarcinoma not otherwise specified [NOS], or mucoepidermoid carcinoma [MEC]), and patient preferences. When the full histological report was available, those same factors were also considered when deciding to perform elective nodal irradiation (ENI). Finally, patients not considered suitable for either END or ENI (due to the absence of high-risk factors), or who refused either of those procedures, were assigned to the observation group.

2.2 | Tissue specimens

Hematoxylin-eosine stained slides were obtained for all patients and examined by an experienced pathologist (J.S.), who was blinded to all clinical data. The pathologist selected the areas of primary tumor for tissue microarray (TMA) construction. Immunohistochemistry was performed to determine expression of the following receptors: androgen (AR), estrogen (ER), and progesterone (PR) receptors; epidermal growth factor receptor (EGFR); human epidermal growth factor receptor 2 (HER2); programmed death ligand 1 receptor (PD-L1); and PD-L1 expression in tumor-infiltrating mononuclear cells (TIMCs).

2.3 | Immunohistochemical analysis

Immunohistochemistry was performed and examined on TMA by a pathologist (A.M.) blinded to the clinical data. The anti-HER-2/neu (clone 4B5) and anti-EGFR (clone 5B7) antibodies, both from Roche, were used.

Antigens were retrieved in Target Retrieval Solution (Dako Denmark A/S, Glostrup, Denmark) with high and low pH (for PD-L1) using a Pt. link machine from Dako at 97°C for 20 minutes. Estrogen alfa receptors were marked with ER clone EP1 radiotracer uptake (RTU) monoclonal antibodies from Dako. Immunohistochemistry was performed with the En Vision FLEX from Dako. Immunoperoxidase staining was performed using the Dako Autostainer Link 48. For PD-L1 (ab205921), monoclonal antibodies by Abcam and Dako (M3562) were used for marker staining and AR. Anti-human AR (clone AR441) antibodies were used to stain anti-PD-L1 (clone 28-8). Specimens were incubated with antibody for 20 minutes in anti-PD-L1 1/100 anti-AR 1/50 dilutions, followed by incubation with Dako En Vision FLEX/HRP for 20 minutes. Next, the specimens were incubated with En Vision FLEX DAB+ Chromogen for 5 minutes. Immunoperoxidase staining was performed manually at room temperature. PRs were stained using monoclonal antibodies (GA090) from Dako. Immunohistochemistry was performed using the Dako OMNIS EnVisionTM FLEX system. Specimen antibodies in paraffin were examined with the Dako OMNIS Target Retrieval Solution, high pH at 97°C for 30 minutes. RTU antibody (PR clone PgR1294) was used to stain the marker. Immunoperoxidase staining was performed with the OMNIS equipment from Dako. The AR, ES, and PR were scored based on positive or negative diffuse nuclear staining. Immunohistochemistry of HER2 was evaluated according to the methods used to assess HER2 in breast cancer and described in detail elsewhere.[8] The EGFR score was based on the percentage of immunopositive cells and staining intensity. Cases with >80% of immunopositive cells and moderate or strong intensity were considered positive. Immunoreactivity of PD-L1 expression was evaluated in the tumor cell membrane, with positivity

defined as >5% of cell membrane staining. PD-L1 immunoreactivity in TIMC was defined as >5% of infiltrating cell membrane staining.

2.4 | Statistical analysis

Statistical analysis was performed using the Statistica software package, v. 12 (StatSoft Inc, Tulsa, Oklahoma). Outcome measures included disease-free survival (DFS) and overall survival (OS). DFS and OS were calculated from the date of surgery until the date of recurrence or last follow-up visit (DFS) or until death or last follow-up visit (OS). Kaplan-Meier methods were used to estimate survival outcomes. The log-rank test was used to compare survival curves.

The chi-square test was used to determine the influence of receptor positivity on clinical variables including pathological T classification, N classification, margin status, treatment failure (local, regional, distant, or combination), DFS, and OS. A value of $P < .05$ was considered statistically significant.

TABLE 1 Clinical characteristics and treatment outcomes

Number of patients	124 (100%)
Sex, male	65 (52.4%)
Tumor localization	
Parotid gland	75 (60.5%)
Submandibular gland	22 (17.7%)
Minor salivary glands	27 (21.8%)
Grade, number of patients (%)	97 (100%)
Low	36 (37.1%)
Intermediate	23 (23.7%)
High	38 (39.2%)
Stage T1 + T2	78 (62.9%)
Stage T3 + T4	46 (37.1%)
N0	94 (75.8%)
N+	30 (24.2%)
Positive margin status	92 (74.2%)
Adjuvant treatment	78 (62.9%)
Recurrence	35 (28.2%)

3 | RESULTS

A total of 124 patients (65 males; 52.4%) were included in the study. Mean age at diagnosis was 59 years (range, 24-87; SD, 15). Mean (SD) follow-up was 31 (28.9) months (range, 2-110). Most tumors were located in the parotid (75 of 124; 60.5%) or submandibular glands (22 of 124; 17.7%).

The tumor grade was determined in 97 patients, 39.2% of which were high grade (Table 1). Most patients (73%-63%) received postoperative radiotherapy. Adjuvant treatment had no impact on survival.

One hundred and six patients were clinically N0 at presentation. Of these, 27 (25.7%) underwent END, 17 (16.0%) underwent ENI, and 62 (58.5%) underwent observation. Treatment failure was higher (nonsignificantly) in the END group (25.9%) vs the observation (21.0%) and ENI (11.8%) groups. No differences were observed among the three groups in terms of DFS or OS.

Histologically, adenoid cystic carcinoma (ACC) was the most common tumor type (n = 33; 26.6%) followed by MEC (n = 16; 12.9%) and acinic cell carcinoma (AccCC) (n = 15; 12.0%; Table 2).

TABLE 2 Incidence of molecular receptors according to pathological type

Pathology/ receptor	Number of cases	AR+	ER+	PR+	HER2+	EGFR+	PD-L1 tumor	PD-L1 in TIMC
		26/120 (21.0%)	3/121 (2.5%)	3/121 (2.5%)	12/118 (10.2%)	92/119 (78.6%)	22/117 (18.8%)	6/117 (5.1%)
AccCC	15	0%	0%	0%	0%	25%	0%	0%
SDC	16	80%	0%	0%	43.8%	75%	6.3%	12.5%
MEC	16	18.8%	6.3%	18.8%	0%	87,5%	18.8%	6.3%
ACC	33	6.5%	0%	0%	0%	80.6%	30%	0%
Adeno-NOS	13	41.7%	16.7%	0%	9,1%	90.9%	9.1%	0%
CaExPA	13	23.1%	0%	0%	23.1%	100%	38.5%	0%
CaMioEp	9	0%	0%	0%	0%	100%	0%	0%
Other	9	11.1%	0%	0%	0%	66.7%	33.3%	33.3%

Note: Not all specimens were tested for all receptors.

Abbreviations: ACC, adenoid cystic carcinoma; AccCC, acinic cell carcinoma; adeno NOS, adenocarcinoma not otherwise specified; AR, androgen receptor; CaExPA, carcinoma ex pleomorphic adenoma; CaMioEp, myoepithelial carcinoma; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MEC, mucoepidermoid carcinoma; PR, progesterone receptor; SDC, salivary duct carcinoma.

TABLE 3 Receptor status and clinical data

Receptor status	Total number of cases (%)	Locally advanced disease (T3/T4)	Node positive status	Positive surgical margin	Treatment failure
AR+	26/120 (21.7%)	16/26 (61.5%)	15/26 (57.7%)	20/26 (76.9%)	8/26 (30.8%)
AR−	94/120 (78.3%)	26/94 (27.7%)	13/94 (13.8%)	67/94 (71.3%)	25/94 (26.6%)
P value		.003	<.001	NS	NS
HER2+	12/118 (10.2%)	7/12 (58.3%)	5/12 (41.7%)	6/12 (50.0%)	5/12 (41.7%)
HER2(−)	106/118 (89.8%)	35/106 (33.0%)	23/106 (21.7%)	81/106 (76.4%)	29/106 (27.4%)
P value		NS	NS	NS	NS
EGFR+	92/117 (78.6%)	32/92 (34.8%)	20/92 (21.7%)	65/92 (70.7%)	24/92 (26.1%)
EGFR(−)	25/117 (21.4%)	10/25 (40.0%)	6/25 (24.0%)	21/25 (84.0%)	8/25 (32.0%)
P value		NS	NS	NS	<.001
PD-L1+	22/117 (18.8%)	3/22 (13.6%)	2/22 (9.1%)	14/22 (63.6%)	3/22 (13.6%)
PD-L1(−)	95/117 (81.2%)	39/95 (41.1%)	25/95 (26.3%)	72/95 (75.8%)	30/95 (31.6%)
P value		.02	NS	NS	<.001

Abbreviations: AR, androgen receptor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; NS, not significant; PD-L1, programmed death ligand 1 receptor.

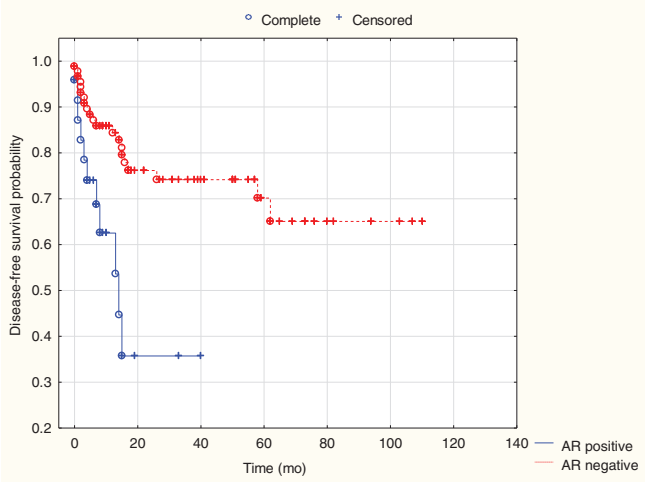

FIGURE 1 Disease-free survival as a function of androgen receptor (AR) positivity [Color figure can be viewed at wileyonlinelibrary.com]

AR status was determined by immunohistochemistry in 120 specimens; of these, 26 were AR+ (21.0%). AR positivity was detected in 12 of the 15 (80%) salivary duct carcinomas (SDC) and 5 of the 12 adenocarcinomas (41.7%; Table 2). Patients with locally advanced disease were more likely to be AR+ than AR− (61.5% vs 27.7%; $P = .004$) and also more likely to present regionally-advanced disease (N+) (57.7% vs 13.8%; $P < .001$; Table 3). AR positivity was significantly more common in males (73.1% vs 26.9%; $P = .03$). AR positivity had a significant negative impact on both DFS ($P = .005$; Figure 1) and OS ($P = .02$; Figure 4). However, positive AR status had no statistically significant impact on margin status or treatment failure (Table 3).

PR and ER expressions were examined in 121 cases. Three cases were PR+ (all of 3 cases of mucoepidermoid carcinoma) and three cases were ER+ (2 adenocarcinomas and 1 mucoepidermoid carcinoma; Table 2). Immunohistochemical findings for HER2 status were available in 118 cases, 12 of which (10.2%) were positive. By tumor type, 7 of 16 cases (43.8%) with SDC were HER2 positive whereas all three cases (100%) with carcinoma ex pleomorphic adenoma (CaExPA) were positive (Table 2). There were no statistically significant differences in terms of HER2 positivity or negativity between patients with locally or regionally advanced cancer, nor for margin status or disease failure (Table 3). However, HER2 positivity had a significant negative impact on DFS ($P = .02$, Figure 2) and OS ($P = .02$; Figure 5).

EGFR analysis was performed in 117 cases. Moderate and strong staining (>80% of cells positive) was present in 92 cases (78.6%). The tumor type was immunopositive in all 13 tumors (100%) obtained from patients with CaExPA, in all 7 (100%) cases with myoepithelial carcinoma, and in 10 of 11 cases (90.9%) diagnosed with adenocarcinoma NOS (Table 2). EGFR expression was significantly associated with favorable prognosis, with only 18.9% of EGFR+ patients developing a recurrence vs 75.0% of EGFR-negative patients ($P < .001$). EGFR status had no significant effect on disease stage, margin status, DFS, or OS (Table 3).

PD-L1 expression was determined in 117 cases, 22 of which (18.8%) were positive. Positivity was most common in ACC (9 of 30; 30%) and CaExPA (5 of 13; 38.5%; Table 2). Locally advanced disease was more common in PD-L1 negative cases (41.5% vs 14.3%; $P = .02$), and these patients presented significantly higher recurrence rates (38.5% vs 0%;

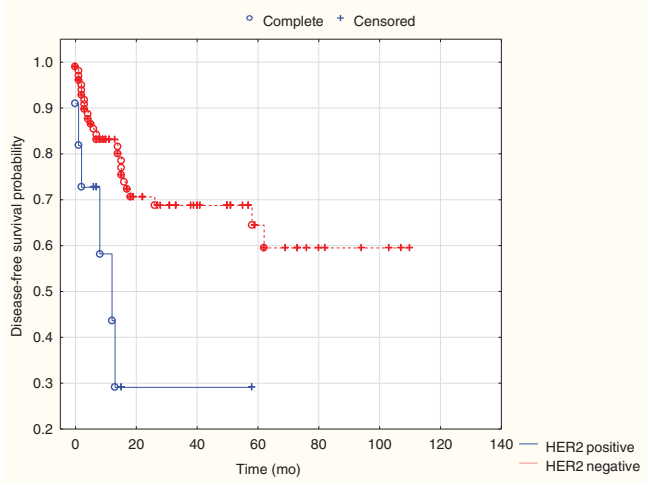

FIGURE 2 Disease-free survival as a function of human epidermal growth factor receptor 2 (HER2) positivity [Color figure can be viewed at wileyonlinelibrary.com]

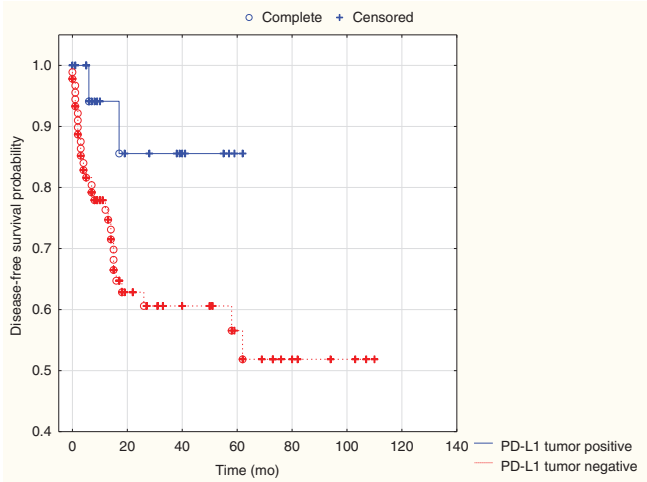

FIGURE 3 Disease-free survival as a function of programmed death ligand 1 receptor (PD-L1) positivity [Color figure can be viewed at wileyonlinelibrary.com]

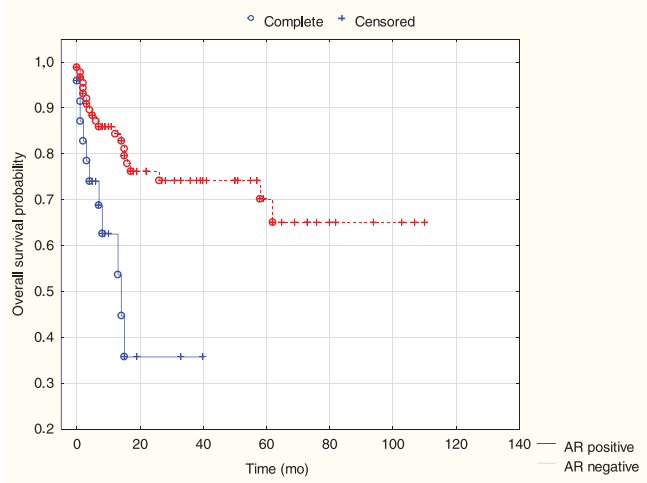

FIGURE 4 Overall survival as a function of androgen receptor (AR) positivity [Color figure can be viewed at wileyonlinelibrary.com]

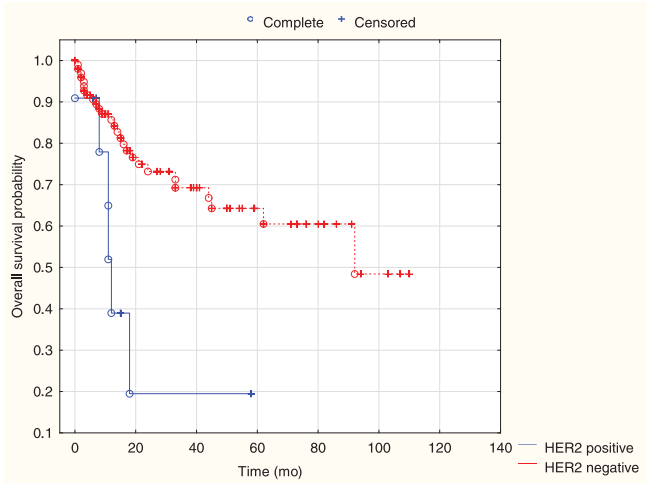

FIGURE 5 Overall survival as a function of human epidermal growth factor receptor 2 (HER2) positivity [Color figure can be viewed at wileyonlinelibrary.com]

$P < .001$; Table 3). PD-L1(−) was associated with a significant decrease in DFS ($P = .04$) but did not influence OS (Figure 3).

PD-L1 positivity in TIMC was found in only 6 out of 117 cases (5.1%) overall. However, both of the cases with lymphoepithelial carcinoma were PD-L1+ (2/2; 100%), as were 2 of the 16 cases (12.5%) with SDC (Table 2).

4 | DISCUSSION

In the current study, we examined tissue specimens from 124 patients treated surgically for SGC to determine the presence of a range of different protein receptors. We found that several receptors were overexpressed in these tumors, most notably AR and EGFR, which were positive in 21.0% and 78.6%, respectively, of the tissue samples. In addition, 12 of the 15 specimens (80%) resected from patients with SDC were AR+. With regard to the influence of marker status on clinical outcomes, a significantly higher percentage of patients with locally advanced disease was AR+ (61.5% vs 27.7%). In addition, DFS and OS outcomes were significantly worse in AR+ and HER2+ patients (Figures 1 and 2). By contrast, EGFR expression was significantly associated with favorable prognosis and EGFR status had no significant effect on disease stage, margin status, DFS, or OS. Patients with PD-L1-negative tumors were more likely to present locally advanced disease (41.5% vs 14.3%), and had significantly higher recurrence rates (38.5% vs 0%) and worse DFS. These findings underscore the important influence of specific markers on clinical outcomes.

4.1 | Overexpression of ARs

The poor results of treatment for malignant SGC has given rise to an ongoing search for novel, personalized therapeutic approaches. In many cancers, overexpression of certain proteins has been shown to play a crucial role in cancer pathogenesis and disease progression.[9,10] For example, in prostate cancer, there is substantial evidence for the overexpression of AR.[11] Although only limited data are available in SGC, the studies published to date suggest that, similar to prostate cancer, AR is the most commonly expressed receptor in these tumors. Dalin et al[12] and Udager et al[13] reported AR overexpression in patients with SGC, particularly in those with the SDC histological subtype (>80% and 98%, respectively). Our data, which show that 80% of patients with SDC were AR+, confirm those findings. In the available literature, adenocarcinoma NOS is the second most common histological SGC subtype with AR overexpression, with prevalence rates ranging from 21% to 33%.[14,15] In our series, we observed a slightly higher rate (41.6%) of AR+, although this could be attributed to the limited number of patients with adenocarcinoma NOS in our study.

In our series, AR+ was significantly more common in males, present in nearly three-fourths of male patients (73%) vs approximately only one in four (27%) females. Other authors have reported similar findings, indicating—consistent with our data—a higher rate among men.[16] However, some studies have not found any difference between men and women with regard to AR status.[17]

Only a few studies, most with a limited number of cases, have investigated treatment results and prognosis in patients with AR+ SGC. Aquino et al[18] found a nonsignificant trend toward worse survival in AR+ patients. In our series, we found a significant association between AR positivity and worse DFS and OS. The other studies that have examined the results and prognosis in patients with SGC have done so only for patients with SDC, and even in those cases the findings regarding the impact of AR positivity on outcomes are contradictory, with some studies reporting a benefit[19] and others a negative impact.[17] In our study, the patients with AR+ disease had significantly worse DFS and OS. Given the disparate results of the studies published to date, more research will be needed to determine the true impact of AR+ in this patient population.

Several single-center studies and case reports have shown a positive response to androgen deprivation therapy (ADT) in AR+ patients, mostly in SDCs and adenocarcinomas NOS.[12] For example, in a small single-center study (n = 17) conducted by Locati et al, the overall response was 64.7%, with a 5-year OS rate of 19.3%.[20] Boon et al[21] retrospectively reviewed 34 patients with advanced AR+ SDC treated with first-line ADT, finding a clinical benefit (partial response or stable disease) in 50% of the patients. Importantly, the median OS was significantly better in the ADT group—17 months vs 5 months—than the control group (best supportive care). However, as those authors noted, since not all patients responded to ADT, future research should focus on identifying predictors of treatment response.

Locati et al are currently conducting an EORTC-sponsored clinical trial (NCT01969578) to evaluate the efficacy and safety of ADT (experimental arm) vs chemotherapy (standard arm) in patients with recurrent and/or metastatic AR+ SDC and adenocarcinoma NOS. Those authors hope to demonstrate a 15% improvement in progression-free survival (PFS) at 6 months in the ADT group. The results of that trial should provide valuable data to clarify the role of ADT in patients with AR+ SGC.

4.2 | Overexpression of PR and ER

The overexpression of PR and ER described in the literature is consistent with the findings of our study, where only 2.5% of cases were ER and PR positive.[18,22,23] However, some authors, such as Kolude et al,[24] have reported higher rates. Those authors found that 28% and 8%, respectively, of tumors were ER+ and PR+, although these findings were not statistically significant.

Although the value of administering sex hormone antagonists has been well documented in the treatment of various cancer types,[18] scant data are available regarding the influence of these hormone receptors in patients with SGC. However, in their case series (n = 69), Aquino et al[18] reported that cytoplasmic ER-β expression was strongly associated with tumor grade, underscoring the potential prognostic value of this marker.

4.3 | HER2 expression

The HER2 receptor is among the most widely studied receptors in cancer research, and anti-HER2 treatment is routinely prescribed in the treatment of breast cancer.[25] However, in SGC, most of the studies that have evaluated HER2 immunoreactivity are small single center studies.[26] Alotaibi et al[26] performed a meta-analysis of 39 studies conducted to evaluate HER2 expression in SGC. Those studies included a heterogenous group of patient profiles (ie, with a wide range of pathological types and localizations); not surprisingly, the percentage of patients with HER2 immunopositivity varied widely from study to study. Nevertheless, 11 of those studies found that HER2 overexpression was associated with poor prognosis and aggressive tumor behavior. Moreover, most of the studies included in that meta-analysis reported that a higher proportion of patients with SDC were HER2+ compared to other pathological subtypes.[26]

In our study, HER2 overexpression was present in slightly more than 10% of the whole cohort, but in nearly 50% of the cases diagnosed with SDC (7 of the 16 patients) and 23% of patients (3 of the 13 cases) with carcinoma ex pleomorphic

adenoma. Interestingly, although HER2 positivity was not associated with disease stage, margin status, or treatment failure in our series, it was associated with lower DFS and OS. This finding suggests that anti-HER2 treatment could potentially benefit patients with HER2+ SGC. Several studies have assessed the value of anti-HER2 treatment in these patients. Takahashi et al[27] recently reported results from a phase II clinical trial to assess the efficacy and toxicity of trastuzumab plus docetaxel in patients (n = 57) with locally advanced and/or recurrent or metastatic HER2+ SDC. In that study, the overall response rate was 70.2%, with a clinical benefit rate of 84.2%, leading the authors to conclude that this combined treatment is a promising therapy for HER2+ SDC. Most of the other studies that have evaluated the role of the anti-HER2 agent trastuzumab in SGCs are case reports and case series. Haddad et al and Perissinotti et al[28,29] evaluated anti-HER2 treatment as a single agent, finding that anti-HER2 treatment had no effect on outcomes in the HER2+ patients. By contrast, some of the other studies included in the meta-analysis by Alotaibi et al reported a positive effect for anti-HER2 on complete response rates when this treatment was combined with cytotoxic drugs.[26] Nonetheless, due to the small number of patients in the treatment groups of those studies, it is difficult to draw any clear conclusions. Further research is needed; ideally a multi-institutional randomized controlled trial in which HER2+ patients are treated with chemotherapy, with or without anti-HER2 treatment.

4.4 | EGFR expression

EGFR plays an important role in cellular proliferation, survival, and differentiation. This receptor is overexpressed in a wide range of tumor types, including lung, head and neck, breast, and particularly colon cancer (in which 50% to 70% of tumors express EGFR).[30] EGFR overexpression is associated with lower survival rates in head and neck, ovarian, cervical, and esophageal cancer.[31] However, the impact of EGFR positivity in SGC is unclear.

The reported prevalence of EGFR overexpression in patients with SGC ranges from 36%[32] to 71%.[14] In the study by Ettl et al, EGFR was overexpressed in 36% of 106 patients with SGC, and EGFR+ disease was significantly associated with worse survival.[33] By contrast, Monteiro et al[32] found that EGFR positivity had no significant impact on survival in a study involving 88 patients with SGC (36% of whom were EGFR+). In our study, EGFR was overexpressed in >78% of cases and significantly associated only with treatment failure, but not any of the other outcome variables. Nonetheless, the findings reported by Bonner et al suggest that targeted therapy is beneficial in EGFR+ head and neck patients.[34] Locati et al found that cetuximab had a clinical benefit rate of 50% in 30 patients with EGFR+ recurrent and/or metastatic SGC.[35] Given the mixed findings reported to date regarding the role of EGFR positivity in SGC, it is clear that more data will be needed to better elucidate the impact of this marker and the value of anti-EGFR agents on clinical outcomes.

4.5 | PD-L1 expression

PD-L1 is expressed in resting T cells, B cells, dendritic cells, and various tumor cells. When the PD-1 molecule binds to the PD-L1 receptor ligand, this complex inhibits cytotoxic T cells, thus leading to apoptosis and tumor escape.[36] PD-L1 overexpression in renal cell carcinoma, esophageal cancer, and melanoma is associated with worse prognosis and outcomes.[37-39] However, the role of PD-L1 overexpression in SGC remains unclear due to insufficient data. Harada et al evaluated 49 patients diagnosed with malignant SGC, finding that PD-L1 overexpression (present in 51% of patients) was associated with higher disease stage, higher rates of recurrence and metastasis, and worse survival.[40] A study conducted by Haderlein et al in a more homogenous group of 67 patients with SDC found that 22% of patients overexpressed PD-L1; although those authors did not assess the impact of PD-L1 overexpression on survival, no differences in disease stage were observed between the two subgroups.[41] The largest study conducted to date (n = 219) in patients with malignant SGC was performed by Mukaigawa et al,[42] who found PD-L1 overexpression in 22.8% of patients, with a significant negative influence on pT and pN status and on survival. However, because those authors used a low cutoff value (1%) for PD-L1 positivity (based on ROC analysis), it is difficult to compare their results to our study or other studies in which a 5% cutoff value was used.[42] In our study, PD-L1 overexpression was observed in 18.8% of the series; surprisingly, PD-L1+ patients were significantly less likely to present advanced local disease or treatment failure and had significantly better DFS outcomes. These findings were unexpected given that they contradict previously reported findings.[40,42] The reason for this discrepancy is not clear, but could be due to the small sample size (only 22 patients were PD-L1+) and short follow-up in our study.

To date, only one study has examined PD-L1 expression in TIMC.[41] In that study, Harada et al found PD-L1 expression in 42.6% of patients, with a significant negative impact on survival. By contrast, in our series, PD-L1 TIMC positivity was observed in only 5.1% of patients, and with a significant difference in margin status between the two groups (positive and negative). Moreover, PD-L1 TIMC+ patients were significantly older, had more treatment failures, and presented a trend ($P = .05$) toward significance with regard to positive nodal status.

Nivolumab, a PD-L1 inhibitor, has been shown to increase objective response rates in a range of cancer types, including metastatic and recurrent melanoma, lung cancer, and renal cell carcinoma.[43-45] The reported benefits of nivolumab have increased interest in this drug, as evidenced by the numerous phase III clinical trials currently underway (39 trials as of October 2018). In SGC, nivolumab is currently being evaluated in phase II trials including patients with recurrent or metastatic SGC (NCT03132038 and NCT03012581). One recent study found that the combination of nivolumab with ipilimumab (a CTLA4 blocker) yielded longer PFS than either approach alone in patients with melanoma.[46] Currently, three trials (NCT03172624, NCT02834013, and NCT03146650) are underway to assess the value of this combined approach in SGC. In addition, another trial is evaluating pembrolizumab combined with docetaxel in patients with metastatic SGC (NCT03360890).

4.6 | Implications of these findings and future directions

Due to the relative rarity of SGC, data on the role of molecular aberrations in patients with SGC are limited. Moreover, few studies—most involving only limited numbers of patients—have been conducted to assess the value of molecular targeted therapy on clinical outcomes in patients with SGC. However, a growing body of evidence, both for SGC as well as other cancer localizations, suggests that molecular therapy can improve treatment outcomes. This is particularly relevant to SGC given the unsatisfactory outcomes offered by standard care (ie, surgery and/or radiotherapy). Given the low prevalence of SGC, it is clear that large multi-institutional studies and randomized trials are needed to better determine the prevalence of these markers in patients with SGC and their relative impact on clinical outcomes. In this regard, several clinical trials are currently underway, and more will likely be initiated in coming years.

4.7 | Study strengths and limitations

This study has several important limitations, primarily the retrospective nature of the study, with all the limitations inherent to that design. In addition, the sample size was limited, making it difficult to reach any definitive conclusions regarding the role of marker overexpression on clinical outcomes. However, given the rarity of SGC, obtaining large patient samples is difficult. For this reason, multi-institutional studies with large numbers of patients and longer follow-up are needed to more clearly determine the association between these markers and survival. Ideally, randomized clinical trials would help to definitively establish the value of treatment on survival outcomes.

5 | CONCLUSIONS

The results of the present study reveal that a wide range of proteins is expressed in patients with salivary gland cancer. Consistent with previous reports, our data show that most patients with SGC were AR+. AR+ was significantly more common in males, affecting nearly three-fourths of these patients. Relevantly, AR+ and HER2+ patients both had significantly worse survival outcomes (DFS and OS), a finding that suggests that such patients would be good candidates to receive anti-AR or anti-HER2 therapy. In contrast to previous reports, our data suggest that PD-L1+ patients may have better outcomes than those with PD-L1(−) disease, and thus the reported negative predictive value of PD-L1 positivity may be less than previously thought. Patients with PD-L1-negative tumors had significantly worse clinical outcomes on numerous measures, suggesting a predictive value for this biomarker. Finally, our data suggest that the presence of certain receptors—notably ER and PR—do not appear to influence treatment outcomes.

Given the heterogenous data reported to date in small cases series, multi-institutional prospective trials are needed to definitively determine the prognostic and therapeutic value of these markers in patients with SGC.

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How to cite this article: Szewczyk M, Marszałek A, Sygut J, Golusiński P, Golusiński W. Prognostic markers in salivary gland cancer and their impact on survival. *Head & Neck*. 2019;41:3338–3347. https://doi.org/10.1002/hed.25857