Multicentre, retrospective study of the efficacy and safety of nivolumab for recurrent and metastatic salivary gland carcinoma

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Although immune-checkpoint inhibitors (ICIs) are effective against various cancers, little is known regarding their role in salivary gland carcinoma (SGC) treatment. Therefore, we evaluated the efficacy and safety of nivolumab monotherapy in patients with recurrent and/or metastatic SGC. In this multicentre retrospective study, nivolumab (240 mg) was administered every 2 weeks. The overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and safety were examined; the correlation between treatment outcomes and clinicopathological factors was analysed. Twenty-four patients were enrolled; the most common histopathology was salivary duct carcinoma. Eleven tumours were PD-L1-positive; no tumour was microsatellite instability-high. The ORR was 4.2%, and the median PFS and OS were 1.6 and 10.7 months, respectively. One patient continued nivolumab for 28 months without disease progression. One patient showed grade 4 increase in creatine phosphokinase levels and grade 3 myositis. Biomarker analysis revealed significantly increased OS in patients with performance status of 0; modified Glasgow prognostic score of 0; low neutrophil-to-lymphocyte ratio, lactate dehydrogenase, and C-reactive protein; and high lymphocyte-to-monocyte ratio and in patients who received systemic therapy following nivolumab. Although nivolumab’s efficacy against SGC was limited, some patients achieved long-term disease control. Further studies are warranted on ICI use for SGC.

Salivary gland carcinoma (SGC) is a rare type of cancer accounting for only 0.14% of all malignant neoplasms; it is estimated that 1.4 in 100,000 individuals are diagnosed with SGC per year1. According to the histological classification of salivary gland tumours by the World Health Organization, there are 20 histopathological types of SGC; their prognosis and biological characteristics considerably vary with the histological type2. Resection is the standard treatment for SGC regardless of the histopathological type and postoperative radiotherapy is
recommended for patients at a high risk of recurrence. Numerous clinical trials of cytotoxic chemotherapies have been conducted in patients with recurrent and/or metastatic (R/M) SGC\textsuperscript{23} and several potential targets for systemic therapy have been reported\textsuperscript{24–26}; however, there were no randomised controlled trials\textsuperscript{27}. Moreover, unlike lung metastasis of adenoid cystic carcinoma (AdCC), majority of which show indolent growth\textsuperscript{3,18,19}, the progression of salivary duct carcinoma (SDC) and adenocarcinoma, not otherwise specified (NOS) is aggressive\textsuperscript{3,20,21}. Hence, there is a need for systemic therapeutic strategies based on the histological characteristics for SGC.

Immune-checkpoint inhibitors (ICIs) have demonstrated durable antitumor effects against multiple cancer types, including head and neck squamous cell carcinoma\textsuperscript{22,23}. Two prospective studies on pembrolizumab monotherapy\textsuperscript{24} and pembrolizumab combined with vorinostat\textsuperscript{25} for SGC have been published. Furthermore, two studies on nivolumab in patients with non–HNSCC, including six\textsuperscript{26} and two\textsuperscript{27} patients with SGC, respectively, have been published. However, these studies were not conducted exclusively in patients with SGC; to the best of our knowledge, there are no studies on the efficacy of nivolumab for SGC.

As a significant number of patients fail to benefit from ICIs; studies have been conducted to identify biomarkers to predict the response of patients to ICIs. Programmed death-ligand 1 (PD-L1) expression\textsuperscript{22,23,28,29} and microsatellite instability (MSI)\textsuperscript{28,30} are used as companion diagnostic markers; tumour mutation burden (TMB) is also one of the potential biomarkers of ICI response\textsuperscript{26,28,31}. While most SGCs are microsatellite stable\textsuperscript{22–24}, there are no consistent data on PD-L1 expression in SGCs due to the use of different antibodies and evaluation methods among studies\textsuperscript{32–34}. Several case series studies of PD-L1 expression in SGCs including multiple histopathological types have shown a significantly higher positivity of PD-L1 in SDC than in other histopathological types of SGC\textsuperscript{35–39}. Moreover, SDC is reported to harbour a higher mutational burden than other types of SGC\textsuperscript{22,24,30–31}. Overall, these findings suggest that ICIs may have a higher efficacy against SDCs than other histopathological types of SGCs.

Recently, inflammatory markers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), serum C-reactive protein (CRP), modified Glasgow prognostic score (mGPS), absolute eosinophil count, and serum lactate dehydrogenase (LDH) have been reported to be useful predictors of progression of cancer including SDC\textsuperscript{42,43}. Although it is largely unknown how systemic inflammation affects the survival of patients with cancer, neutrophils are known to promote tumour growth and distant metastasis by releasing cytokines that promote neovascularisation. As the number of lymphocytes reflects antitumor immunity, increased NLR due to neutrophilia and lymphocytopenia is associated with worse prognosis in patients treated with ICIs\textsuperscript{44–46}. The LMR, PLR, CRP level, mGPS, absolute eosinophil count, and LDH have also been reported to correlate with the therapeutic effects of ICIs in patients with melanoma, non–small-cell lung cancer, and head and neck squamous cell carcinoma\textsuperscript{46–51}. Therefore, in this retrospective multicentre study, we aimed to evaluate the efficacy of nivolumab monotherapy in patients with SGC including SDC. Additionally, we conducted a database analysis to determine the correlation between clinical profiles including PD-L1 positivity, MSI, and inflammatory biomarkers and the survival of patients with SGC treated with nivolumab.

Results

Patient characteristics and treatment. Twenty-four patients, identified in the cancer registry of the participating institutions during the study period, were enrolled in this study (Table 1). Written informed consent was obtained from all participants. The median follow-up period for all patients was 6.5 (range 0.6–28.2) months. All patients had received systemic therapy before nivolumab. The most common histopathological type of cancer was SDC (n = 20, 83%). Eleven tumours (46%) presented PD-L1 expression at a rate of ≥1%. Among them, three (13%) presented ≥50% PD-L1 positivity. Among 23 evaluable patients, none was classified as MSI–H.

The median number of cycles of nivolumab administered was 8 (range 1–57). As of the cut-off date, 30 January 2020, two patients (8%) continued to receive nivolumab for 28 and 6 months, whereas 22 patients (92%) discontinued treatment due to PD (n = 19, 79%) and AEs (n = 3, 13%). Six patients (25%) received one or more of the following systemic therapy regimens after nivolumab treatment: cetuximab plus paclitaxel (n = 5, 21%), carboplatin plus docetaxel, trastuzumab plus docetaxel, abiraterone, and S-1 (n = 1, 4%, respectively).

Response and survival outcomes. The therapeutic efficacy of nivolumab are shown in Table 2. None of the patients achieved CR; 1 (4%) and 2 (8%) patients showed PR, SD, and PD, respectively. The ORR was 4.2% (95% CI 0.1–21.1%). Two patients with SD maintained the status for more than 24 weeks. Thus, both CBR and DCR were 12.5% (95% CI 2.7–32.4%). The Kaplan–Meier survival curves of PFS and OS of all patients are shown in Fig. 1; the median PFS was 1.6 (95% CI 1.2–4.4) months and the median OS was 10.7 (95% CI 5.1–19.8) months. The therapeutic effects observed in 20 patients with SDC were as follows: ORR, 5.0% (95% CI 2.7–24.9%); median PFS, 1.5 (95% CI 1.1–2.7) months; and median OS, 11.3 (95% CI 3.8–19.8) months. Figure 2 shows the waterfall, spider, and swimmer plots of all patients based on the histopathological diagnosis. Figure 3 shows the representative images of tumour before and during nivolumab monotherapy in two patients.

Safety. All AEs reported are listed in Table 3. Twenty-two patients (92%) experienced at least one AE during the treatment. Six patients (25%) showed grade 3 or 4 AEs; 1 patient showed grade 4 increase in creatine phosphokinase (4%); 3 patients (13%) showed grade 3 anaemia, and 1 patient each showed an increase in alkaline phosphatase, amylase, aspartate aminotransferase, alanine transaminase, hyponatraemia, and myositis (4%). No treatment-related death was observed. Frequent AEs of all grades included anaemia (n = 17, 71%), increased alkaline phosphatase (n = 10, 42%), and hypoalbuminemia (n = 9, 38%). Five patients (21%) had an irAE; and only one of these patients (4%) had grade 4 increase in creatine phosphokinase and grade 3 myositis. Other
| Age (years)        | n (%)          |
|-------------------|----------------|
| Median (range)    | 56 (29–82)    |
| Sex               |                |
| Male              | 19 (79)       |
| Female            | 5 (21)        |
| Primary site      |                |
| Parotid gland     | 19 (79)       |
| Submandibular gland | 3 (13)    |
| Minor salivary gland | 1 (4)        |
| Accessory parotid gland | 1 (4)    |
| Histopathology    |                |
| Salivary duct carcinoma | 20 (83) |
| Adenocarcinoma, NOS | 2 (8)       |
| Adenoid cystic carcinoma | 1 (4) |
| Mucoepidermoid carcinoma | 1 (4) |
| Immunohistochemistry |             |
| HER2-positivea    | 11 (46)       |
| AR-positiveb      | 20 (83)       |
| PD-L1 (28–8) < 1% | 13 (54)       |
| PD-L1 (28–8) 1–9% | 5 (21)        |
| PD-L1 (28–8) 10–49% | 3 (13) |
| PD-L1 (28–8) ≥ 50% | 3 (13)       |
| MSI-H (n = 23)    | 0 (0)         |
| Prior treatment   |                |
| None              | 0 (0)         |
| Surgery           | 18 (75)       |
| Radiotherapy      | 19 (79)       |
| Concomitant radiotherapy (cisplatin) | 9 (38) |
| Concomitant radiotherapy (carboplatin) | 2 (8) |
| Systemic therapy  | 24 (100)      |
| Systemic therapy for RM disease | 22 (100) |
| Leuprorelin + bicalutamide | 10 (42) |
| Carboplatin + paclitaxel | 7 (29) |
| Trastuzumab + docetaxel | 7 (29) |
| Carboplatin + docetaxel | 5 (21) |
| S-1               | 5 (21)        |
| Trastuzumab + S-1 | 5 (21)        |
| Othersc           | 13 (54)       |
| Systemic therapy after nivolumab | 6 (25) |
| Cetuximab + paclitaxel | 5 (21) |
| Trastuzumab + docetaxel | 1 (4) |
| Carboplatin + docetaxel | 1 (4) |
| Abiraterone       | 1 (4)         |
| S-1               | 1 (4)         |
| Platinum refractory | 12 (50)     |
| Target lesion     |                |
| Locoregional      | 4 (17)        |
| Locoregional + distant metastasis | 1 (4) |
| Distant metastasis only | 19 (79) |
| Site of metastasis|                |
| Lung              | 10 (42)       |
| Liver             | 5 (21)        |
| Lymph nodes       | 4 (17)        |
| Soft tissue (skin, muscle) | 3 (13) |
| Bone              | 1 (4)         |
| Brain, meninges   | 1 (4)         |
| Continued         |                |
irAEs reported were grade 2 pneumonitis (n = 1, 4%), grade 1 hyperthyroidism (n = 2, 8%), and hypothyroidism (n = 1, 4%). Three patients discontinued treatment due to AEs including grade 3 myositis (n = 1) and grade 2 pneumonitis (n = 2).

Exploratory analysis of biomarkers of ICI response. Table 4 and Fig. 4 show the results of the exploratory analysis of the biomarkers. As all patients were microsatellite stable, no analysis was performed according to the MSI status. There was no association between PD-L1 positivity and prognosis. In the univariate analysis, higher NLR, higher serum LDH and CRP, and lower LMR were significantly associated with shorter PFS. The significant predictors of a shorter OS were the ECOG PS ≥ 1, mGPS ≥ 1, higher NLR, higher serum LDH, and higher serum CRP. Systemic therapy following nivolumab and higher LMR were significantly associated with a longer OS. The Kaplan–Meier curves of the OS and PFS, waterfall, spider, and swimmer plots according to the biomarkers are presented in Fig. 3, Supplementary Figs. S1 and S2, respectively. The 1-year OS of patients with the ECOG PS 0, with systemic therapy after nivolumab, mGPS 0, lower NLR, higher LMR, lower LDH level, and lower CRP level was 59.3%, 83.3%, 65.0%, 85.7%, 52.5%, 55.5%, and 68.6%, respectively (Fig. 3).

In the present retrospective study of nivolumab monotherapy in 24 patients with R/M SGC, the ORR was 4.2%, with the median PFS and OS of 1.6 and 10.7 months, respectively. The ORR of the 20 patients with SDC was 5.0% and the median PFS and OS were 1.5 and 11.3 months, respectively. Nivolumab was well tolerated by patients with SGC, and AEs associated with nivolumab was comparable with those associated with pembrolizumab. In this study, the therapeutic effects were limited; however, some patients achieved considerably long-term disease control.

Prospective studies on pembrolizumab24 and pembrolizumab combined with vorinostat25 reported that the ORR of patients with multiple histopathological types of SGC was 12% and 16%, respectively, with the median PFS and OS of 4–6.9 and 13–14 months, respectively. The ORR and the median OS of patients with SGC to nivolumab in this study were comparable with those observed in patients with advanced SGC treated with pembrolizumab24,25. However, the median PFS of this study was shorter than that reported by the previous study on pembrolizumab. This could be because only patients with obvious progression within 6 months were included in this study, whereas the previous studies with pembrolizumab did not adopt this criterion.

In the present study, the results of the biomarker analysis revealed that most factors related to PS and inflammatory biomarkers such as the NLR, LMR, LDH, and CRP levels were associated with the prognosis of tumour in
patients treated with nivolumab. To the best of our knowledge, this is the first study to demonstrate an association between inflammatory biomarkers and prognosis in patients with SGC treated with nivolumab. In particular, NLR showed an apparent negative dose–response relationship with the OS; the 1-year OS of patients with lower NLR was 85.7%. The NLR, LMR, PLR, CRP level, mGPS, and LDH level have also been reported to correlate with the therapeutic effects of ICIs in various cancers. As a result, NLR, LMR, PLR, LDH, CRP, and mGPS are suggested to reflect the general conditions (immunological competence) of the host, and they can be used as biomarkers of ICI treatment response.

In malignant melanoma, non-small cell lung cancer, and head and neck squamous cell carcinoma, PD-L1 immunohistochemistry including the presence of PD-L1-expressing immune cells in the tumour microenvironment, mismatch repair (MMR)/MSI, and TMB have been reported as biomarkers of ICI response. Based on the findings of previous studies, which showed significantly higher PD-L1 expression and

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**Figure 1.** Kaplan–Meier curves of progression-free and overall survival. Kaplan–Meier curves of (A) progression-free survival and (B) overall survival. The vertical lines indicate censored events.
Figure 2. Characteristics of responses in patients with salivary gland carcinoma treated with nivolumab according to Response Evaluation Criteria in Solid Tumours (version 1.1) based on histopathological diagnosis. (A) The highest reduction from the baseline in target lesions. Tumour shrinkage relative to the baseline was observed in four patients (16.7%). The upper dotted lines represent the threshold for progressive disease (a 20% increase in the sum of the longest diameter of the target lesions) and the lower dotted lines represent the threshold for a partial response (a 30% decrease in the sum of the longest diameter of the target lesions). (B) Change from the baseline (%) in the sum of the target lesions over time to progressive disease. The upper dotted lines represent the threshold for progressive disease (a 20% increase in the sum of the longest diameter of the target lesions) and the lower dotted lines show the threshold for a partial response (a 30% decrease in the sum of the longest diameter of the target lesions). (C) Time to response and the duration of survival. Each bar represents an individual patient, with the length of the bar corresponding to the time of overall survival based on the disease status. SDC salivary duct carcinoma, MEC mucoepidermoid carcinoma, AdCC adenoid cystic carcinoma.
Figure 3. Representative images of the tumor before and during nivolumab monotherapy in two patients with recurrent and/or metastatic salivary gland carcinoma. (A–D) Pre-treatment of a patient with lung, liver, and hilar lymph node metastasis, (E–H) 40 days after the initiation of nivolumab treatment, (I–L) 96 days after the initiation of nivolumab treatment. Tumor shrinkage was observed in this patient following PD diagnosis due to a new lesion. The yellow arrows indicate lung metastases (A, E, I), new lung lesion (F), hilar lymph node metastasis (C, G, K), and liver metastases (D, H, L). (M–O) Pre-treatment of a patient with cervical skin metastasis, (P–R) 57 days after the initiation of nivolumab treatment, (S–U) 204 days after the initiation of nivolumab treatment. Tumor shrinkage was observed in this patient after an increase in skin tumor thickness, which was in the SD range.
TMB in SDC than in other tumours, higher therapeutic effects of ICIs are being expected. However, the efficacy of ICI monotherapy for SDC was limited in our cohort. This might be since no tumour was MSI-H and the PD-L1-positivity rate in the tumour cells was low in our SDC cohort.

While previous studies on anti-HER2 antibody including trastuzumab and androgen deprivation therapy (e.g., bicalutamide and leuprorelin) for HER2- or AR-positive patients with SDC showed the ORR was 20–89%, the response rate of patients to nivolumab in this study was unsatisfactory. Thus, nivolumab monotherapy is not recommended for patients with HER2- or AR-positive advanced SDCs before anti-HER2- or AR-targeted therapy. In contrast, ICIs might be tried in patients with SGC without targetable molecules instead of conventional cytotoxic anticancer agents. Recent studies reported that cytotoxic anticancer agents seemed to achieve a higher ORR with higher toxicity than the ICIs. However, it is difficult to directly compare those therapies as patient background (e.g., histological type) might differ. As our data suggest low nivolumab ORR and shorter survival in patients with increased systemic inflammatory markers (e.g., NLR), the use of cytotoxic anticancer agents may be prioritised in symptomatic patients (e.g., patients with pain and/or aggressive tumour growth) and patients with increased systemic inflammatory markers. Currently, a clinical trial on the combination of pembrolizumab and docetaxel in patients with thyroid cancer or SGC without standard-of-care treatment is under progress (ClinicalTrials.gov Identifier: NCT03360890). Other ongoing clinical trials targeting patients with SGC include the combination of pembrolizumab and lenvatinib (ClinicalTrials.gov Identifier: NCT04209660), two ICIs (nivolumab and ipilimumab; ClinicalTrials.gov Identifier: NCT02834013, NCT03146650 and NCT03172624), and ICIs and AR-targeted therapy (pembrolizumab and goserelin acetate; ClinicalTrials.gov Identifier: NCT03942653).

This study had some limitations. First, owing to the retrospective nature and small sample size of the study, the superiority of nivolumab over other drugs was not examined. Second, the biomarkers identified in this study including NLR might be merely prognostic factors, which are associated with survival and might not predict outcome.

| Event                        | Any grade | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|------------------------------|-----------|---------|---------|---------|---------|
| Any                          | 22 (92)   | 19 (79) | 8 (33)  | 6 (25)  | 1 (4)   |
| Anaemia                      | 17 (71)   | 11 (46) | 3 (13)  | 3 (13)  | 0       |
| ALP increased                | 10 (42)   | 8 (33)  | 1 (4)   | 1 (4)   | 0       |
| Hyperalbuminemia             | 9 (38)    | 7 (29)  | 2 (8)   | 0       | 0       |
| Hyperkalaemia                | 6 (25)    | 3 (13)  | 3 (13)  | 0       | 0       |
| Heart failure                | 6 (25)    | 6 (25)  | 0       | 0       | 0       |
| Serum amylase increased      | 5 (21)    | 3 (13)  | 1 (4)   | 1 (4)   | 0       |
| AST increased                | 5 (21)    | 4 (17)  | 0       | 1 (4)   | 0       |
| Hynonatremia                 | 4 (17)    | 3 (13)  | 0       | 1 (4)   | 0       |
| CPK increased                | 3 (13)    | 0       | 2 (8)   | 0       | 1 (4)   |
| ALT increased                | 3 (13)    | 2 (8)   | 0       | 1 (4)   | 0       |
| Cre increased                | 3 (13)    | 1 (4)   | 2 (8)   | 0       | 0       |
| Chronic kidney disease       | 2 (8)     | 0       | 2 (8)   | 0       | 0       |
| γ-GTP increased              | 2 (8)     | 1 (4)   | 1 (4)   | 0       | 0       |
| Pneumonitis                  | 2 (8)     | 0       | 2 (8)   | 0       | 0       |
| Hyperthyroidism              | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| Hypertriglyceridaemia        | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| Hypokalaemia                 | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| APTT prolonged               | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| Blood LDH increased          | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| Hyperglycaemia               | 2 (8)     | 2 (8)   | 0       | 0       | 0       |
| Myositis                     | 1 (4)     | 0       | 1 (4)   | 0       | 0       |
| Lymphocyte count decreased   | 1 (4)     | 0       | 1 (4)   | 0       | 0       |
| INR increased                | 1 (4)     | 0       | 1 (4)   | 0       | 0       |
| White blood cell decreased   | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Platelet count decreased     | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Hypothyroidism               | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Cholesterol high             | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Hyperuricaemia               | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Hypophosphataemia            | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| Arthralgia                   | 1 (4)     | 1 (4)   | 0       | 0       | 0       |
| **Table 4.** Exploratory analysis of the biomarkers. **HR** hazard ratio, **CI** confidence interval, **irAE** immune-related adverse event, **PD-L1** programmed death-ligand 1, **mGPS** modified Glasgow prognostic score, **LDH** lactate dehydrogenase, **CRP** C-reactive protein. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | **N** | **Progression-free survival** |                  | **Overall survival** |                  |                  |                  |                  |
|                                |      | **HR** | **95% CI** | **P-value** | **HR** | **95% CI** | **P-value** |                  |
| **Age**                        |      |   |          |           |   |          |           |                  |
| < 65 years                     | 14  | 1.00 |          |          | 1.00 |          |          |                  |
| ≥ 65 years                     | 10  | 1.81 | 0.76–4.30 | 0.181 | 1.05 | 0.37–2.96 | 0.931 |                  |
| **Sex**                        |      |   |          |           |   |          |           |                  |
| Male                           | 19  | 1.00 |          |          | 1.00 |          |          |                  |
| Female                         | 5   | 0.53 | 0.15–1.85 | 0.322 | 1.27 | 0.40–4.00 | 0.686 |                  |
| **ECOG PS**                    |      |   |          |           |   |          |           |                  |
| 0                              | 13  | 1.00 |          |          | 1.00 |          |          |                  |
| ≥ 1                            | 11  | 1.99 | 0.85–4.68 | 0.115 | 2.87 | 1.08–7.61 | 0.034 |                  |
| **Primary site**               |      |   |          |           |   |          |           |                  |
| Parotid gland                  | 19  | 1.00 |          |          | 1.00 |          |          |                  |
| Others                         | 5   | 0.62 | 0.21–1.86 | 0.393 | 1.04 | 0.33–3.25 | 0.945 |                  |
| **Prior systemic therapy**     |      |   |          |           |   |          |           |                  |
| −                              | 2   | 1.00 |          |          | 1.00 |          |          |                  |
| +                              | 22  | 2.95 | 0.37–23.28 | 0.305 | 2.72 | 0.33–22.11 | 0.350 |                  |
| **irAE**                       |      |   |          |           |   |          |           |                  |
| −                              | 19  | 1.00 |          |          | 1.00 |          |          |                  |
| +                              | 5   | 1.99 | 0.70–5.64 | 0.196 | 2.27 | 0.69–7.47 | 0.178 |                  |
| **Systemic therapy after nivolumab** |      |   |          |           |   |          |           |                  |
| −                              | 15  | 1.00 |          |          | 1.00 |          |          |                  |
| +                              | 7   | 0.82 | 0.32–2.08 | 0.673 | 0.06 | 0.01–0.48 | 0.008 |                  |
| **Histopathology**             |      |   |          |           |   |          |           |                  |
| Salivary duct carcinoma        | 20  | 1.00 |          |          | 1.00 |          |          |                  |
| Others                         | 4   | 0.36 | 0.08–1.58 | 0.176 | 0.81 | 0.23–2.88 | 0.746 |                  |
| **PD-L1**                      |      |   |          |           |   |          |           |                  |
| 0%                             | 13  | 1.00 |          |          | 1.00 |          |          |                  |
| ≥ 1%                           | 11  | 0.85 | 0.36–2.03 | 0.716 | 0.73 | 0.25–2.10 | 0.558 |                  |
| **mGPS**                       |      |   |          |           |   |          |           |                  |
| 0                              | 14  | 2.69 | 0.99–7.30 | 0.052 | 30.06 | 3.66–246.95 | 0.002 |                  |
| ≥ 1                            | 9   | 2.04 | 0.72–5.79 | 0.179 | 8.90 | 1.81–43.80 | 0.007 |                  |
| **Neutrocyte-to-lymphocyte ratio** |      |   |          |           |   |          |           |                  |
| < 2.6                          | 9   | 1.00 |          |          | 1.00 |          |          |                  |
| 2.6–5.0                        | 8   | 2.04 | 0.72–5.79 | 0.179 | 8.90 | 1.81–43.80 | 0.007 |                  |
| 5.9–19.8                       | 6   | 4.02 | 1.24–13.07 | 0.021 | 15.48 | 2.82–85.04 | 0.002 |                  |
| **Platelet-to-lymphocyte ratio** |      |   |          |           |   |          |           |                  |
| < 22,563.8                     | 10  | 1.00 |          |          | 1.00 |          |          |                  |
| 22,563.8–26,816.1              | 6   | 1.50 | 0.51–4.41 | 0.463 | 4.69 | 1.16–18.97 | 0.030 |                  |
| 30,770–131,016                 | 7   | 2.94 | 0.95–9.09 | 0.061 | 5.67 | 1.43–22.38 | 0.013 |                  |
| **Lymphocyte-to-monocyte ratio** |      |   |          |           |   |          |           |                  |
| < 2.7                          | 9   | 1.00 |          |          | 1.00 |          |          |                  |
| 2.7–4.2                        | 6   | 1.44 | 0.49–4.18 | 0.507 | 0.24 | 0.07–0.84 | 0.025 |                  |
| 4.3–6.3                        | 8   | 0.11 | 0.02–0.57 | 0.008 | 0.14 | 0.04–0.56 | 0.006 |                  |
| **LDH**                        |      |   |          |           |   |          |           |                  |
| 118–211                        | 17  | 1.00 |          |          | 1.00 |          |          |                  |
| 236–586                        | 7   | 3.09 | 1.06–8.98 | 0.039 | 3.42 | 1.11–10.50 | 0.032 |                  |
| **CRP**                        |      |   |          |           |   |          |           |                  |
| < 0.18                         | 8   | 1.00 |          |          | 1.00 |          |          |                  |
| 0.18–1.14                      | 8   | 3.92 | 1.08–14.23 | 0.038 | 1.18 | 0.31–4.48 | 0.810 |                  |
| 1.25–5.10                      | 7   | 5.08 | 1.29–20.05 | 0.020 | 10.58 | 2.25–49.89 | 0.003 |                  |

Note: Ptrend is the trend test for continuous variables.
response to nivolumab. Moreover, the optimal cut-off value for NLR was unknown. Thus, future clinical trials with a larger sample size should be performed to address these issues.

In the present study, the efficacy of nivolumab monotherapy for SGC was limited. However, some patients achieved long-term disease control with nivolumab. Further studies are warranted to elucidate a predictive factor of ICI in patients with advanced SGC.
Materials and methods

Patients and treatment. This was a multicentre retrospective cohort study conducted in Japan. Following approval from the ethics committee of the participating institutions (Approval number of each institution: International University of Health and Welfare, Mita Hospital, 5-18-50; Nihonkai General Hospital, 30-(4)-3; Niigata University, 2019-0056; Tokyo Medical University, T2018-0059; Nagoya City University, 60-20-0049), data of patients with resectable R/M SGC treated with nivolumab between May 2017 and September 2019 were extracted from the database of the nation-wide cancer registry of each participating institution. This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient and/or their legal guardians. Additionally, we obtained informed consent of the patients for publication of identifying images and photographs.

Patients with ≥20% tumour growth within 6 months prior to treatment detected by computed tomography (CT) scan, magnetic resonance imaging, and/or positron emission tomography-CT were treated with nivolumab (240 mg) every 2 weeks. The treatment dose and duration were determined in accordance with the Japanese guidelines for head and neck cancer, including salivary gland cancer. A pathological review of all patients was performed by a pathologist with expertise in SGCs (T.N.). Carcinoma ex pleomorphic adenomas were classified into different histopathological types according to each carcinoma component instead of a separate category. Imaging tests were performed every 6–8 weeks.

Immunohistochemical and gene alteration analyses. The expression status of PD-L1, MSI, HER2, and androgen receptor (AR) in patients administered nivolumab was also obtained from the database of the participating institutes. The expression level of PD-L1 in the resected or biopsy specimens of tumours was analysed using the rabbit antihuman PD-L1 clone 28-8 pharmDx; Dako–Agilent Technologies, Santa Clara, CA, USA). PD-L1 expression was defined as the percentage (instead of intensity) of tumour cells exhibiting plasma membrane staining.

The MSI test kit (product code: 4987931010017; FALCO Biosystems, Kyoto, Japan) was used to evaluate MSI as described previously. Briefly, a polymerase chain reaction (PCR) of microsatellite markers at five loci (BAT25, BAT26, NR21, NR24, and MONO27) was conducted using DNA extracted from tumour specimens. In normal patients, the PCR products were in the quasi-monomorphic variation range (QVR). Specimens with the PCR products outside the QVR were classified as MSI-positive. Specimens with more than one positive locus were classified as MSI-high (MSI-H).

HER2 and AR statuses were assessed as described previously. Briefly, specimens with 3+ HER2 immunoreactivity or HER2 gene amplification were classified as HER2-positive according to the guidelines for breast cancer by the American Society of Clinical Oncology and the College of American Pathologists. AR was classified as positive if ≥20% of nuclei in tumour cells were immunoreactive.

Analysis of biomarkers of ICI response. An exploratory analysis of potential biomarkers of ICI response was performed. The associations between prognosis and age, sex, the Eastern Cooperative Oncology Group (ECOG) performance status (PS), prior systemic therapy (present or absent), immune-related adverse events (irAE; present or absent), systemic therapy after nivolumab (present or absent), histopathological type (SDC or non-SDC), PD-L1 status, HER2 status, AR status, MSI status, mGPS, NLR, PLR, LMR, serum CRP, LDH, and absolute eosinophil count were examined.

Statistical analysis. The therapeutic effect of nivolumab was evaluated according to the overall response rate (ORR), which was defined as the percentage of patients who achieved complete response (CR) or partial response (PR), clinical benefit rate [CBR, defined as the percentage of patients who achieved CR, PR, or stable disease (SD) for at least 24 weeks], disease control rate (DCR, defined as the percentage of patients who achieved CR, PR, or SD regardless of duration), median progression-free survival (PFS), and median overall survival (OS). Treatment efficacy was evaluated according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). PFS was defined as the time from the start of nivolumab treatment to the diagnosis of progressive disease (PD). OS was defined as the time from the start of nivolumab treatment to death from any cause. Safety was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) ver. 5.0. The Kaplan–Meier method was used to estimate PFS and OS. The Cox proportional hazards model was used to calculate the hazard ratio (HR) with 95% confidence interval (CI). The results with a P value of <0.05 were considered statistically significant. All analyses were performed using STATA ver. 16 (StataCorp, College Station, TX, USA).

Data availability

The datasets generated in the current study are available from the corresponding author on request.

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References

1. Cancer Registry and Statistics. Cancer Information Service, National Cancer Center, Japan (Ministry of Health, Labour and Welfare, National Cancer Registry.) https://ganjo ho.jp/data/reg_stat/statistics/dl/cancer_incidenceNCR(2016-2017).xls. Accessed 28 September 2020.
2. El-Naggar, A. K. et al. WHO Classification of Head and Neck Tumours 4th edn. (IARC, Lyon, 2017).
Mechanism-driven biomarkers to guide immune checkpoint blockade

11. Boon, E.
10. Fushimi, C.
8. Tsurutani, J.
13. Hong, D. S.
4. Uijen, M. J. M.
6. Jhaveri, K. L.
18. Mimica, X.
17. Ferrarotto, R.
19. Hanna, G. J.
21. Nakaguro, M.
22. Ferris, R. L.
24. Cohen, R. B.
23. Burtness, B.
27. Kokkali, S.
28. Topalian, S. L., Taube, J. M., Anders, R. A. & Pardoll, D. M. Mechanism-driven biomarkers to guide immune checkpoint blockade

First line androgen deprivation therapy vs. chemotherapy for patients with androgen receptor positive recurrent or metastatic salivary gland carcinoma — A retrospective study. Front. Oncol. 9, 701 (2019).

Hong, D. S. et al. Larotrectinib in patients with TRK fusion-positive solid tumours: A pooled analysis of three phase 1/2 clinical trials. Lancet Oncol. 21, 531–540 (2020).

Doebele, R. C. et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: Integrated analysis of three phase 1–2 trials. Lancet Oncol. 21, 271–282 (2020).

Tchekmedyan, V. et al. Phase II study of lenvatinib in patients with progressive, recurrent or metastatic adenoid cystic carcinoma. J. Clin. Oncol. 37, 1529–1537 (2019).

Locati, L. D. et al. Patients with adenoid cystic carcinomas of the salivary glands treated with lenvatinib: Activity and quality of life. Cancer 126, 1888–1894 (2020).

Ferrarotto, R. et al. ACCURACY a phase (P) II trial of AL101, a pan-Notch inhibitor, in recurrent/metastatic (R/M) adenoid cystic carcinoma (ACC) patients (pts) with Notch activating mutations (Notch act mut): Preliminary safety and efficacy data. Ann. Oncol. 30, v465–v466 (2019).

Mimica, X. et al. Distant metastasis of salivary gland cancer: Incidence, management, and outcomes. Cancer 126, 2153–2162 (2020).

Hanna, G. J. et al. Long-term outcomes and clinicogenomic correlates in recurrent, metastatic adenoid cystic carcinoma. Oral Oncol. 106, 104690 (2020).

Nagao, T., Licitra, L., Loening, T., Vielh, P. & Williams, M. D. Salivary duct carcinoma. In WHO Classification of Head and Neck Tumours 4th edn (eds Naggar, A. K. et al.) 173–174 (Lyon, IARC, 2017).

nakaguro, M. et al. Salivary duct carcinoma: Updates in histology, cytology, molecular biology, and treatment. Cancer Cytol. https://doi.org/10.1002/cncr.22288 (2020).

Ferris, R. L. et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 375, 1856–1867 (2016).

Burtness, B. et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): A randomised, open-label, phase 3 study. Lancet 394, 1915–1928 (2019).

Cohen, R. B. et al. Pembrolizumab for the treatment of advanced salivary gland carcinoma: Findings of the phase 1b KEYNOTE-028 study. Am. J. Clin. Oncol. 41, 1083–1088 (2018).

Rodriguez, C. P. et al. A Phase II Trial of pembrolizumab and vorinostat in recurrent metastatic head and neck squamous cell carcinoma and salivary gland cancer. Clin. Cancer Res. 26, 837–845 (2020).

Hori, R. et al. Real-world outcomes and prognostic factors in patients receiving nivolumab therapy for recurrent or metastatic head and neck carcinoma. Cancers (Basel) https://doi.org/10.3390/cancers11091317 (2019).

Kokkali, S. et al. Nivolumab in patients with rare head and neck carcinomas: A single center's experience. Oral Oncol. 101, 104359 (2020).

Topalian, S. L., Taube, J. M., Anders, R. A. & Pardoll, D. M. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat. Rev. Cancer. 16, 275–287 (2016).

Ott, P. A. et al. T-Cell-infamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. J. Clin. Oncol. 37, 318–327 (2019).

Le, D. T. et al. PD-1 blockade in tumours with mismatch-repair deficiency. N. Engl. J. Med. 372, 2509–2520 (2015).

Rizvi, N. A. et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science 348, 124–128 (2015).

Ho, A. S. et al. Genetic hallmarks of recurrent/metastatic adenoid cystic carcinoma. J. Clin. Investig. 129, 4276–4289 (2019).

Nakanou, T. et al. Prognostic value of programmed death ligand-1 and ligand-2 co-expression in salivary gland carcinomas. Oral Oncol. 90, 30–37 (2019).

Gargano, S. M. et al. Novel therapeutic targets in salivary duct carcinoma uncovered by comprehensive molecular profiling. Cancer Med. 8, 7322–7329 (2019).

Mukaigawa, T. et al. Programmed death ligand-1 expression is associated with poor disease free survival in salivary gland carcinomas. J. Surg. Oncol. 114, 36–43 (2016).

Chang, H. et al. Overexpression of PD-L2 is associated with shorter relapse-free survival in patients with malignant salivary gland tumors. Oncol. Targets Ther. 10, 2983–2992 (2017).

Szweczyk, M. et al. Prognostic markers in salivary gland cancer and their impact on survival. Head Neck. 41, 3338–3347 (2019).

Vital, D. et al. The expression of PD-L1 in salivary gland carcinomas. Sci. Rep. 9, 12724 (2019).

Linxweiler, M. et al. The immune microenvironment and neoantigen landscape of aggressive salivary gland carcinomas differ by subtype. Clin. Cancer Res. https://doi.org/10.1158/1078-0432.CCR-19-3758 (2020).

Dalén, M. G. et al. Comprehensive molecular characterization of metastatic salivary duct carcinoma reveals actionable targets and similarity to apocrine breast cancer. Cancer Res. Clin. Cancer Res. 22, 4623–4633 (2016).

Ross, J. S. et al. Comprehensive genomic profiles of metastatic and relapsed salivary gland carcinomas are associated with tumor type and reveal new routes to targeted therapies. Ann. Oncol. 28, 2539–2546 (2017).

Terzić, J., Grivennikov, S., Karin, E. & Karin, M. Inflammation and colon cancer. Gastroenterology 138, 2101–2114.e5 (2010).

Kawakita, D. et al. Impact of hematological inflammatory markers on clinical outcome in patients with salivary duct carcinoma: A multi-institutional study in Japan. Oncotarget. 8, 1083–1091 (2017).

Templeton, A. J. et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. J. Natl Cancer Inst. 106, dju124 (2014).
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Competing interests
The authors declare no competing interests.

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