Abstract

Objectives

Recent advances in systemic therapy for salivary duct carcinoma (SDC) have been driven by the development of HER2- and androgen receptor (AR)-targeted therapies. Trastuzumab deruxtecan has proven effective not only in HER2-positive but also HER2-low breast and gastro-esophageal cancers. However, the significance of HER2-low expression in SDC remains unknown. This study aimed to investigate the clinicopathologic characteristics, prognostic implications, and impact on efficacy to AR-targeted therapy in HER2-low SDC.

Materials and Methods

This was a multi-center, observational study. HER2 status was reclassified as follows: HER2-positive (IHC3+ or 2+/ISH+), HER2-low (IHC1+ or 2+/ISH-), and HER2-zero (IHC0). The subjects were compared in three groups: total population, curative treatment cohort, and AR-targeted therapy cohort.

Results

The total population consisted of 526 patients, of whom, 271 (52%), 184 (35%), and 71 (13%) had HER2-positive, -low, and -zero tumors, respectively. Sex, M category, histological origin, Ki67, and p53 expression differed significantly between the HER2-low and HER2-positive cases. No differences in relapse-free or overall survival were observed for HER2 status in the curative treatment cohort; however, in the AR-targeted therapy cohort, the HER2-low group had significantly better response rates (41.6% vs. 18.9%, Odds ratio = 0.30, *P* = 0.012) and longer median progression-free survival (6.9 vs. 4.2 months, Hazard ratio = 1.61, *P* = 0.029) than those of the HER2-positive group.

Conclusion

HER2-low showed different clinicopathologic features from HER2-positive cases, with no prognostic differences observed in patients who underwent curative treatment. Still, HER2-low may be associated with the efficacy of AR-targeted therapy.

Keywords

Salivary duct carcinoma; HER2; HER2-low; Androgen receptor; Combined androgen blockade; Salivary gland cancer; Head and neck cancer

Abbreviations

HER2, human epidermal growth factor receptor 2

SDC, salivary duct carcinoma

AR, androgen receptor

IHC, immunohistochemistry

ISH, *in situ* hybridization

SGC, salivary gland carcinoma

T-DXd, trastuzumab deruxtecan

BC, breast cancer

CXPA, carcinoma ex pleomorphic adenoma

EN, extreme negative

EP, extreme positive

NE, non-extreme

CAB, combined androgen blockade

RFS, relapse-free survival

OS, overall survival

ORR, objective response rate

CR, complete response

PR, partial response

CBR, clinical benefit rate

SD, stable disease

PFS, progression-free survival

PD, progression disease

CI, confidence interval

Tmab, trastuzumab

DTX, docetaxel

HR, hormone receptor

HR, hazard ratio

Introduction

Salivary duct carcinoma (SDC), which accounts for 10 - 20% of all salivary gland carcinomas (SGCs), is defined as an aggressive carcinoma, histologically resembling mammary ductal carcinoma [1,2]. Most SDCs are characteristically positive for androgen receptor (AR), and approximately 40% are reported to overexpress human epidermal growth factor receptor 2 (HER2) [1,3,4]. As patients with SDC frequently develop distant metastasis after surgery and postoperative radiotherapy, SDC has been recognized as the histologic type demonstrating the worst prognosis among SGCs [5-8].

Recent advances in systemic therapy for SDC have been driven by the development of HER2-targeted therapies [9,10]. The latest NCCN guidelines recommend trastuzumab [11], trastuzumab emtansine [12], trastuzumab plus pertuzumab [13], trastuzumab plus docetaxel [14] or trastuzumab deruxtecan (T-DXd) [15] for HER2-positive SGCs. Recently, T-DXd, which is an anti-HER2 antibody-drug conjugate, has been shown to be effective not only in HER2-overexpressing breast (BC) [16], gastric [17], colorectal [18], and lung [19] and HER2-mutant lung [20] cancers, but also in tumor-agnostic HER2-overexpressing or -mutant cancers

[21,22]. In addition, DESTINY-Breast04 in BC [23], DESTINY-Gastric01 in gastroesophageal adenocarcinoma [24], and the STATICE trial in uterine carcinosarcoma [25] have shown that T-DXd is also effective in HER2-low (HER2 1+ or 2+ expression by immunohistochemistry [IHC] and absence of *HER2* gene amplification) patients. Thus, HER2-low is a newly defined category that has emerged for BC therapy and is now the focus of attention with regard to many types of cancer.

The latest NCCN guidelines also recommend leuprorelin [26], bicalutamide [26], abiraterone [27], or goserelin [28] for recurrent, unresectable, or metastatic AR-positive SGCs. Although AR-targeted therapy has a lower response rate than HER2-targeted therapy [12-15,26-29], AR-targeted therapy has better tolerability than HER2-targeted therapy. It is desirable to establish an effective biomarker to aid in selecting the appropriate treatment for patients with SDC harboring both HER2 and AR. On the other hand, for HER2-negative and AR-positive SDC cases, AR-targeted therapy is regarded as the only promising treatment; however, there has been no accurate biomarker to date for predicting treatment response.

In this study, we reclassified HER2-low SDC from conventional HER2-negative SDC and investigated the clinicopathologic characteristics, prognosis, and impact on treatment response to AR-targeted therapy in HER2-low SDC.

Materials and methods

Patients and tissue specimens

This was a retrospective study of patients with SDC treated at the 11 institutions participating in the multi-institutional Joint SDC Study Group in Japan. All cases were histopathologically confirmed by expert pathologists according to rigorous histomorphological criteria for SDC [2] (Supplemental Figure S1). Clinical data were collected from the patients' medical records in each facility.

To analyze the HER2-low population among SDC patients and the association between clinicopathologic factors and HER2 status, we included the total population registered in our collaborative study (Figure 1). On the other hand, we analyzed the patients with stage IVA or below who are surgically treated with curative intent in the primary disease setting to clarify the significance of HER2-low expression in the curative

therapy cohort. Furthermore, among cases where curative surgery was not feasible as the initial treatment due to T4b, N3b, M1, or other conditions, or cases in which recurrence or metastasis occurred after curative surgery, those in which AR-targeted therapy was administered as salvage drug treatment were defined as the AR-targeted therapy cohort (Figure 1). We also assessed the clinical outcomes according to HER2 status in this cohort.

Assessment of HER2 status

HER2 status was assessed in accordance with the 2018 ASCO Guidelines for BC using IHC and/or *in situ* hybridization (ISH) by central pathological review [30]. IHC staining was performed using 4-μm-thick formalin-fixed, paraffin-embedded tissue sections. HER2-positive was defined as IHC 3+ or IHC 2+ with ISH-confirmed gene amplification. HER2-negative was further divided into two sub-groups; HER2-zero was defined as IHC 0 and HER2-low was IHC 1+, or IHC 2+ without gene amplification (Supplemental Figure 1).

Clinicopathologic factors

The following epidemiological and clinicopathologic parameters were analyzed and compared between the HER2-low and HER2-positive or HER2-zero groups: age at diagnosis; sex; primary site; TNM category; clinical stage; histological origin (*de novo* or carcinoma ex pleomorphic adenoma: CXPA), and AR, Ki67, CK5/6, and p53 expression via IHC. The histological origin of SDC was determined by expert pathologists through a central review of whole-section hematoxylin-eosin-stained specimens from resected primary tumors. The methods used for analyzing immunopositivity for AR, Ki67, CK5/6, and p53 were as described previously [4,31]. AR was considered overexpressed if ≥ 70% of tumor cell nuclei showed strong staining [4,27,28,31,32]. For Ki67, a value of < 40% was classified as Ki67-low, while ≥ 40% was classified as Ki67-high [4]. CK5/6 was considered positive when > 11% of cells were immunopositive for CK5/6. p53 staining results were interpreted based on expression pattern and classified into three groups as follows: extreme negative (EN), complete confluent negativity of staining; extreme positive (EP), strong diffuse confluent positivity; and non-extreme (NE), all intermediate expression of any intensity. We previously reported significant correlations

between p53-EN and TP53 truncating mutations, as well as between p53-EP and TP53 missense mutations [31]. Furthermore, we also reported that SDC cases with p53-EN/EP have worse overall survival (OS) than those with p53-NE [4].

AR-targeted therapy

The regimen of AR-targeted therapy consisted of a combination of leuprorelin acetate and bicalutamide 80 mg/day (combined androgen blockade; CAB), as previously described [26,29,33]. Of the anti-AR targeted therapy cohort, 34 cases were enrolled in a clinical trial conducted in Japan [26]. Tumor response was determined based on the Response Evaluation Criteria in Solid Tumors version 1.1.

Statistical analysis

To compare patient characteristics, *HER2* gene amplification and AR expression among the different HER2 status tumors, Fisher's extract test and Mann-Whitney U test were used for comparisons between two groups, and Chi-square test and Kruskal-Wallis test were used for comparisons among three or more groups.

In the curative treatment cohort, the Kaplan-Meier method with log-rank test and the Cox proportional hazards model were applied to estimate the association of HER2 status with relapse-free survival (RFS) and overall survival (OS). RFS was defined as the duration between the date of treatment initiation and the date of recurrence identified on imaging or pathology, or death from any cause. OS was defined as the duration from starting day of treatment (the start date of measurement) and death from any cause as an event.

In the AR-targeted therapy cohort, binomial logistic regression analysis, Kaplan-Meier method with log-rank test, and Cox proportional hazards model were performed to examine the relationship between HER2 status and CAB treatment results: objective response rate (ORR) [the proportion of patients who experienced the best overall response of complete response (CR) or partial response (PR)], clinical benefit rate (CBR) [the proportion of patients who experienced a best overall response of CR, PR, or stable disease (SD) for more than 24 weeks], progression-free survival (PFS) [the time from the start of CAB therapy to progression

disease (PD) or death from any cause], and OS [the time from initiation of CAB therapy to death from any cause].

A 2-tailed p-value < 0.05 was considered statistically significant. Statistical analyses were performed using JMP Pro 17.0.0 (SAS Campus Drive, Cary, NC, USA).

Results

Patient characteristics

Between August 1992 and December 2022, 607 patients with SDC were treated at the 11 institutions participating in a joint SDC study group in Japan, and 526 cases were finally included in this study after excluding cases with unknown HER2 status (Figure 1). The data cut-off date was 30 October 2023. Table 1 shows the patient characteristics in this study.

The total population included 450 males and 76 females, with a median age of 63 years (range, 26-93 years). As for histological origin, 142 were classified as *de novo,* 293 as CXPA, and 91 as undefined.

In the curative treatment cohort, all 363 patients underwent curative surgery, of whom 246 received postoperative radiotherapy. No cases received anti-AR or anti-HER2 therapy as neoadjuvant or adjuvant

treatment. The median follow-up period of the curative treatment cohort was 48 months (range, 0-248 months).

Of the 305 patients, including 142 with recurrent or metastatic disease after curative treatment and 163 without curative treatment, 167 patients received AR-targeted therapy (AR-targeted therapy cohort). All patients in this cohort were treated with CAB therapy, with 14 administrated Tmab/DTX prior to CAB therapy. The median follow-up period of the AR-targeted therapy cohort was 26 months (range, 1-105 months).

The frequency of HER2-low expression in the total population

The 526 patients were classified into three groups according to HER2 status; 71 (13%) were HER2-zero, 184 (35%) were HER2-low, and 271 (52%) were HER2-positive (Supplemental Table S1, Supplemental Figure S1). In detail, the number of cases by HER2 IHC score for the total 526 patients was 71 (13%) for 0, 90 (17%) for 1+, 112 (21%) for 2+, and 253 (48%) for 3+. Of the 398 cases analyzed by ISH, 177 (44%) had *HER2* amplification. The correlation between HER2 IHC score and *HER2* gene amplification is shown in Supplemental Table S2 and Supplemental Figure

S2. The mean ± standard deviation of the *HER2*/CEP17 ratio in the IHC 0, 1+, 2+ and 3+ patients was 1.1 ± 0.3, 1.1 ± 0.3, 1.5 ± 1.0 and 5.1 ± 2.8, respectively. When a *HER2*/CEP17 ratio ≥2, as determined by ISH, is considered to represent *HER2* gene amplification positivity, the positive rates of *HER2* gene amplification in the IHC 0, 1+, 2+, and 3+ patients were 2%, 3%, 16%, and 91%, respectively. A high concordance was observed between IHC3+ and FISH positivity.

Correlation between clinicopathological factors and HER2 status in the total population

Analysis of the correlations between clinicopathologic factors and HER2 status showed that there were significantly less females and distant metastasis and more cases of *de novo* origin, Ki67-low and NE p53 expression in the HER2-low than in the HER2-positive group. On the other hand, there was no significant difference in any variables between the HER2-zero and HER2-low groups.

Our analysis of the relationship between HER2 status and AR expression level (Supplemental Figure S3) showed that the mean ± standard deviation of the AR expression level in the HER2-zero, HER2-

low, and HER2-positive groups was 65 ± 34%, 70 ± 32%, and 69 ± 30%, respectively. No significant differences were observed among the three groups.

Survival analysis for the curative treatment cohort

The characteristics of 363 patients in the curative treatment cohort according to HER2 status are shown in Supplementary Table S3. Regarding the treatment method, there were no differences among the three groups in terms of neck dissection, postoperative radiotherapy, and adjuvant chemotherapy. Figure 2 shows the OS and RFS curves of the curative treatment cohort stratified by HER2 status. The median OS (95% confidence interval, CI) in the HER2-zero, HER2-low, and HER2-positive groups was 70.5 (25.6–115), 83.5 (58.3–109), and 81.7 (32.5–130.9) months, respectively. The median RFS (95% CI) in the HER2-zero, HER2-low and HER2-positive groups was 25.3 (9.8–40.9), 17.9 (12.5–23.2), and 14.2 (10.0–18.5) months, respectively.

Therapeutic effects and progression-free survival for the AR-targeted therapy cohort

Patient characteristics in the AR-targeted therapy cohort are shown in Supplemental Table S4. HER2-positive patients were more likely to have received chemotherapy and/or Tmab/DTX before CAB therapy. Supplemental Table S5 shows the best overall response to CAB therapy according to HER2 status. A CR was observed in 9 patients (12%) in the HER2-low group, but only 2 patients (5%) in the HER2-zero group and 1 patient (2%) in the HER2-positive group. ORR (95% CI) in the HER2-zero, HER2-low, and HER2-positive groups was 32.4% (17.4–47.5), 41.6% (30.6-52.6), and 18.9% (8.3–29.4), respectively. CBR (95% CI) in the HER2-zero, HER2-low, and HER2-positive groups was 43.2% (27.3–59.2), 61.0% (50.2-71.9), and 35.9% (22.9–48.8), respectively. In both univariate and multivariate analyses, the ORR and CBR values for CAB therapy were significantly better in the HER2-low group than in the HER2-positive group (Table 3).

Figure 3A shows the PFS curves for patients treated with CAB therapy stratified by HER2 status. The median PFS (95% CI) in the HER2-zero, HER2-low and HER2-positive groups was 5.4 (2.7–8.1), 6.9 (4.3–

9.5), and 4.2 (2.9–5.6) months, respectively. In both univariate and multivariate analyses, PFS was significantly better in the HER2-low group than in the HER2-positive group (Table 3, Figure 3A). Waterfall and spider plots for CAB therapy in three groups are shown in Figures 3B and 3C. On the other hand, there were no significant differences in OS among three groups (Table 3).

Discussion

With the promising efficacy of novel anti-HER2 antibody-drug conjugates in BC, HER2-low is a newly defined category of HER2 expression. This is the first study to examine the frequency of HER2-low SDC, its association with clinicopathologic factors, and its prognostic impact using data based on a large number of SDC patients. This study revealed that 35% of the total population in our joint SDC study group in Japan showed HER2-low expression. HER2-low was not related to prognosis in the patients with curative stage SDC, but was associated with the efficacy of CAB therapy in patients with recurrent or metastatic SDC.

Although the frequency of HER2-low expression has been reported in a variety of solid tumors, it has been difficult to compare the frequency of

HER2-low among different cancer types as there are no standardized HER2 IHC testing procedures or guidelines except those for breast and gastric cancers. However, Uzunparmak et al. recently reported HER2 expression as assessed by IHC in 4,701 various tumors at a single institution using consistent scoring criteria. They reported that the frequency of HER2-low (1+ or 2+) was found across multiple tumor types: 47.1% in breast, 46.9% in lung, 46.5% in endometrial, 46% in urothelial, 45.5% in gallbladder, and 34.6% in gastric/gastroesophageal junction, with the highest frequency at 50.0% observed in salivary gland (*n* = 24/48; histopathologic diagnosis not available) [34]. Regarding SDC, the only reported frequency of HER2-low was 57% (*n* = 30/53) [35], which was higher than that in our cohort (35%). To determine a more accurate frequency of HER2-low in SDC, it would be necessary to establish criteria for HER2-low in SDC and undertake an analysis of a large number of cases.

In this study, both IHC and ISH were performed on the same tumor and the same tissue section. However, although rare, cases of IHC 3+ with ISH negativity and IHC 0/1+ with ISH positivity were observed

(Supplemental Table S2). Similar findings have also been reported in breast cancer [36]. One possible explanation for this phenomenon could be epigenetic influences. However, since such cases are rare, we consider ISH to be unnecessary for IHC 0/1+ or 3+ cases from a cost-effectiveness perspective, in accordance with the Breast ASCO/CAP Guidelines 2018 [30].

Regarding the correlation between clinicopathologic factors and HER2 status, there were significantly less females and distant metastasis, and more cases of *de novo* origin, Ki67-low and NE p53 expression in the HER2-low than in the HER2-positive group. On the other hand, there were no significant differences between HER2-zero and HER2-low in any variables. We previously reported that HER2-positive is more common in females or in tumors of CXPA origin [4,31], and that extreme p53 positivity and negativity were associated with *TP53* missense and truncating mutations, respectively [31]. The results that HER2-low tumors had more Ki67-low and less *TP53* mutations than did HER2-positive tumors were similar to those reported for BC [37,38]. Therefore, the less distant

metastases in patients with HER2-low tumors in this study could be due to the low Ki67 expression and lack of *TP53* mutations.

Similar to HER2, AR is a crucial target in the systemic therapy for SDC. There was no significant relationship between AR positivity and HER2 status in our SDC study. While the conventional biphasic HER2 classification is not related to AR expression in SDC [4,39], recent reports suggest that all cases exhibiting strong or moderate-strong AR staining intensity were found to be HER2-nonamplified [35]. However, the report lacked data regarding the correlation between AR staining intensity and HER2-positive, HER2-low, and HER2-zero disease. In BC, AR expression was more frequent in HER2-low BCs than in HER2-zero BCs [40-44]. It has been observed that the co-expression of AR and HER2 may lead to crosstalk in the downstream signal pathway in BC [45,46] and prostate cancers [47,48]. However, the co-expression of AR and HER2 occurs in various combinations of intensities and positivities in SDC. Further research is needed to clarify how these combinations affect the biology, prognosis, and response to systemic therapy in AR/HER2 co-expressed SDC [49,50].

Various results have been reported comparing the prognosis between HER2-low and HER2-zero in each primary site. In this study, no statistical differences in either OS or RFS were observed in the curative treatment cohort of patients with SDC. This was similar to the results for breast [51], colorectal [52], and gastric cancers [53], while HER2-zero had a better prognosis than did HER2-low in pancreatic [54] and ovarian cancers [55]. In addition, studies of BC have reported that HER2-zero was more sensitive than HER2-low to cyclin-dependent kinase 4/6 inhibitors [56] and neoadjuvant chemotherapy [57].

On the other hand, one study on recurrent/metastatic cases has reported that HER2 positivity is a poor prognostic factor [58]. Cavalieri et al. reported that the HER2-positive group had significantly worse disease-free survival and OS from the onset of recurrent/metastatic disease compared with the HER2-negative group among patients with AR-positive recurrent/metastatic SGC. However, the administration rate of androgen deprivation therapy was 89% in the HER2-negative group and 65% in the HER2-positive group. In this study, we investigated the efficacy of CAB therapy and the prognosis of patients with unresectable, recurrent, or

metastatic disease who underwent CAB therapy. We reported that the HER2-positive group had worse response rates and PFS compared with the HER2-low group. Compared with the report by Cavalieri et al., the uniformity of salvage therapy in our study allows for a more accurate assessment of the relationship between the efficacy of CAB therapy and HER2 status.

In the AR-targeted therapy cohort, PFS in the HER2-low group was significantly better than that in the HER2-positive group, while patients with HER2-low SDC tended to have shorter OS compared to those with HER2-positive SDC (Table 3). This might be due to the administration of Tmab/DTX after the failure of CAB therapy in patients with HER2-positive SDC. In fact, 25 cases with disease progression after CAB therapy underwent anti-HER2 therapy, including Tmab/DTX. Although the response rate to CAB therapy is lower than that to Tmab/DTX, CAB therapy has better tolerability than Tmab/DTX. Therefore, in patients with both HER2- and AR-positive SDC, CAB therapy may be undertaken depending on the patient's age, performance status and disease status. Additionally, in HER2-negative and AR-positive cases, AR-targeted

1 therapy was the only promising treatment. Therefore, it is necessary to
2 clarify the predictive factors for CAB. As the worse predictive factors for
3 CAB, female sex [59], AR-low expression [28,50], EZH2-high expression
4 [60], low AR and Notch pathway activity scores [33,61], *SRD5A1*-low
5 expression [33], high Risk score (*CD3E* and *LDB3*) [62], and HER2-
6 positive [29,50] have been reported. To the best of our knowledge, this is
7 the first study to show that HER2-low, when compared with HER2-positive,
8 is a better predictor of treatment response to CAB therapy. One of the
9 possible reasons is that HER2-low tumors could be driven by the AR
10 pathway rather than HER2. Atallah et al. also concluded that HER2-low
11 BC is mainly driven by hormone receptor (HR) signaling in HR-positive
12 tumors [40]. However, based on this theory, CAB therapy should also be
13 expected to be highly effective for HER2-zero tumors. One reason for this
14 contradiction could be the effect of the downstream signaling of HER2.
15 Genetic mutations for downstream signaling of HER2 activate the HER2
16 pathway in a ligand-independent manner, thus activating the HER2
17 pathway even in the absence of HER2 overexpression. We previously
18 reported that HER2-negative SDCs had statistically significant higher

mutation rates of *PIK3CA, HRAS,* and *BRAF* than did HER2-positive SDCs [31]. Interestingly, in BC, it has been reported that HER2-zero tumors have relatively more alterations in the MAPK and PI3K pathways, which are downstream signaling of HER2, than HER2-low and -positive tumors [37]. If there is similar trend in genetic alterations for the HER2 pathway between HER2-zero and HER2-low in SDC as well as BC, this difference could result in the lower therapeutic effect of CAB due to the predominance of tumor activity in the HER2 pathway over the AR pathway.

The comparison of the efficacy and safety between CAB therapy and chemotherapy has been reported in a phase II trial (EORTC1206) [63]. The CAB regimen used in this trial consisted of bicalutamide 50 mg/day and triptorelin. The mPFS for CAB therapy was 4.0 months (95% CI, 3.6–8.7), and the confirmed ORR was 23.1%, thus failing to demonstrate superiority over chemotherapy. Due to differences in the drug regimen and the lack of clarity regarding patient background, including HER2 status in cases treated with CAB therapy, a direct comparison between the therapeutic effects of this study and the EORTC1206 study is not possible. Regarding safety, the types of adverse events (AEs) observed with CAB

therapy and chemotherapy differed significantly [63], making a simple comparison of AE incidence rates insufficient for determining superiority. However, in general, AEs associated with CAB therapy are highly tolerable [59]. Therefore, it would be best to select either CAB therapy or chemotherapy based on factors such as age, gender, PS, comorbidities, disease progression, and patient preference.

The latest NCCN guidelines recommend HER2-tageted therapies including T-DXd for HER2-positive SGC, but do not recommend the same for HER2-negative, including HER2-low, SGC. On the other hand, T-DXd is recommended in HER2-low BC [23], gastroesophageal adenocarcinoma [24], and uterine carcinosarcoma [25]. Therefore, a therapeutic effect of T-DXd on HER2-low SDC is expected and a clinical trial to test this hypothesis is ongoing (jRCT2011210017). This study has demonstrated the benefit of CAB for HER2-low patients, while T-DXd may also be a candidate for systemic therapy for HER2-low/AR-positive patients, depending on the results of ongoing clinical trials.

Based on the results of this study, there is currently no clinicopathological difference between HER2-low and HER2-zero, and no

significant difference in prognosis within the curative treatment cohort. Therefore, in clinical practice, HER2-low and HER2-zero can be considered equivalent, meaning they can be regarded as the same group; i.e., HER2-negative. However, in terms of treatment efficacy with CAB therapy, HER2-low may serve as a potential biomarker. Furthermore, based on the T-DXd clinical trial results, HER2-low may emerge as a distinct treatment target from that of HER2-negative.

This study has several limitations. First, we applied the criteria in BC to determine HER2 status in SDC. Although BC and SDC have many similarities in terms of histopathology, distinct differences are seen in the intensity and frequency of HER2 expression, and the types of HR expressed. The emerging importance of HER2-targeted therapies in HER2-positive SGC underscores the need to establish optimal HER2 status criteria in SGC to select the appropriate treatment for patients with SGC. Additionally, since AR IHC and HER2 IHC/ISH in this study were retrospectively performed using archival tissues, we cannot exclude the potential impact of using older tissue specimens on the sensitivity of IHC and ISH. When we divided the tissue specimens from 1992 to 2022 into

three time periods and compared HER2 status as well as HER2 and AR IHC results, no difference was observed in HER2 status or HER2 IHC staining intensity. However, a significant difference was found in AR staining intensity, with the frequency of AR positivity being significantly lower in older tissue specimens (Supplemental Table S6). Another limitation is the selection bias in the AR-targeted therapy cohort as not all the patients with recurrent or metastatic AR-positive SDC received CAB therapy. This cohort also included some patients treated with Tmab/DTX or other forms of chemotherapy prior to CAB therapy, which might have affected the tumor response to CAB and survival outcomes. A prospective trial with appropriate eligible criteria is needed to accurately analyze the impact of HER2 status on the efficacy of CAB therapy.

Conclusion

In conclusion, there were significantly lower frequencies of females and distant metastasis, and significantly higher percentages of tumors of *de novo* origin, Ki67-low, and NE p53 expression among those with HE2-low SDC than in those with HER2-positive SDC, while there were no significant differences between HER2-zero and HER2-low. Despite HER2

status not being associated with prognosis in the SDC patients treated with curative surgery, HER2-low was an independent predictive factor for ORR, CBR, and PFS in those treated with CAB therapy when compared with the HER2-positive group. Therefore, HER2-low can serve as a novel biomarker with both predictive and prognostic importance in patients with SDC.

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Figure captions

Figure 1. Flow for defining the study population

Abbreviations: SDC, salivary duct carcinoma; HER2, human epidermal growth factor receptor 2; RT, radiotherapy; NED, no evidence of disease; AR, androgen receptor.

Figure 2. Kaplan-Meier curves for relapse-free and overall survival in the curative treatment cohort stratified by HER2 status.

(A) Relapse-free survival and (B) overall survival. The vertical lines show censored events. Multivariate analysis was adjusted by age, sex, primary tumor site, TNM category, post-operative radiotherapy, and histologic origin (including systemic therapy in overall survival).

Abbreviations: HR, hazard ratio; CI confidence interval.

Figure 3. Characteristics of the responses in the AR-targeted therapy cohort according to the Response Evaluation Criteria in Solid Tumors (version 1.1).

(A) Kaplan-Meier curves for progression-free survival. The vertical lines show censored events. Multivariate analysis was adjustment by age, sex, ECOG PS, primary site, disease status, previous chemotherapy, previous trastuzumab/docetaxel, androgen receptor expression. (B) Best reduction from baseline in target lesions. The upper dotted lines represent the threshold for progressive disease (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 (30% decrease in the sum of the longest diameter of the target lesions). (C) Change from baseline (%) in the sum of the target lesions over time to progressive disease. The upper dotted lines represent the threshold for progressive disease, and the lower dotted lines show the threshold for a partial response.

Abbreviations: HR, hazard ration; CI confidence interval.

Supplementary Figure S1. Histomorphology, HER2 immunohistochemistry (IHC), and *HER2 in situ* hybridization (ISH) of salivary duct carcinoma (SDC).

(A) SDC showing solid growth with comedo necrosis. (B) Tumor cells exhibit high-grade nuclear atypia and abundant eosinophilic cytoplasm. (C) HER2 IHC; score 0, (D) score 1+, (E) score 2+, (F) score 3+, (G) HER2 ISH; not amplified (*HER2*/CEP17 ratio: 1.03, *HER2* copy number: 1.75), (H) HER2 ISH; amplified (*HER2*/CEP17 ratio: 8.60, *HER2* copy number: 13.8)

Supplementary Figure S2. Association of HER2 protein expression with gene amplification in the total population.

The vertical axis represents the *HER2*/CEP17 ratio.

Abbreviations: IHC, immunohistochemistry.

Supplementary Figure S3. The expression level of androgen receptor according to HER2 status in the total population.

The vertical axis represents the expression level of androgen receptor. The vertical lines indicate the mean and mean ± standard deviation.

Figure 1

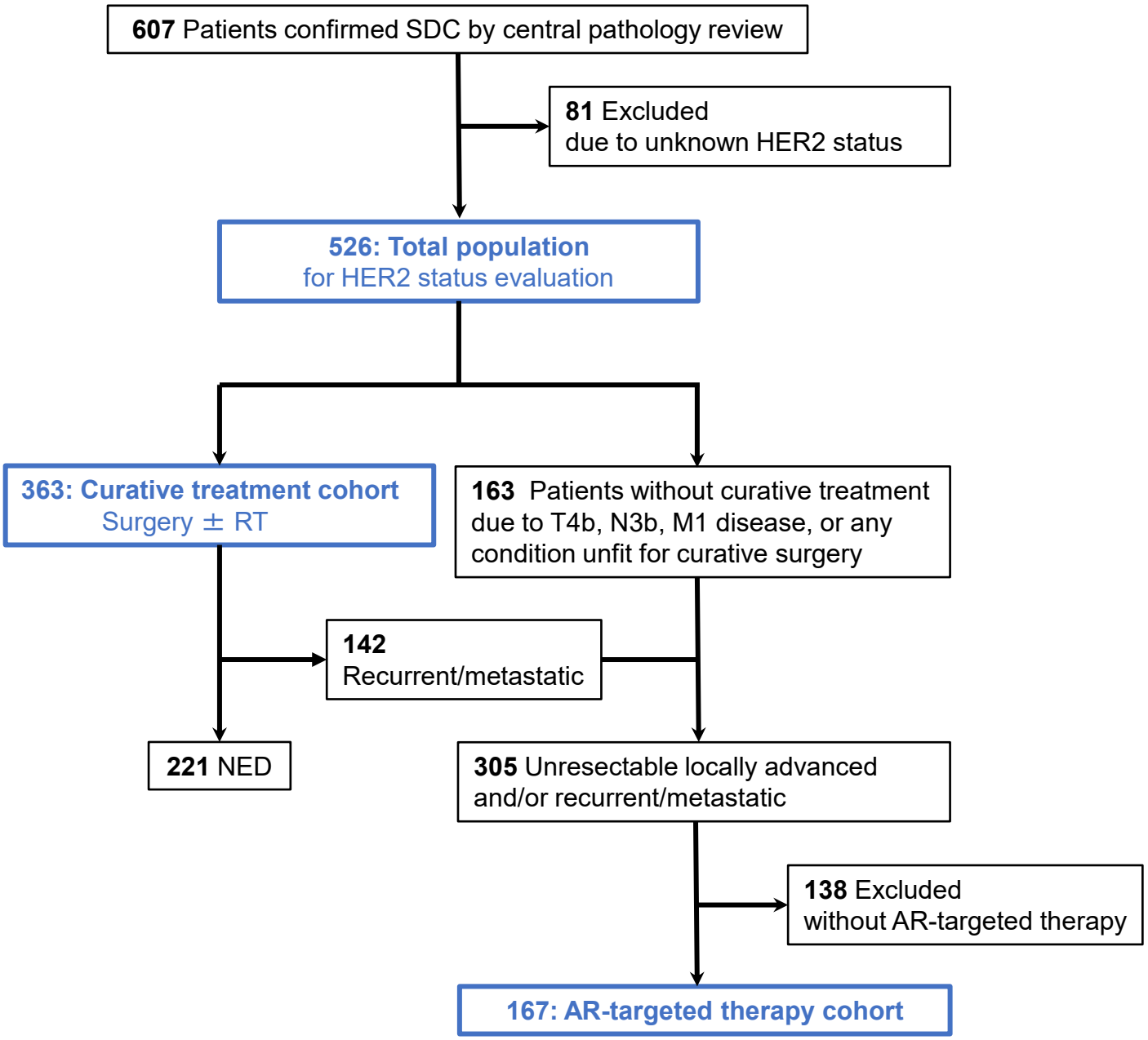
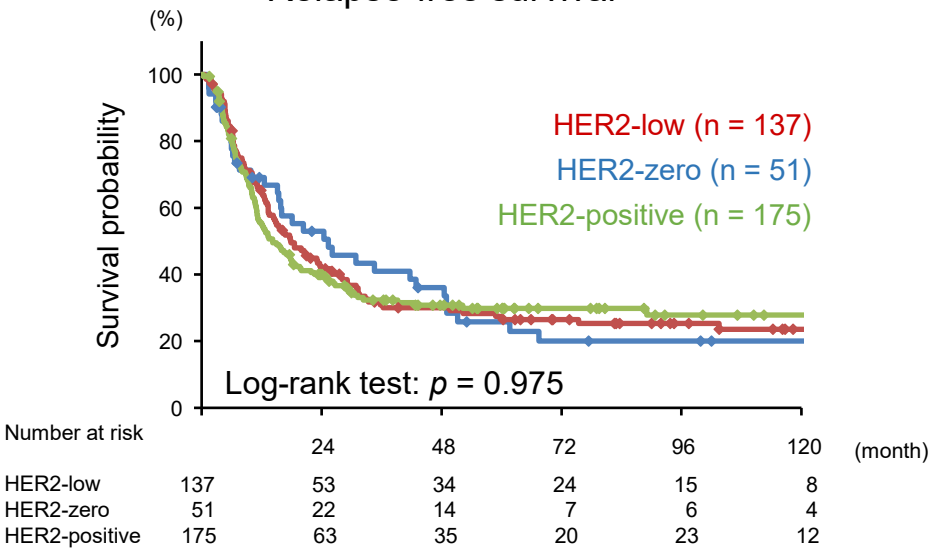

607 Patients confirmed SDC by central pathology review
81 Excluded due to unknown HER2 status
526: Total population for HER2 status evaluation
363: Curative treatment cohort Surgery ± RT
163 Patients without curative treatment due to T4b, N3b, M1 disease, or any condition unfit for curative surgery
142 Recurrent/metastatic
221 NED
305 Unresectable locally advanced and/or recurrent/metastatic
138 Excluded without AR-targeted therapy
167: AR-targeted therapy cohort

Figure 2

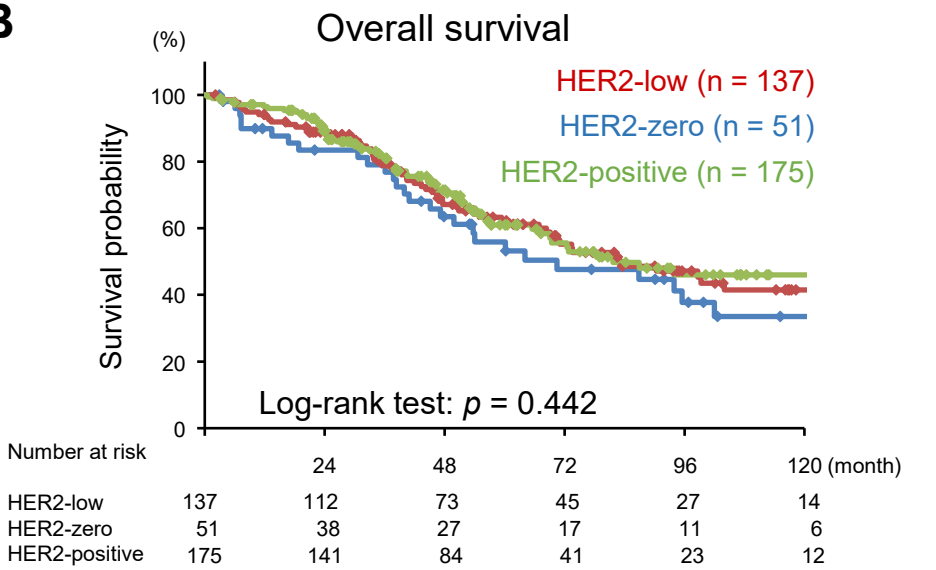
A



Number at risk		24	48	72	96	120 (month)
HER2-low	137	53	34	24	15	8
HER2-zero	51	22	14	7	6	4
HER2-positive	175	63	35	20	23	12

HER2 status	n	Median months (95%CI)	Univariate analysis		Multivariate analysis	
			HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Low	137	17.9 (12.5-23.2)	1.00	-	1.00	-
Zero	51	25.3 (9.8-40.9)	1.02 (0.69-1.48)	0.911	1.03 (0.69-1.54)	0.874
Positive	175	14.2 (10.0-18.5)	1.03 (0.79-1.35)	0.825	1.26 (0.96-1.66)	0.098

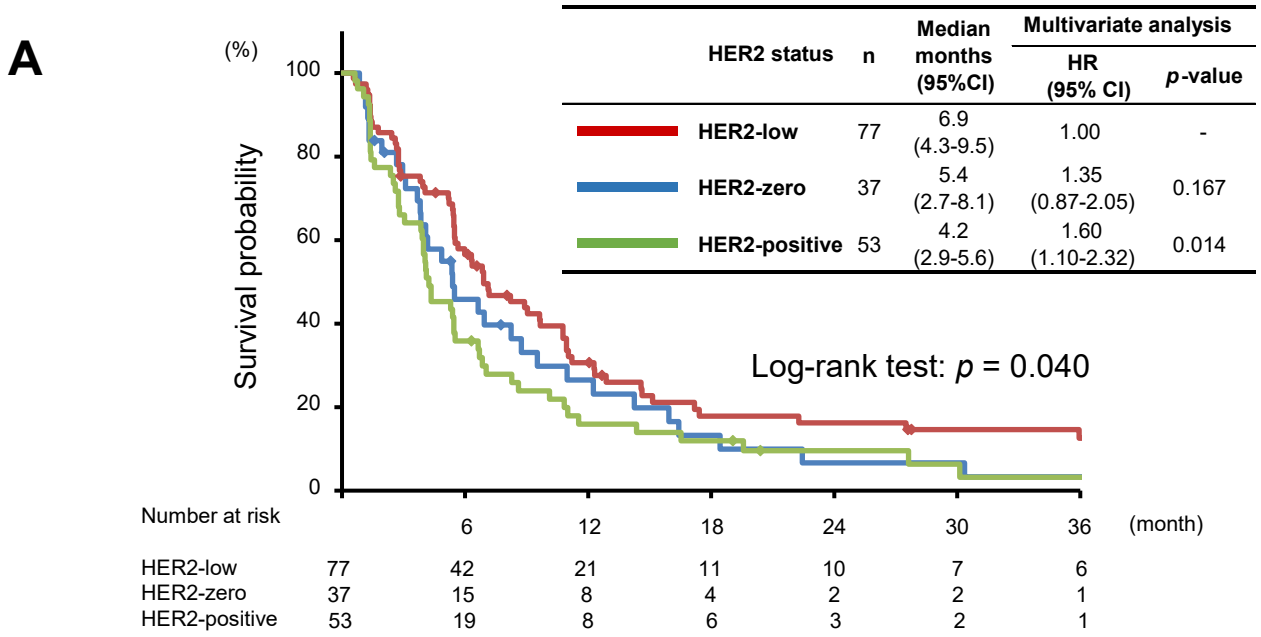
B



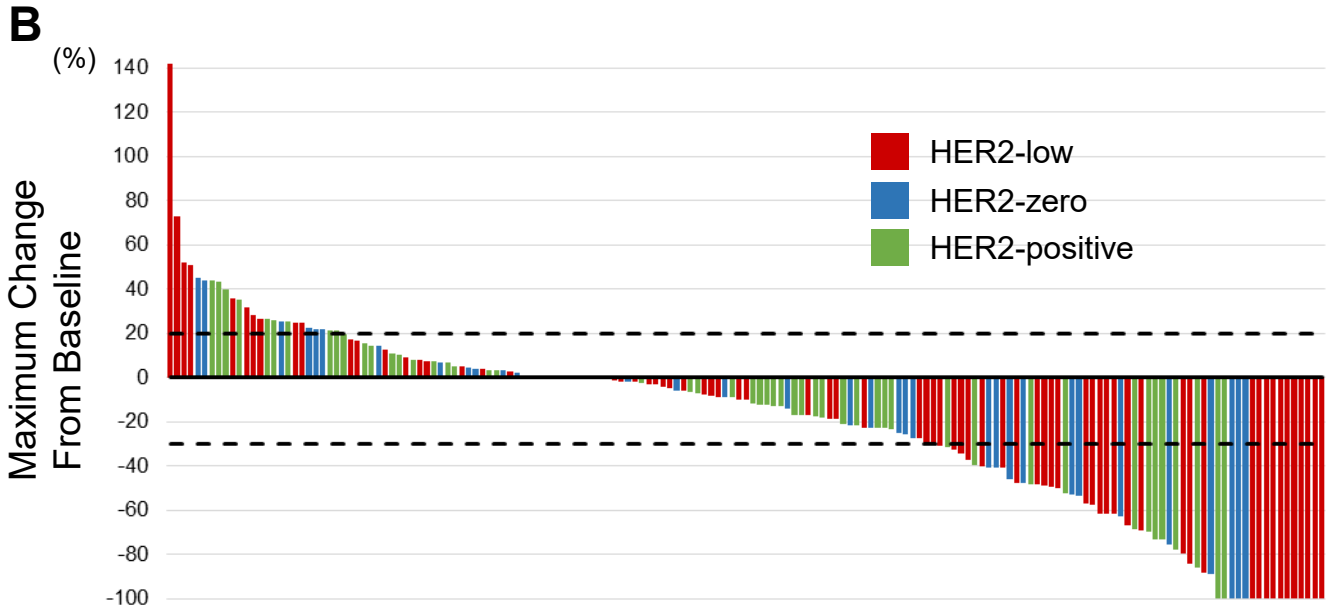
Number at risk		24	48	72	96	120 (month)
HER2-low	137	112	73	45	27	14
HER2-zero	51	38	27	17	11	6
HER2-positive	175	141	84	41	23	12

HER2 status	n	Median months (95%CI)	Univariate analysis		Multivariate analysis	
			HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Low	137	83.5 (58.3-109)	1.00	-	1.00	-
Zero	51	70.5 (25.6-115)	1.24 (0.78-1.92)	0.339	1.42 (0.89-2.25)	0.141
Positive	175	81.7 (32.5-131)	0.93 (0.66-1.32)	0.686	1.23 (0.78-1.93)	0.371

Figure 3



HER2 status	n	Median months (95%CI)	Multivariate analysis HR (95% CI)	Multivariate analysis *p*-value
HER2-low	77	6.9 (4.3-9.5)	1.00	-
HER2-zero	37	5.4 (2.7-8.1)	1.35 (0.87-2.05)	0.167
HER2-positive	53	4.2 (2.9-5.6)	1.60 (1.10-2.32)	0.014



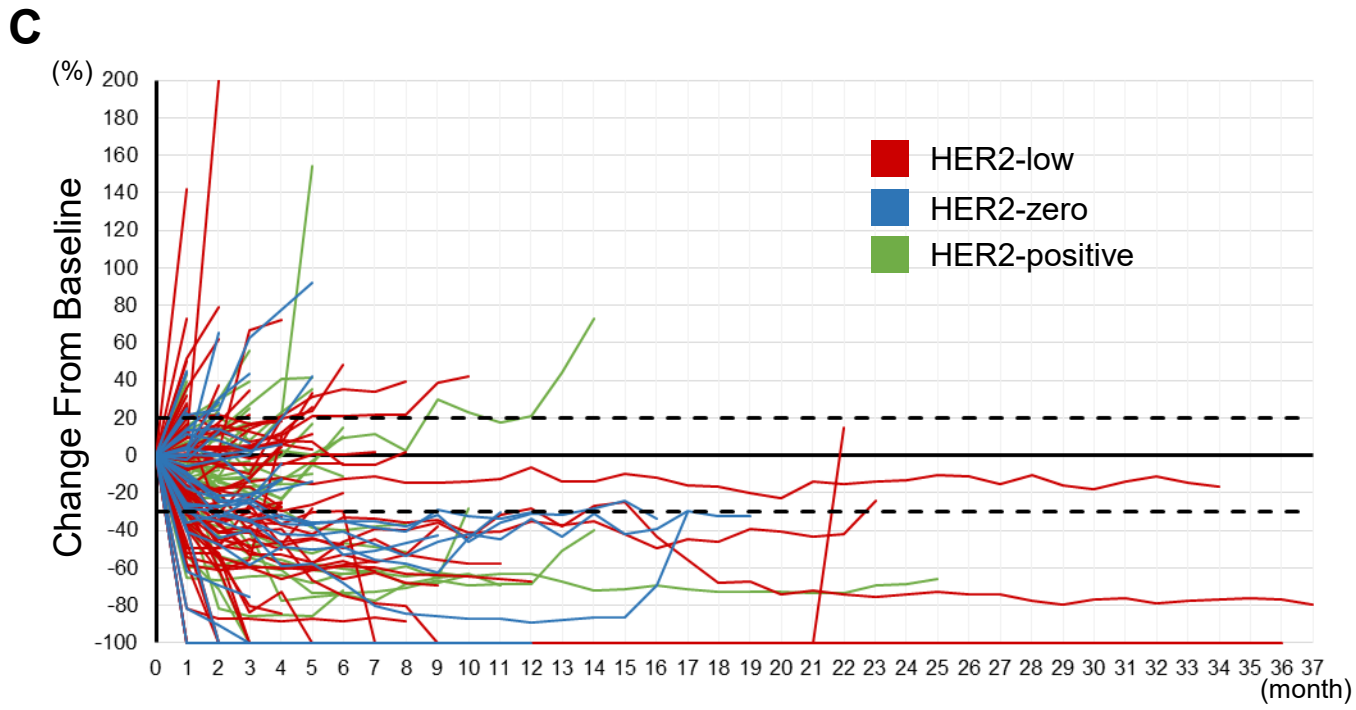


Table 1. Patient characteristics

	Total population (n = 526)	Curative treatment cohort (n = 363)	AR-targeted therapy cohort (n = 167)
Age; years (range)	63 (26-93)	63 (26-93)	66 (27-90)
< 65; n (%)	295 (56)	209 (58)	76 (46)
≥ 65; n (%)	231 (44)	154 (42)	91 (54)
Sex; n (%)			
Male	450 (86)	303 (83)	158 (95)
Female	76 (14)	60 (17)	9 (5)
Primary site; n (%)			
Parotid gland	362 (69)	266 (73)	114 (68)
Submandibular gland	136 (26)	83 (23)	40 (24)
Sublingual gland	2 (0)	1 0	1 (1)
Minor salivary gland	26 (5)	13 (4)	12 (7)
Histological origin; n (%)			
de novo	142 (27)	127 (35)	-
CXPA (intracapsular)	39 (7)	39 (11)	-
CXPA (minimally invasive)	10 (2)	10 (3)	-
CXPA (invasive)	244 (46)	182 (50)	-
undefined	91 (17)	5 (1)	-
T category; n (%)			
1	56 (11)	47 (13)	-
2	144 (27)	111 (31)	-
3	119 (23)	73 (20)	-
4a	176 (33)	132 (36)	-
4b	26 (5)	-	-
Unknown	5 (1)	-	-
N category; n (%)			
0	220 (42)	195 (54)	-
1	34 (6)	26 (7)	-
2	221 (42)	142 (39)	-
3	50 (10)	-	-
M category; n (%)			
0	414 (79)	363 (100)	-
1	112 (21)	-	-
HER2 status; n (%)			

HER2-zero	71	(13)	51	(14)	37	(22)
HER2-low	184	(35)	137	(38)	77	(46)
HER2-positive	271	(52)	175	(48)	53	(32)
AR status; n (%)						
Negative (< 70%)	160	(30)	117	(32)	22	(13)
Positive (≥ 70%)	352	(67)	235	(65)	145	(87)
First-line treatment; n (%)						
Surgery	-		363	(100)	-	
Radiotherapy	-		246	(68)	-	
Systemic therapy for recurrence/metastasis; n (%)						
Tmab/DTX	-		77	(21)	14	(8)
CAB	-		119	(33)	167	(100)
Median follow-up; mo (range)	-		48	(0-248)	26	(1-105)

CXPA: carcinoma ex pleomorphic adenoma,Tmab/DTX: trastuzumab/docetaxel, CAB; combined androgen blockade.

Table 2. Comparison of clinicopathologic characteristics between patients with HER2-zero, low, and positive SDC in the total population

	HER2-zero (n = 71, 13%)	HER2-low (n = 184, 35%)	HER2-positive (n = 271, 52%)	*p*-value	
				low vs. zero	low vs. positive
Age					
Median (range)	62 (27-89)	64 (28-93)	63 (26-88)	0.267	0.788
Sex; n (%)					
Male	65 (92)	164 (89)	221 (82)	0.651	0.034*
Female	6 (8)	20 (11)	50 (18)		
Primary site; n (%)					
Parotid gland	50 (70)	134 (73)	178 (65)	0.756	0.123
Others	21 (30)	50 (27)	93 (34)		
T category; n (%)					
1, 2	23 (32)	71 (39)	106 (39)	0.387	1.000
3, 4	47 (66)	111 (60)	163 (60)		
N category; n (%)					
0	34 (48)	75 (41)	111 (41)	0.325	1.000
1-3	37 (52)	109 (59)	159 (59)		
M category; n (%)					
0	58 (82)	159 (86)	197 (73)	0.334	< 0.001*
1	13 (18)	25 (14)	74 (27)		
Clinical stage; n (%)					
I-III	26 (37)	52 (28)	71 (26)	0.224	0.667
IV	44 (62)	130 (70)	198 (73)		
Histological origin; n (%)					
de novo	29 (41)	81 (44)	32 (12)	0.878	< 0.001*
CXPA	29 (41)	74 (40)	191 (70)		
CXPA; n (%)					
invasive	24 (83)	61 (82)	159 (83)	1.000	0.855
non-invasive	5 (17)	13 (18)	31 (16)		
AR; n (%)					
< 1%	4 (6)	6 (3)	8 (3)	0.448	0.253
≥ 1%, < 10%	5 (7)	11 (6)	12 (4)		
≥ 10%, < 70%	17 (24)	32 (17)	65 (24)		
≥ 70%	43 (61)	129 (70)	180 (66)		

Ki67; n (%)								
< 40%	38	(54)	78	(42)	66	(24)	0.305	< 0.001*
≥ 40%	28	(39)	80	(43)	166	(61)		
CK5/6; n (%)								
0, 1+	39	(55)	101	(55)	149	(55)	0.318	0.352
2+, 3+	21	(30)	39	(21)	72	(27)		
p53; n (%)								
NE	48	(68)	117	(64)	159	(59)	0.427	0.008*
EN/EP	13	(18)	23	(13)	65	(24)		

CXPA: carcinoma ex pleomorphic adenoma, AR: androgen receptor, CK5/6: cytokeratin 5/6, NE: not extreme, EN/EP: extreme negative/extreme positive.

*Statistically significant association ($p < 0.05$).

Table 3. The association between HER2 status and clinical outcomes in patients with SDC treated with CAB

			ORR					CBR				
				Univariate analysis		Multivariate analysis			Univariate analysis		Multivariate analysis	
HER2 status	n	(%)	ORR (%; 95% CI)	Odds Ratio (95% CI)	*p*-value	Odds Ratio (95% CI)	*p*-value	CBR (%; 95% CI)	Odds Ratio (95% CI)	*p*-value	Odds Ratio (95% CI)	*p*-value
Low	77	(46)	41.6 (30.6-52.6)	1.00	-	1.00	-	61.0 (50.2-71.9)	1.00	-	1.00	-
Zero	37	(22)	32.4 (17.4-47.5)	0.68 (0.30-1.54)	0.346	0.68 (0.29-1.61)	0.378	43.2 (27.3-59.2)	0.49 (0.22-1.08)	0.074	0.45 (0.19-1.02)	0.056
Positive	53	(32)	18.9 (8.3-29.4)	0.33 (0.14-0.75)	0.006*	0.30 (0.12-0.77)	0.012*	35.9 (22.9-48.8)	0.36 (0.17-0.74)	0.005*	0.31 (0.14-0.70)	0.005*
			PFS					OS				
				Univariate analysis		Multivariate analysis			Univariate analysis		Multivariate analysis	
HER2 status	n	(%)	Median (months; 95%CI)	Hazard Ratio (95% CI)	*p*-value	Hazard Ratio (95% CI)	*p*-value	Median (months; 95%CI)	Hazard Ratio (95% CI)	*p*-value	Hazard Ratio (95% CI)	*p*-value
Low	77	(46)	6.9 (4.3-9.5)	1.00	-	1.00	-	30.5 (25.6-35.3)	1.00	-	1.00	-
Zero	37	(22)	5.4 (2.7-8.1)	1.35 (0.87-2.05)	0.167	1.36 (0.86-2.10)	0.180	24.0 (16.4-31.5)	1.07 (0.65-1.71)	0.777	0.99 (0.60-1.65)	0.984
Positive	53	(32)	4.2 (2.9-5.6)	1.60 (1.10-2.32)	0.014*	1.61 (1.04-2.47)	0.029*	39.7 (35.8-43.5)	0.86 (0.55-1.32)	0.488	0.77 (0.47-1.26)	0.299

Adjustment by age, sex, ECOG PS, primary site, disease status, Previous chemotherapy, Previous Tmab/DTX, AR expression.

ORR: objective response rate (complete response + partial response), CBR: clinical benefit rate (complete response + partial response + stable disease ≥24 weeks), CI: confidence interval, PFS: progression-free survival, OS: overall survival.

*Statistically significant association ($p<0.05$).