Salivary duct carcinoma (SDC) of the head and neck is an aggressive adenocarcinoma of the salivary glands, first described by Kleinsasser et al in 1968. A diagnosis of SDC for any patient has historically been associated with a poor prognosis, with a high incidence of both locoregional and distant metastases. Radical surgery followed by postoperative radiation and chemotherapy has been the mainstay of treatment, but despite this, SDC is still associated with high mortality. In a review of 104 cases by Barnes, 65% of patients died of their disease, most of them within 4 years.

Immunohistochemical analysis of SDC has revealed a variety of similarities to ductal carcinoma of the breast. Both are seen as an infiltrating ductal carcinoma, with elements of comedonecrosis and a reactive desmoplastic stroma; carcinoembryonic antigen and gross cystic disease fluid protein are often positive in these tumors. Human epidermal growth factor receptor 2 (HER-2) is another protein found on the surface of cells in ductal carcinoma of the breast. The gene coding for this receptor is known as HER2/neu (c-erbB-2); overexpression occurs in 20% to 30% of ductal

Salivary Duct Carcinoma and Herceptin

carcinoma of the breast and is associated with a worse prognosis.\textsuperscript{3} Advances in adjuvant therapy have found that the drug trastuzumab (Herceptin, Genentech, San Francisco, CA) is a monoclonal antibody that binds the extracellular domain of HER-2 and is effective in increasing disease-free interval and prolonging overall survival for patients with ductal carcinoma of the breast.\textsuperscript{4,5} In our review of SDC at the UCLA Medical Center, HER-2 expression confirmed via immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) was explored. Because of striking similarities to ductal carcinoma of the breast, a subset of our patients with recurrent SDC have been started on adjuvant therapy including trastuzumab, and in this study we review the natural history of this aggressive tumor in our patient population and the implications of trastuzumab in altering the course of SDC.

MATERIALS AND METHODS

**Patient Selection.** A retrospective analysis of all patients with SDC of the head and neck seen at the UCLA Medical Center from 1993 to 2006 was performed. A search for patients with this disease entity was completed by accessing the computerized database in the Department of Pathology. Seven patients with SDC of the head and neck were found. Patient charts were obtained from the UCLA Office of Medical Records, and each patient or their next of kin was then contacted for specific information regarding diagnosis, treatment, and long-term follow-up.

**Analysis of Data.** Patients were evaluated for initial stage of tumor, location of primary, facial nerve involvement, treatment modalities, evidence of recurrence, disease-free interval, and overall survival. Tumor specimens were examined for overexpression of HER-2 protein using immunohistochemical staining and FISH, and this was performed on any associated neck dissection specimens as well. Follow-up assessment of current patient status was determined via medical chart review and telephone contact with each patient and/or their next of kin.

**Treatment.** All 7 patients received surgery and postoperative radiation therapy as primary treatment for SDC. Patients who experienced recurrence of disease (3/7 patients) received postoperative chemotherapy consisting of paclitaxel (Taxol) and carboplatin, as well as a regimen including trastuzumab therapy.

**Immunohistochemical Analysis.** After surgical resection, all pathology specimens were analyzed by hematoxylin–eosin (H&E) stain to confirm that the histopathologic criteria for SDC were met. Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections from 7 SDCs using anti-\textit{HER-2/neu} stains which utilized a modified avidin–biotin technique, and these were interpreted following the criteria established by Dako for the HercepTest.\textsuperscript{6} Overexpression of HER-2 protein was defined as strongly positive membrane staining in >10\% of the cancer cells. Membrane staining of tumor cells was scored as 1\+ (negative), 2\+ (weakly positive), and 3\+ (strongly positive). Positive and negative controls stained appropriately.

**Fluorescence In Situ Hybridization.** FISH assays were done using the PathVysion assay (Abbott-Vysis, Downers Grove, IL) using the methods previously described for breast cancer.\textsuperscript{7} Slides were evaluated for \textit{HER-2} gene amplification by determining the \textit{HER-2}/CEP17 (chromosome 17 centromere) signal ratio. If the ratio was <2.0, the specimen was considered to lack gene amplification; if the ratio was \geq 2.0, the specimen was considered to show \textit{HER-2} gene amplification. All results were verified by independent pathologist slide review.

RESULTS

**Clinical Analysis.** Table 1 shows information regarding the age and sex of each patient, site of tumor, stage, facial nerve involvement, evidence of recurrence, treatment modalities, and the current status of the patient with postoperative follow-up ranging from 8 to 118 months. Median duration of follow-up was 26 months. Six of seven (86\%) patients were women. Average age at diagnosis was 68 years. Four of seven (57\%) had primary tumors arising in the parotid gland. There was evidence of metastatic disease to the neck in 5/7 (71\%) of patients. The majority of patients (4/7) presented with facial weakness or paralysis, indicative of perineural invasion at the time of diagnosis. After treatment with surgery and radiation, 3/7 (43\%) patients had recurrence of disease at either a local, regional, or distant site. Two patients (29\%) died, 1 from metastatic SDC and the other from colon cancer. Three patients received trastu-
zumab therapy as part of the chemotherapy protocol after recurrence of disease was documented. Of these, 1 died from SDC, while the other 2 are still alive at 19 and 36 months, respectively, since the date of their diagnosis. The latter patient has shown disappearance of pulmonary nodules via repeat CT and positron emission tomographic studies and has remained disease-free for 3 years on trastuzumab therapy.

**Histology.** Microscopically, all SDCs were found to be very similar to ductal carcinoma of the breast. All cases showed an extensive intraductal and infiltrating ductal carcinoma pattern. Neoplastic ducts exhibited solid, cribiform, and papillary proliferations. Intraductal carcinoma with central comedonecrosis with or without dystrophic calcifications was seen (Figure 1A). The stroma appeared desmoplastic such as that seen in scirrhous carcinoma of the breast. Tumor cells with large pleomorphic hyperchromatic nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm were noted. In addition, some tumor cells had an apocrine appearance replete with apical snouts. Mitoses as well as lymphatic and perineural invasion were noted frequently. (Figure 1B).

On immunohistochemical analysis, all 7 cases of SDC stained positive for HER-2 protein. Staining was strong and diffuse (3+) in all cases (Figure 1C). FISH was also performed confirming +HER-2 overexpression in 3 cases (Figures 2A–2C).

**DISCUSSION**

SDC is an aggressive malignancy of the head and neck. Seventy-one percent of the patients in our study had evidence of metastasis to the neck, with perineural invasion of the facial nerve present in the majority of patients. Despite multimodality therapy, 43% of patients experienced a recurrence of disease. These findings are consistent with previous reviews of this entity.2,8,9

In a review of over 100 patients by Barnes,2 the tumor was found to be 3 times more common in men and occurred primarily in patients older than 50 years of age. In contrast, in our study, SDC was found overwhelmingly (86% in women patients, with an average age of 68. Tumor location is primarily in the parotid gland (57% in the current study, over 85% in the review by Barnes), although a minority of cases are found in the submandibular and minor salivary glands of the head and neck.

On H&E staining, SDC has remarkable similarity to ductal carcinoma of the breast, as previously described. However, estrogen and progesterone receptors, which are commonly present in ductal breast carcinoma, are rarely found in SDC.2,10 In other studies, the presence of androgen receptors in many cases of SDC raises the possibility of treatment with antiandrogen therapy, although there has been some variability in results from study to study.10–13 Therefore, the role for hormonal therapy in the treatment of SDC is still under scrutiny.

The significance of trastuzumab therapy in the treatment of both breast cancer and SDC may be appreciated by an understanding of the role of the surface protein HER-2 found on the cell membranes of these cancer cells. Growth and replication of cells are regulated, in part, by polypeptide growth factors that bind to high-affinity receptors typically found on the plasma membrane of cells. These receptors are transmembrane proteins composed of an extracellular portion which is the ligand-binding site, and an intracellular portion which is the active site. Activation of the receptor

| Age, y (sex) Tumor site | Lymph node* Facial nerve Recurrence Radiation Chemo Herceptin Status |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 69 (F) Parotid          | +49 of 51 (IV)  | +               | + (local)       | +               | +               | +               | Alive           |
| 50 (F) Retromolar trigone space | 0 (I) | –               | –               | –               | –               | –               | Alive           |
| 86 (F) Parapharyngeal space | +25 of 38 (IV) | –               | –               | +               | –               | –               | Alive           |
| 66 (F) Parotid          | +1 of 5 (IV)    | +               | + (local, regional, distant) | +               | +               | +               | Died            |
| 55 (F) Parotid          | +10 of 19 (IV)  | +               | + (regional, distant) | +               | +               | +               | Alive           |
| 66 (M) Parotid          | +0 of 17 (IV)   | –               | –               | –               | –               | –               | Alive           |
| 84 (F) Submandibular gland | +6 of 28 (IV)  | –               | –               | –               | –               | –               | Died (other causes) |

Abbreviations: F, female; M, male.
*I and IV represent stages (given within parentheses).
with a ligand results in phosphorylation of multiple enzymes, which in turn causes cell signaling through a complex series of events. One such receptor is the 185-kDa HER-2, which is a member of the c-erbB family of tyrosine kinases. The gene for HER-2 is a proto-oncogene located on chromosome 17 known as \( HER-2/neu \) (or \( c-erbB-2 \)). Amplification and overexpression of HER-2 occurs in 20% to 30% of breast cancers and is an independent adverse prognostic indicator of sur-

**FIGURE 1.** (A) Salivary duct carcinoma with comedonecrosis and dystrophic calcifications visualized at low power (hematoxylin–eosin stain, original magnification x10). (B) Perineural invasion by tumor cells (original magnification x20). (C) Strong and diffuse membranous staining by \( HER-2/neu \) immunohistochemistry.

**FIGURE 2.** (A) Salivary duct carcinoma (SDC) with positive fluorescence in situ hybridization (FISH) for \( HER-2/neu \). \( HER-2 \) (red) to chromosome 17 centromere (green) ratio is 11.1. (Note: a ratio \( \geq 2.0 \) is significant for presence of \( HER-2/neu \) gene amplification.) (B) SDC with positive FISH for \( HER-2/neu \) with a ratio of 12.2. (C) SDC with positive FISH for \( HER-2/neu \) with a ratio of 9.4.
vival.\textsuperscript{14} It confers more aggressive characteristics to the malignant breast cancer cells, shorter survival, resistance to chemotherapy and hormonal therapy, and a higher risk of metastatic disease.\textsuperscript{3–5,15}

One way to block cellular growth is to block growth factor receptors. Trastuzumab (Herceptin), a recombinant, humanized, murine monoclonal antibody that binds the extracellular domain of HER-2 with high affinity, has been introduced for the treatment of patients with advanced breast cancer that overexpress HER-2.\textsuperscript{4,5} It has had substantial and reproducible antitumor activity in both preclinical and clinical studies. In HER-2-overexpressing tumor specimens, trastuzumab has had marked antiproliferative effects, has demonstrated synergy with a number of cytotoxic drugs, and has potentiated radiation therapy.\textsuperscript{3}

Clinically, it has shown significant efficacy in the treatment of HER-2-positive breast cancer, inducing tumor responses when used as either monotherapy or in combination with a number of chemotherapeutic agents.\textsuperscript{5,15,16} Trastuzumab as a single agent is associated with an objective clinical response in 11% to 23% of extensively pretreated patients with metastatic breast cancer overexpressing HER-2, as well as a response rate of 25% to 62% in previously untreated patients.\textsuperscript{5,17,18} The median duration of response ranged from 6.6 to 9.1 months.\textsuperscript{19,20} Vogel et al\textsuperscript{21} found an objective response rate of 26% in these patients, with 57% of the responders free of disease progression at 12-months follow-up.

A randomized phase III clinical trial has shown that the combination of chemotherapy and trastuzumab had higher remission response rates and 25% longer survival duration (increased 4–6 months) than chemotherapy alone.\textsuperscript{19,22,23} This combination tripled the median time to disease progression compared with standard chemotherapy and significantly improved the duration of response and time to treatment failure. Slamon et al\textsuperscript{24} compared chemotherapy alone with chemotherapy with trastuzumab, and found that the addition of trastuzumab increased time to disease progression from 4 to 7.4 months and increased the rate of overall response from 32% to 50%. It increased the median survival from 20.3 to 25.1 months and decreased the rate of death at 1 year from 33% to 22%.\textsuperscript{14} It appears to provide greater benefit if used earlier.\textsuperscript{15} It also demonstrates a favorable safety profile; the primary adverse side effect is cardiac dysfunction, which occurs in less than 5% of patients.\textsuperscript{21,24} It does not have the additional side effect profile such as alopecia, mucositis, and neutropenia that is seen with traditional chemotherapeutic agents.

Clinical trials studying the effects of trastuzumab on other epithelial malignancies with overexpression of HER-2 are already underway for lung cancer, prostate cancer, and ovarian cancer.\textsuperscript{25} As described previously, the benefits of trastuzumab in the treatment of HER-2-overexpressing breast cancer is well documented in the literature. Because SDC shows many immunohistochemical similarities to breast carcinoma, it is likely that patients with SDC will have a beneficial clinical response to trastuzumab.

Breast cancer patients with IHC 3+ or FISH-positive disease gain the greatest clinical benefits from trastuzumab.\textsuperscript{5,16} However, controversy exists as to whether IHC or FISH is superior in detection of HER-2 and in predicting effective response to trastuzumab. The FDA has approved selected IHC (antigen retrieval) methods and FISH for HER-2 detection and candidacy for trastuzumab therapy. Press et al\textsuperscript{26} demonstrated the superiority of FISH in a large-scale study of breast cancer patients showing a third positive for FISH and candidates for trastuzumab therapy. They argue that IHC is unpredictable with inconsistent controls during processing of archival formalin-based samples, leading to erroneous false-positive and false-negative detections of HER-2 protein.\textsuperscript{26} In this study, all 7 cases of SDC showed strong IHC staining for HER-2. Using archival, formalin-based paraffin-embedded samples, it is unclear as to the validity of the IHC method of detection. As such, we further applied FISH-based analysis to all of the samples, and only 3 of the 7 cases were positive for HER-2. These 3 cases were patients who had recurrent disease, received chemoradiotherapy, and trastuzumab. Our findings are in agreement with studies pertaining to breast cancer in which FISH-positive HER-2 overexpression may identify patients with more aggressive variants of carcinoma and likely to respond to trastuzumab.

At our institution, a multidisciplinary protocol has been introduced to treat those patients with only FISH-positive HER-2-overexpressing SDC with trastuzumab after conventional therapy has been instituted. This protocol includes a loading dose of 4 mg/kg with maintenance therapy of 2 mg/kg per week, similar to the dose given for advanced breast cancer. Consultation with a medical oncologist is essential in planning and continuing adjuvant therapy with trastuzumab. In
the current study, 2 of the 3 patients who received trastuzumab therapy are still alive at 19 and 36 months after surgery, although both have had recurrence of disease. One of these patients has shown disappearance of lung nodules while being on trastuzumab therapy and has remained disease-free for 36 months. The significance of this promising finding, as it pertains to the role of trastuzumab, remains to be determined. Disease progression is still being monitored. Further prospective trials are needed to evaluate the efficacy of trastuzumab therapy in treating SDC. We hope that this study serves as an introduction to the possibility of an adjuvant therapy for SDC. Should trastuzumab have similar treatment results as those seen for HER-2-overexpressing breast cancer, one may expect it to be a promising chemo-therapeutic agent that will be regularly included for treatment of FISH-based HER-2-positive SDCs of the head and neck.

REFERENCES