

HER2 Expression in Salivary Gland Carcinomas: Dependence on Histological Subtype

Bonnie Glisson,¹ A. Dimitrios Colevas,²
Robert Haddad,² Jeffrey Krane,²
Adel El-Naggar,¹ Merrill Kies,¹
Rosemary Costello,² Carmen Summey,¹
Matthew Arquette,³ Corey Langer,⁴
Philip C. Amrein,⁵ and Marshall Posner²

¹The University of Texas M. D. Anderson Cancer Center, Houston, Texas; ²Dana Farber Cancer Institute, Boston, Massachusetts;

³Washington University, St. Louis, Missouri; ⁴Fox Chase Cancer Center, Philadelphia, Philadelphia; and ⁵Massachusetts General Hospital, Boston, Massachusetts

ABSTRACT

Purpose: Previous evaluation of HER2 overexpression in salivary gland cancers indicated an incidence varying between 7 and 56%, with no clear difference among three histologically different subtypes. As part of a Phase II trial of trastuzumab for treatment of incurable salivary gland cancer, we screened 137 tumors for HER2 expression.

Experimental Design: Unstained sections of paraffin-embedded tumor samples were stained with p185/HER2 receptor antibody. Tumors with moderate (2+) to strong (3+) complete membrane staining in at least 10% of the tumor cells were scored as positive for overexpression.

Results: The overall frequency of overexpression for HER2 was 17% (23 of 137), whereas it was only 8% in the three most common histological subtypes screened. Overexpression was distinctly rare in the most common subtype screened, adenoid cystic carcinoma (4%, 3 of 70). Overexpression was very common in salivary duct cancers; 10 (83%) of 12 were positive for HER2. This observation is consistent with the typical high-grade histological features and aggressive behavior of this subtype as well as with its histogenetic similarity to breast cancer. Analysis based on histogenesis (intercalated duct *versus* excretory duct) indicated a higher frequency of overexpression in the latter (55%) than in the former (7%).

Conclusions: Our overall results suggest that trastuzumab will not have a major role in treatment of salivary gland cancers of intercalated duct origin. Further systematic evaluation of trastuzumab in subtypes of excretory duct origin could be supported.

INTRODUCTION

Salivary gland neoplasms are relatively rare, accounting for 7% of the tumors arising in the head and neck region. The frequency of these neoplasms is ~0.9 of 100,000 with 2,500 new cases annually in the United States. The majority of these arise in the parotid (70%) and, of these, ~25% are malignant. The incidence of malignancy at other sites, such as submandibular, sublingual, or minor salivary glands, is higher, approximating 50% (1).

Histopathologically they are composed of widely varied morphological subtypes with heterogeneous clinical behavior. In general, cancers of intercalated duct origin (adenoid cystic, adenocarcinoma, acinic cell, polymorphous low-grade adenocarcinoma, and myoepithelial) are low grade and biologically indolent compared with those derived from the secretory duct (salivary duct, mucoepidermoid, and squamous; Refs. 2, 3).

Salivary gland malignancies are managed primarily with surgical resection and, if indicated by postoperative pathological findings of stage or grade, or perineural invasion, adjunctive radiation. Systemic therapy is generally reserved for locoregional recurrence and/or metastatic disease, the risk of which is correlated with advanced stage, higher grade, and certain histological subtypes (1). Although cytotoxins such as doxorubicin, cisplatin, 5-fluorouracil, paclitaxel, and navelbine clearly have some degree of activity in this disease, response rates are generally in the range of 15–30%, and responses are relatively short in duration (4–8). Given the rarity of these tumors and the variability in natural history of metastatic disease, with prolonged indolent courses in many patients, it has been difficult to define the survival benefits of cytotoxic agents. Therefore, there is a need to better understand the biology of these cancers and to develop therapeutic approaches based on relevant targets.

In that context, we evaluated expression of HER2 in recurrent salivary gland carcinomas as part of a clinical trial to define the clinical activity of trastuzumab, a recombinant monoclonal antibody directed against HER2, in these tumors. The *HER2* proto-oncogene on chromosome 17q is expressed or overexpressed in a variety of epithelial malignancies (9). The gene encodes a membrane receptor protein that is one of four members of the epidermal growth factor receptor family. HER2 has no known ligand but is capable of heterodimerization with any of the other three HER proteins, and can, thus, participate in effecting a signal transduction cascade with diverse effects that potentiate the malignant phenotype (10). In breast cancer, overexpression of HER2 is correlated with gene amplification, conferring a poor prognosis and relative resistance to cytotoxics

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Notes: A. D. C. is currently at National Cancer Institute, NIH, Bethesda, Maryland.

Requests for reprints: Bonnie Glisson, The University of Texas M. D. Anderson Cancer Center, Department of Thoracic/Head & Neck, Box 432, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-6363; Fax: (713) 796-8655; E-mail: bglisson@mdanderson.org.

Table 1 Case accrual by institution

Dana Farber Cancer Institute	66
M. D. Anderson Cancer Center	60
Washington University	5
Fox Chase Cancer Center	3
Massachusetts General Hospital	2
Yale–New Haven Hospital	1

(11, 12). Trastuzumab is active as a single agent and enhances the rate of response to cytotoxics in HER2-positive breast cancers (13).

In previous reports, frequency of HER2 expression in salivary gland carcinomas, which was detected by either immunostaining or fluorescence *in situ* hybridization, has been variable. Rates of 7 and 56% in adenoid cystic (14–16), 30–38% in mucoepidermoid (14, 17), and 23% in terminal duct adenocarcinoma (14) subtypes have been documented. In two reports, overexpression and/or amplification of HER2 was an independent marker of poor prognosis in mucoepidermoid and adenoid cystic carcinomas (14, 17). These data and the availability of trastuzumab provided the rationale for our prospective evaluation of HER2 expression in patients with incurable salivary gland cancer.

Patients with tumors that overexpressed the protein were evaluated for participation in a Phase II clinical trial with trastuzumab, the complete results of which are reported separately (18). Patients were accrued into two strata on the basis of presumed tumor origin from the intercalated or excretory duct.

MATERIALS AND METHODS

Tumor Tissue. One hundred thirty-five salivary gland carcinomas were screened at six participating institutions between October 1999 and August 2001 (Table 1). Histologically confirmed diagnosis of one of the following malignancies was required: adenoid cystic carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma, malignant mixed tumor, polymorphous low-grade adenocarcinoma, undifferentiated carcinoma, salivary duct carcinoma, myoepithelial carcinoma, carcinoma ex pleomorphic adenoma, squamous cell carcinoma, or adenocarcinoma, not otherwise specified.

Immunostaining. Five- μ m-thin sections from formalin-fixed, paraffin-embedded tumor tissue blocks were mounted on glass slides, deparaffinized, and rehydrated. Antigens were retrieved by boiling in citrate buffer. Slides were incubated with the primary antibody (rabbit anti-p185/HER2, Herceptest; DAKO A/S, Copenhagen, Denmark) and then with the secondary antibody (peroxidase-conjugated goat antirabbit). Diaminobenzidine was applied to develop the stain, and the sections were then counterstained with H&E. Positive and negative controls were run for each screen.

Assessment of HER2 Expression. Slides were scored on a 0–3+ scale, as follows: 0, staining in <10% of tumor cells or no staining; 1+, faint and partial membrane staining in \geq 10% of tumor cells; 2+, weak to moderate complete membrane staining in \geq 10% of tumor cells; or 3+, moderate to strong complete membrane staining in \geq 10% of tumor cells. Scores of either 2+ or 3+ were defined as overexpression. Two

independent observers scored the slides, and discrepancies were resolved by consensus.

RESULTS

Of the six centers participating, the Dana-Farber Cancer Institute and The University of Texas M. D. Anderson Cancer Center contributed 126 (93%) of the cases accrued (Table 1). Table 2 presents HER2 expression by duct of origin and descending order of tumor frequency in the screened population. Adenoid cystic carcinoma was the most common subtype, representing 51% of the total cases. Adenocarcinoma, mucoepidermoid, and salivary duct carcinoma constituted 14, 10, and 9% of the cases, respectively. The other subtypes were infrequent, each accounting for \leq 6% of the specimens analyzed. In addition to the tumors listed in Table 2, one poorly differentiated cancer was 3+. Tumors of intercalated duct origin were far more common than those of excretory duct origin, representing 77% of the series.

The overall frequency of HER2 overexpression was 17% (23 of 137; Table 2). Expression of HER2 scored as 1+ was observed in two adenocarcinomas and one acinic cell carcinoma; all others not specifically listed in Table 2 did not express HER2. Overexpression was rare in adenoid cystic carcinoma (4%). The frequencies in the next most common subtypes, adenocarcinoma and mucoepidermoid were 14 and 21%, respectively. Although the sample size was small at 12, the frequency of HER2 overexpression in salivary duct carcinomas is striking at 83%. Nine (75%) of 12 were strongly positive for HER2 (3+).

Analysis based on origin indicates that 55% (16 of 31) of excretory duct origin tumors were HER2 positive, whereas only 7% (7 of 105) of tumors thought to originate from the intercalated duct were HER2 positive.

The results of weekly trastuzumab therapy in 14 patients from the screened population are reported by Haddad *et al.* (18). To summarize, one of three patients with mucoepidermoid cancer had a partial response that is ongoing at 45 months. Two additional patients with salivary duct cancer had stable disease for 24 and 40 weeks. These two patients are grouped with adenocarcinoma by Haddad *et al.* (18). Although no other patients had response or stable disease beyond 12 weeks, the

Table 2 HER-2 overexpression by tumor origin and histologic type

	No. 2+	No. 3+	No. screened	% positive
Intercalated duct subtypes				
Adenoid cystic	2	1	70	4
Adenocarcinoma	0	3	21	14
Acinic cell carcinoma	0	0	8	0
Carcinoma ex pleomorphic adenoma	0	1	4	25
Myoepithelial	0	0	2	0
Excretory duct subtypes				
Mucoepidermoid	1	2	14	21
Salivary duct	1	9	12	83
Squamous	0	3	5	60
Total	4	19	136 ^a	17

^a One poorly differentiated cancer was 3+.

numbers of patients within each subtype are too small to exclude trastuzumab as an active agent.

DISCUSSION

Our series of 137 cases represents the largest experience yet in the evaluation of *HER2* gene expression in salivary gland carcinomas. Similar to findings in other solid tumors using the Herceptest, our data indicate a much lower frequency of *HER2* positivity than the historical experience cited in the literature. The frequency of overexpression in the three most common subtypes (adenoid cystic, adenocarcinoma, and mucoepidermoid), was only 8% (8 of 103). In contrast, salivary duct carcinoma appears to strongly overexpress *HER2* in the majority of cases. Notably, this relatively rare subtype is a high-grade neoplasm with histogenetic similarities to mammary ductal carcinoma and is well recognized as one of the most aggressive of the salivary gland malignancies.

The clinical trial with trastuzumab was originally designed to accrue patients in two strata based on tumor origin: group 1 (intercalated duct) including adenoid cystic, acinic cell, adenocarcinoma, malignant mixed, and myoepithelial subtypes; and group 2 (excretory duct), including mucoepidermoid, squamous, and salivary duct subtypes. In the first stage of a Simon two-stage design, response assessment in 15 patients of each stratum was planned, and 1 response of 15 would support accrual to a total of 25 patients in that group. The low frequency of *HER2* positivity in the screened population, especially in the most common adenoid cystic subtype, led to an impression of low feasibility and closure of the trial when only 14 patients total had been accrued (7 in each group). The one patient with partial response and the two with prolonged stability all had tumor origin from the excretory duct.

In retrospect, the pattern of *HER2* positivity suggests a higher frequency in malignancies of excretory duct origin than in those of intercalated duct origin: 55% (16 of 31) versus 7% (7 of 106). In fact, the one response to trastuzumab was in a tumor of excretory duct origin. This response is quite intriguing because of its long duration. An argument to further evaluate trastuzumab in this particular group can be made on the basis of the original statistical design of the trial and the screening data. Because of the rarity of recurrent salivary gland cancer, and especially tumors of excretory duct origin, which represented only 22% of the screened cases, a trial such as this will have low feasibility without collaboration among multiple large tertiary centers or through the intergroup mechanism.

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