Polymorphous Low Grade Adenocarcinoma

A Clinicopathologic Study of 164 Cases

James T. Castle, D.D.S., LCDR, DC, USN[1]
Lester D. R. Thompson, M.D., LCDR, MC, USNR[2]
R. Allen Frommelt, M.S.[3]
Bruce M. Wenig, M.D.[2]
Harvey P. Kessler, D.D.S.[4]

[1] Department of Oral and Maxillofacial Pathology, Armed Forces Institute of Pathology, Washington, DC.

[2] Department of Endocrine and Otorhinolaryngic-Head and Neck Pathology, Armed Forces Institute of Pathology, Washington, DC.

[3] Department of Repository and Research Services, Armed Forces Institute of Pathology, Washington, DC.

[4] Division of Oral and Maxillofacial Pathology and Oncology, College of Dentistry, University of Florida, Gainesville, Florida.

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Address for reprints: Lester D. R. Thompson, M.D., Department of Endocrine and Otorhinolaryngic-Head and Neck Pathology, Building 54, Room G066-11, Armed Forces Institute of Pathology, 6825 16th Street, N.W., Washington, DC 20306-6000.

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BACKGROUND. Polymorphous low grade adenocarcinomas (PLGA) are minor salivary gland neoplasms with a predilection for intraoral sites.

METHODS. One hundred sixty-four cases of PLGA diagnosed between 1970–1994 were retrieved from the files of the Armed Forces Institute of Pathology, Washington, DC. Histologic features were reviewed, immunohistochemical studies and prognostic markers were performed, and patient follow-up was obtained. The data were analyzed statistically.

RESULTS. The patients included 109 women and 55 men, ages 23–94 years (average, 57.6 years). The patients usually presented clinically with a palatal mass that ranged in size from 0.4–6 cm (average, 2.2 cm). The tumors were infiltrative and characterized by a polymorphous growth pattern, with individual tumors demonstrating multiple patterns, including solid, ductotubular, cribriform, trabecular, and single file growth. Neurotropism was identified frequently. The neoplastic cells were isomorphic with vesicular nuclei. Mitotic activity was inconspicuous. At an average of 115.4 months after presentation, approximately 97.6% of all patients were either alive or had died without evidence of recurrent disease after treatment with surgical excision only. Four patients had evidence of disease at last follow-up; three had died with evidence of tumor, and one patient was alive with tumor.

CONCLUSIONS. PLGA is a neoplasm of minor salivary gland origin that must be separated from adenoid cystic carcinoma and benign mixed tumor for therapeutic and prognostic considerations. Conservative but complete surgical excision is the treatment of choice for these slow-growing tumors with a low proliferation index; adjuvant therapy does not appear to alter the prognosis. ***Cancer* 1999;86:207–19.**

KEYWORDS: polymorphous low grade adenocarcinoma, prognosis, salivary gland carcinoma.

Polymorphous low grade adenocarcinomas (PLGA) are distinctive salivary gland neoplasms with an almost exclusive propensity to arise from minor salivary glands. Previously used terms for PLGA include lobular carcinoma and terminal duct carcinoma.[1,2] Although the frequency of the tumor is unknown, the awareness of PLGA as a distinct tumor has increased with the establishment of specific histopathologic criteria characterizing the tumor. The clinicopathologic features and immunophenotypic profile of PLGA have been outlined previously in the literature.[3–15] We undertook this study of 164 cases of PLGA, representing, to our knowledge, the largest single series of its kind to date (MEDLINE 1966–1998) to catalogue the clinicopathologic characteristics of this neoplasm in a single comprehensive study.

TABLE 1
Immunohistochemical Panel

Antibody	Primary antibody	Company	Dilution	Antigen unmasking
Cytokeratins AE1/AE3	Mm	Boehringer Mannheim Biochemicals (Indianapolis, IN) and	1:50	Protease treatment
CK1	Mm	Dako (Carpinteria, CA)	1:200	Protease treatment
CEA	Mm	Sanbio BV (Uden, Netherlands)	1:400	Protease treatment
MSA	Mm	Sigma ImmunoChemicals (St. Louis, MO)	1:8000	None
S-100 protein	—	—	1:800	None
GFAP	Rp	—	1:2000	Protease treatment
bcl-2 protein	Mm	Dako	1:20	Microwave pretreatment
p53	Mm	—	1:400	Microwave pretreatment
Ki-67 (MIB-1)	Mm	Immunotech (Westbrook, ME)	1:20	Microwave pretreatment

CEA: carcinoembryonic antigen; MSA: muscle specific antigen; GFAP: glial fibrillary acidic protein; Mm: mouse monoclonal; Rp: rabbit polyclonal.

MATERIALS AND METHODS

One hundred sixty-four cases of PLGA were selected for which adequate follow-up data were obtainable. The cases were retrieved from the files of the Oral and Maxillofacial and Otorhinolaryngic-Head and Neck Tumor Registries of the Armed Forces Institute of Pathology, Washington, DC, between 1970 and 1994. These 164 cases were selected from 172 cases identified as PLGA in a review of 11,236 (1.5%) benign or malignant primary salivary gland tumors seen in consultation during this period. However, we were unable to obtain complete follow-up information in 8 cases, which were excluded from the study. Cases diagnosed prior to 1984 had originally been coded as "adenocarcinoma, not otherwise specified (NOS)"; however, in review, all of these cases met the criteria for PLGA as defined previously.[1,2] One hundred thirty-eight cases were received from civilian sources, including university medical centers and foreign contributors, 19 cases were from military hospitals, and seven cases were from Veterans Administration medical centers.

Hematoxylin and eosin-stained slides from all cases were reviewed to confirm that established histopathologic criteria were met.[1,2] To date, there are no examples in the files of the Armed Forces Institute of Pathology (AFIP) of PLGA arising within major salivary glands.

Adequate follow-up was a prerequisite for inclusion in the study, as noted above. Materials within the files of the AFIP were supplemented by a review of the patients' demographics, symptoms at presentation, history of previous irradiation, surgical pathology and operative reports, cancer registry records, and written questionnaires or oral communication with the treating physician(s) or patient. Follow-up data included information regarding the location of the primary site, the specific treatment modalities used, and the current status of the disease and patient.

Immunophenotypic analysis was performed in 38 cases with suitable material. The standardized avidin-biotin method of Hsu et al.[16] was employed, using 4-μm- thick, formalin fixed, paraffin embedded sections. Table 1 documents the pertinent, commercially available immunohistochemical antibody panel chosen. Preparation for cytokeratin cocktail, p-carcinoembryonic antigen (p-CEA), and glial fibrillary acicic protein (GFAP) required predigestion for 3 minutes with 0.05% Protease VIII (Sigma Chemical Co., St. Louis, MO) in a 0.1 M phosphate buffer, pH 7.8, at 37°C. Antigen enhancement (recovery) was performed for bcl-2, Ki-67, and p53 by using formalin fixed, paraffin embedded tissue treated with buffered citric acid solution and heating for 20 minutes in a calibrated microwave oven. Appropriate standard positive and negative tissue controls were used throughout.

The antibody reactions were graded as weak (1+), moderate (2+) and strong (3+) staining. The fraction of positive cells was determined by separating the percentage of positive cells into four groups: $<$10%, 10–50%, 51–90%, and $>$90%.

Categorical variables were analyzed using chi-square tests to compare observed and expected frequency distributions. Comparison of means between groups were made with unpaired *t*-tests or one-way analysis of variance, depending on whether there were two groups or more than two groups, respectively. Multiple comparisons were analyzed using the Tukey method. Linear regression was used to investigate two measured variables, and Pearson correlation coefficients were generated to measure the strength of the association. Confidence intervals of 95% were generated for all positive findings. The alpha level was set at $P < 0.05$. All analyses were conducted using SPSS software (version 8.0 for PC; SPSS, Inc., Chicago, IL).

TABLE 2
Clinical Characteristics

Characteristic	Polymorphous low grade adenocarcinoma
Total number	164
Females	109
Males	55
Age (yrs)	
Range	23–94
Average	57.6
Females (average)	56.5
Males (average)	59.8
Duration of symptoms (mos)	
Range	0.1–480
Average	27
Mass	113
Slowly enlarging mass	17
Mass with pain and/or ulceration	13
Dentures not fitting	7
Asymptomatic	14

TABLE 3
Average Size and Average Duration of Symptoms by Anatomic Site

Anatomic site	No.	Size (cm)	Duration of symptoms (mos)
Alveolar ridge[a]	13	1.8	30
Buccal mucosa	16	2.3	36
Lip	22	1.3	32
Mucosal, NOS	7	1.4	6
Palate, NOS	52	2.6	37
Hard palate	26	2.3	18
Soft palate	28	1.6	11
Total	164	2.2	27

NOS: not otherwise specified.
[a] Alveolar mucosa or the lateral hard palate area as it curves into the alveolar ridge.

RESULTS

Clinical

A summary of the clinical information is provided in Table 2. The patients included 109 females and 55 males, with a female:male ratio of 2:1. Their ages ranged from 23 years to 94 years, with an average age at presentation of 57.6 years. The average age at presentation for females was slightly younger than males, at 56.5 years and 59.8 years, respectively, but the difference was not statistically significant (t = 1.421; P = 0.157). In decreasing order of frequency, the tumors occurred in the palate, NOS (n = 52; 32%); the soft palate (n = 28; 17%); the hard palate (n = 26; 16%); the lip (n = 22; 13%); the buccal mucosa (n = 16; 10%); the alveolar ridge (n = 13; 8%); and at mucosal sites, NOS (n = 7; 4%). One hundred forty-three patients (87%) presented clinically with a mass lesion, 17 of whom described a slowly enlarging mass and another 13 of whom presented with a mass accompanied by pain, bleeding, or ulceration. Seven patients complained of their "dentures not fitting," whereas the remaining 14 patients' lesions were discovered incidentally during routine dental examination. There was no statistically significant difference in the clinical presentation and the outcome of the patient, including the development of a recurrent tumor (chi-square = 4.446; P = 0.349). The duration of symptoms ranged from a few days to as long as 480 months, with an average duration of symptoms of 27 months. There was, on average, a much shorter duration of symptoms for patients with tumors of the intraoral/cheek mucosa (6 months), soft palate (11 months), or hard palate (18 months) compared with the average for all locations combined (27 months) or compared with the other sites within the oral cavity (Table 3), but this difference was not statistically significant (F = 0.987; P = 0.437).

Pathology

Macroscopic findings

The tumors involved only minor salivary gland sites, all within the oral cavity. Macroscopically, they were described as firm to solid, ovoid masses, typically lying in close proximity to the overlying surface epithelium, and were characteristically unencapsulated (Fig. 1A). The cut surface revealed light yellow to tan parenchyma, infrequently demonstrating central tumor necrosis.

The tumors ranged in size from 0.4–6 cm in greatest dimension, with an average of 2.2 cm (Table 3). There is a statistically significant difference between tumor size at initial presentation when comparing distinct anatomic locations (F = 3.308; P = 0.004). Specifically, tumors of the lip were significantly smaller than tumors located in the palate, with tumors of the palate measuring about 1.1 cm larger (±0.3 cm) than tumors of the lip. However, the ease of clinical visualization of a tumor of the lip versus one in the palate might account more accurately for this statistically significant difference rather than a difference in the overall biology of the tumor.

Microscopic findings

Characteristically, PLGAs are well circumscribed, but not encapsulated, tumors (Fig. 1A,B). They are seen to infiltrate into perisalivary gland adipose connective tissue, but true skeletal muscle invasion is uncommon. When present, skeletal muscle involvement usually presents as a compression of the muscle fibers. Infiltration into the adjacent salivary gland is quite

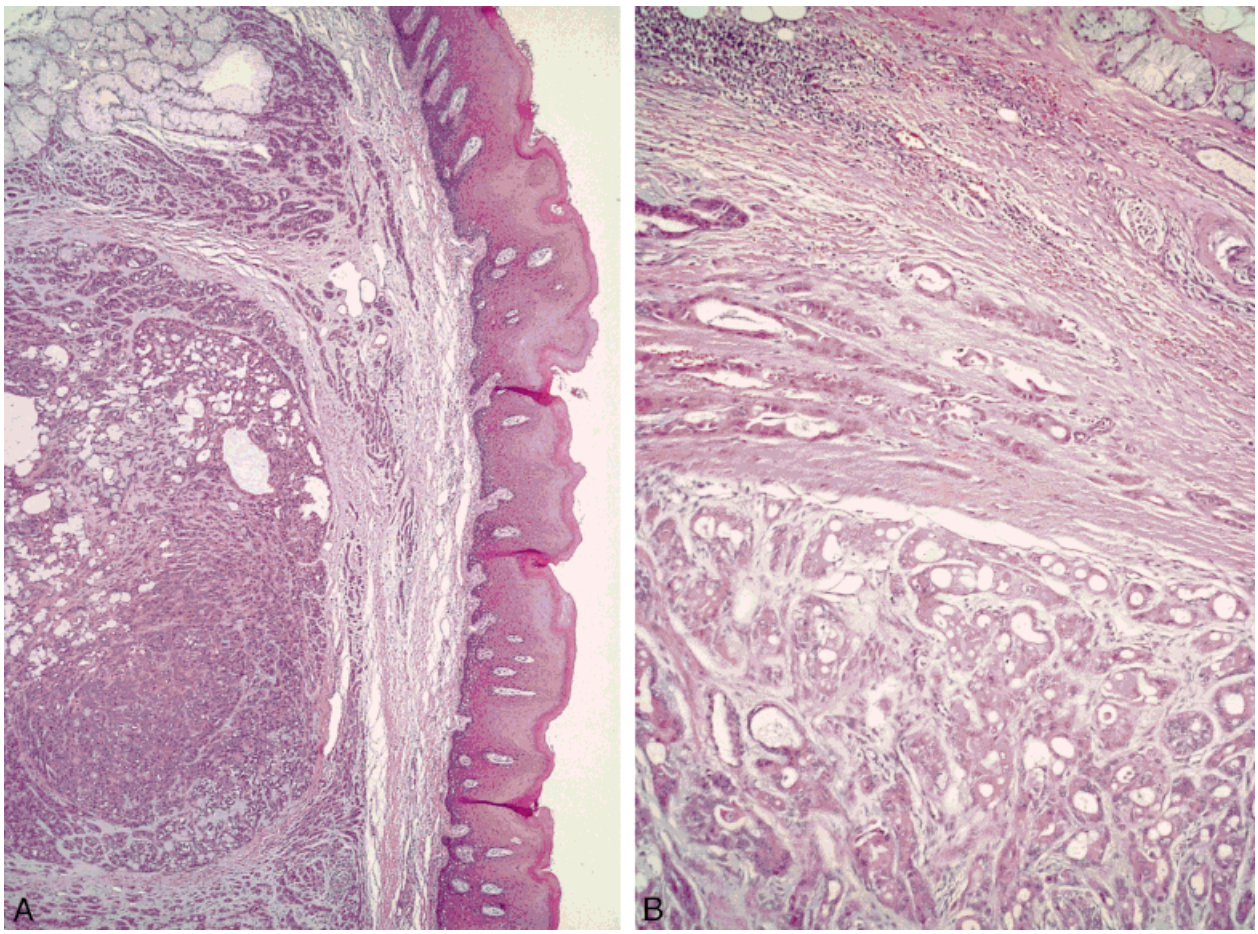

FIGURE 1. (A) The oral squamous mucosa is identified overlying the unencapsulated tumor growth. Residual minor salivary gland tissue is seen surrounded by tumor islands, although it is not infiltrated directly. (B) Fibrous connective tissue concentration about a polymorphous low grade adenocarcinoma with infiltrating tubular glands extending into the adjacent minor salivary gland parenchyma.

common, however. Tumor cells were noted invading into and separating the lobular units of the residual minor salivary gland parenchyma as well as wrapping around the acini, but usually not invading individual acini or duct structures (Fig. 1A). Intact, normal acini and ducts often can be identified completely surrounded by tumor. This may occur in the center of the neoplasm, but it is seen more commonly at the periphery of PLGA. The surface epithelium usually is intact but is ulcerated on occasion. When it is intact, the surface epithelium usually is not involved by tumor (Fig. 1A), although, in a few cases, the neoplastic infiltrate could be seen involving the surface epithelium.

PLGA may display a mixture of growth patterns within a single tumor, including solid islands; glandular profiles (Fig. 2A); tubules (Fig. 2B); trabeculae; cribriform nests (Fig. 2C); and linear, single-cell, "Indian-file" infiltration (Fig. 1B). Tubular areas were lined by one or two cell layers of cuboidal to columnar cells. There were no differences in the cell types found in these two layers. Tumor cells often were arranged concentrically around a central nidus, creating a targetoid appearance (Fig. 3A). The nidus often was found to be a small nerve bundle (neurotropism) and was quite characteristic for PLGA (Fig. 3B). Perineural invasion was identified in nearly all cases, although it was accentuated more frequently in the cases with the targetoid growth pattern. Papillary foci were seen rarely and, when present, represented only a minor component and never the dominant pattern.

The tumor cells were uniformly round to polygonal, of small to medium size, with indistinct cellular borders and with abundant pale to eosinophilic cytoplasm (Fig. 4A). The nuclei were particularly distinctive, being uniformly round to ovoid and containing open, vesicular nuclear chromatin and inconspicuous to small nucleoli (Fig. 4B). Nuclear pleomorphism was negligible, and mitotic figures were present occasionally, generally inconspicuous, and never numerous.

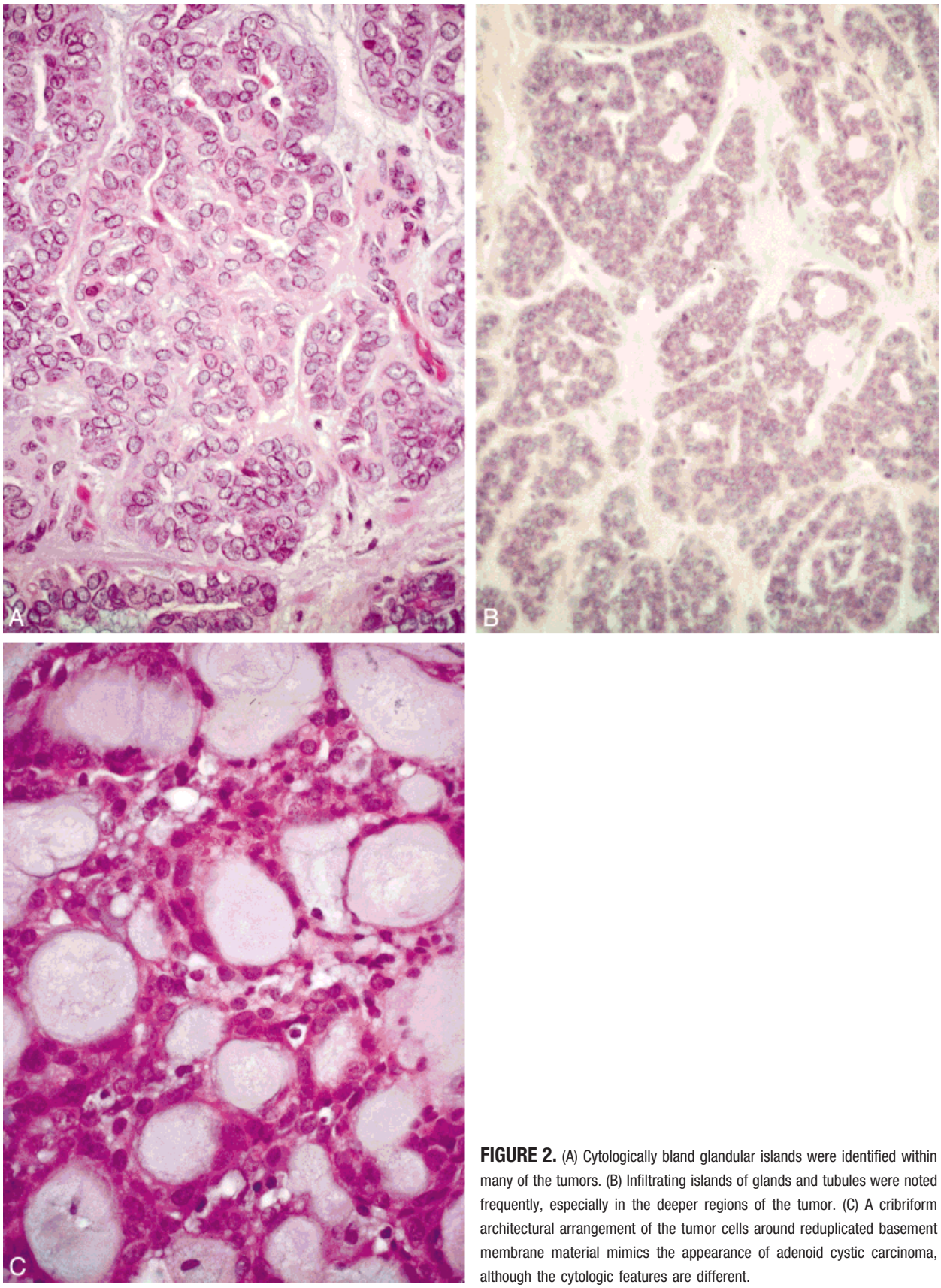

FIGURE 2. (A) Cytologically bland glandular islands were identified within many of the tumors. (B) Infiltrating islands of glands and tubules were noted frequently, especially in the deeper regions of the tumor. (C) A cribriform architectural arrangement of the tumor cells around reduplicated basement membrane material mimics the appearance of adenoid cystic carcinoma, although the cytologic features are different.

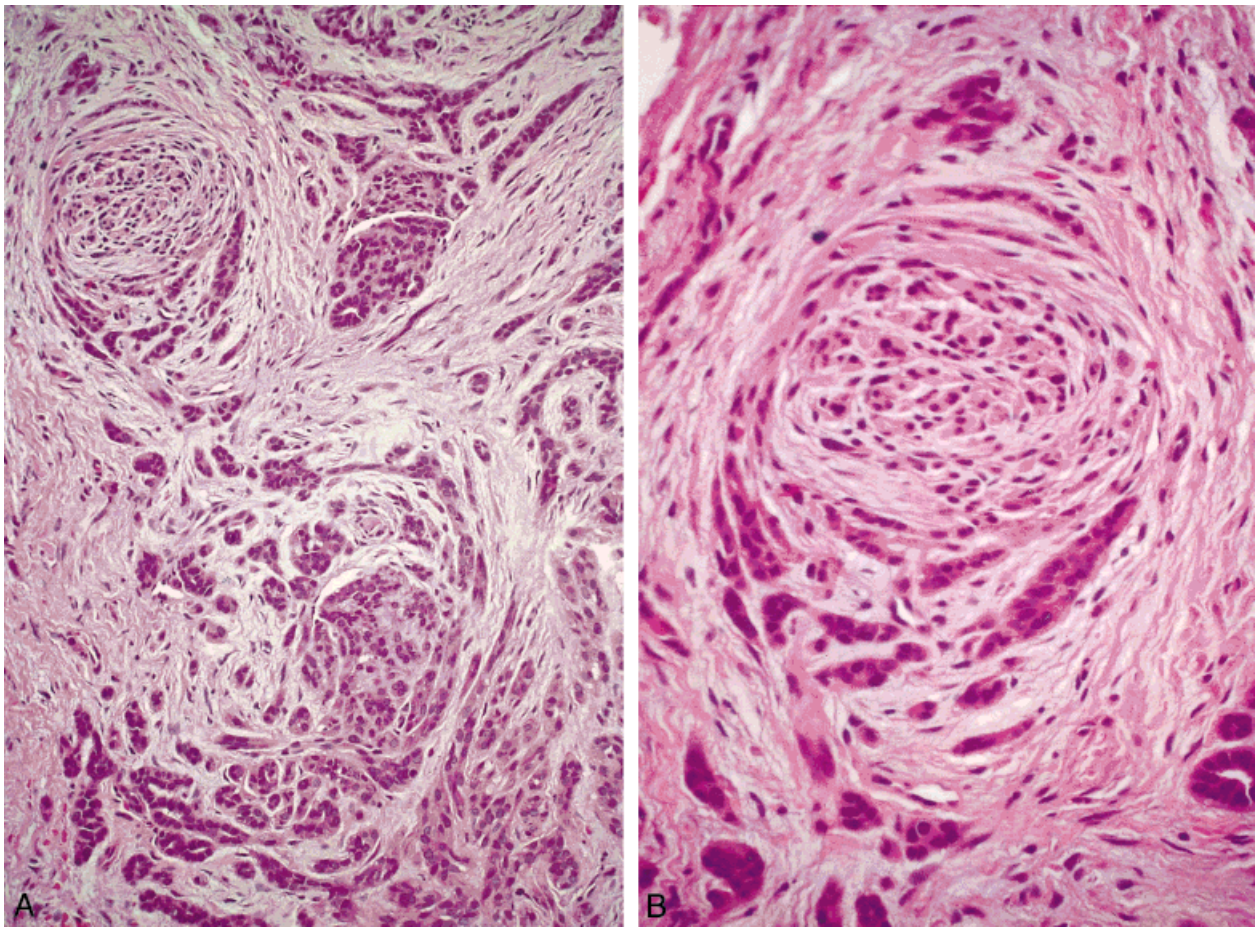

FIGURE 3. (A) Intermediate power demonstrating a central tumor nidus with concentrically arranged tumor cells in thin strands. Perineural invasion is identified (top). (B) High power photomicrograph of tumor cells infiltrating a peripheral nerve, creating a targetoid appearance.

Metaplastic changes, including squamous, sebaceous, or oncocytic alteration, were not identified in our tumors. Occasionally, a slight oxyphilia or granularity to the cytoplasm was noted and, rarely, developed the full character of oncocytic cells. Luminal crystalloids were not typical, although eosinophilic crystalline material was noted in glandular spaces in isolated cases. The tumor cells were surrounded by a hyalinized, slightly eosinophilic stroma that occasionally displayed myxoid degeneration (Fig. 5A). A characteristic slate gray-blue stroma also was encountered frequently (Fig. 5B). When identified, this background stroma was characteristic for PLGA but did mimic the mucoid-myxoid matrix of pleomorphic adenoma.

Immunohistochemical Results

The immunohistochemical results are listed in Table 4. Briefly, there was moderate to strong, diffuse immunoreactivity for cytokeratin, CEA, S-100 protein, and bcl-2. There was focal, weak reactivity with anti-smooth muscle actin (anti-SMA) and anti-GFAP. There was a remarkable variability in the intensity and percentage of cells that reacted with the proliferation markers Ki-67 and p53, although, in general, most of the cells demonstrated nuclear reactivity of a weak intensity.

Treatment and Follow-Up

Complete surgical excision was the treatment of choice (Table 5). Initially, 33 patients (20%) underwent incisional biopsy, whereas the remaining 131 patients (80%) underwent either an excisional biopsy or complete surgical excision. Two of the patients who had an incisional biopsy opted for no additional therapy. Patients treated with incisional or excisional biopsy were treated almost uniformly by subsequent wide local excision of the tumor once the diagnosis of PLGA was determined. Seventeen patients were treated with a combination of biopsy (incisional, excisional, or wide excision) followed by postoperative radiation therapy,

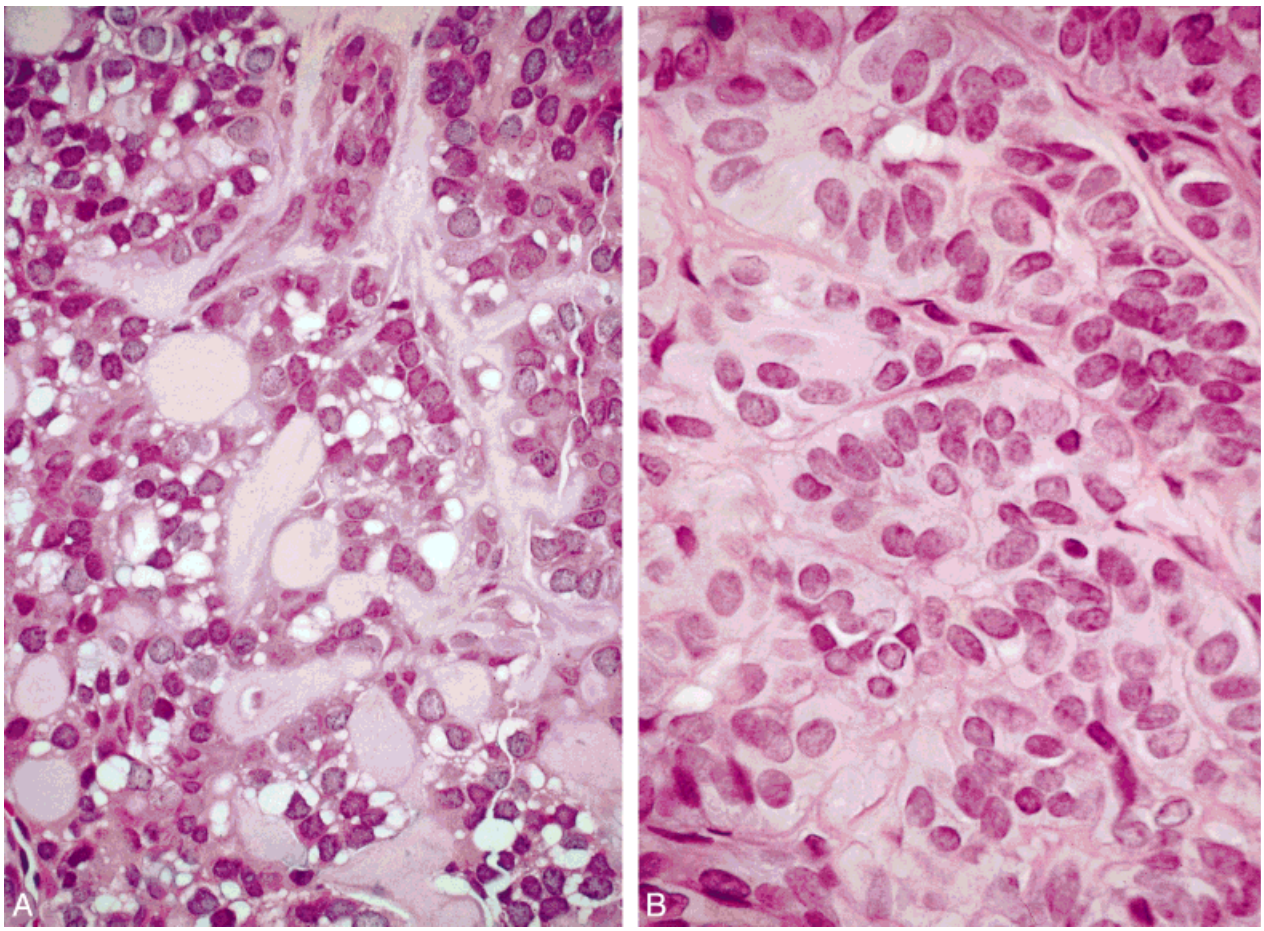

FIGURE 4. (A) Clear cytoplasm surrounded round to oval nuclei with open nuclear chromatin. Replicated basement membrane material also is present. (B) Intercellular borders are indistinct between these cells with eosinophilic cytoplasm. There is an even distribution to the finely granular to open nuclear chromatin. Nucleoli are inconspicuous.

whereas only a single patient received postoperative chemotherapy.

The treatment specific survival is tabulated in Table 5. There was no statistically significant difference in the overall patient outcome (average months of follow-up) based on the type of initial treatment given or for any additional treatment rendered (additional surgery, radiation therapy, chemotherapy) ($F = 1.767$; $P = 0.174$). Adjuvant radiation therapy did not alter survival ($t = 0.362$; $P = 0.718$). In fact, it seems that patients who were treated with radiation therapy were more likely to have evidence of disease at last follow-up when compared with patients who did not have radiation therapy (chi-square = 6.390; $P = 0.011$). We do not have an explanation for this finding.

The overall survival for PLGA was excellent (Table 6). One hundred sixty patients (97.6%) were alive or had died of unrelated causes without evidence of disease, with an average follow-up of 115.4 months (range, 3.0–428.8 months). One patient is still alive with evidence of disease 97.1 months after initial biopsy. This patient refused additional surgery and, instead, received radiotherapy (60 CG) to the head and neck region with subsequent adjuvant chemotherapy. The three remaining patients died with disease, with an average survival of 71.5 months (range, 19.6–131.1 months). The first patient was treated by biopsy only of the hard palate tumor, without any additional therapy, and died of complications of chronic obstructive pulmonary disease and hypertension 19.6 months after the initial biopsy of the palatal tumor. Tumor was still present at the time of death but, presumably, was not the cause of death. The second patient died with disease 63.8 months after the initial surgery. He had suffered two local recurrences, each of which was treated by wide surgical excision without adjuvant therapy. At the time of death, he was reported to have local disease in the oral cavity and "throat." The third

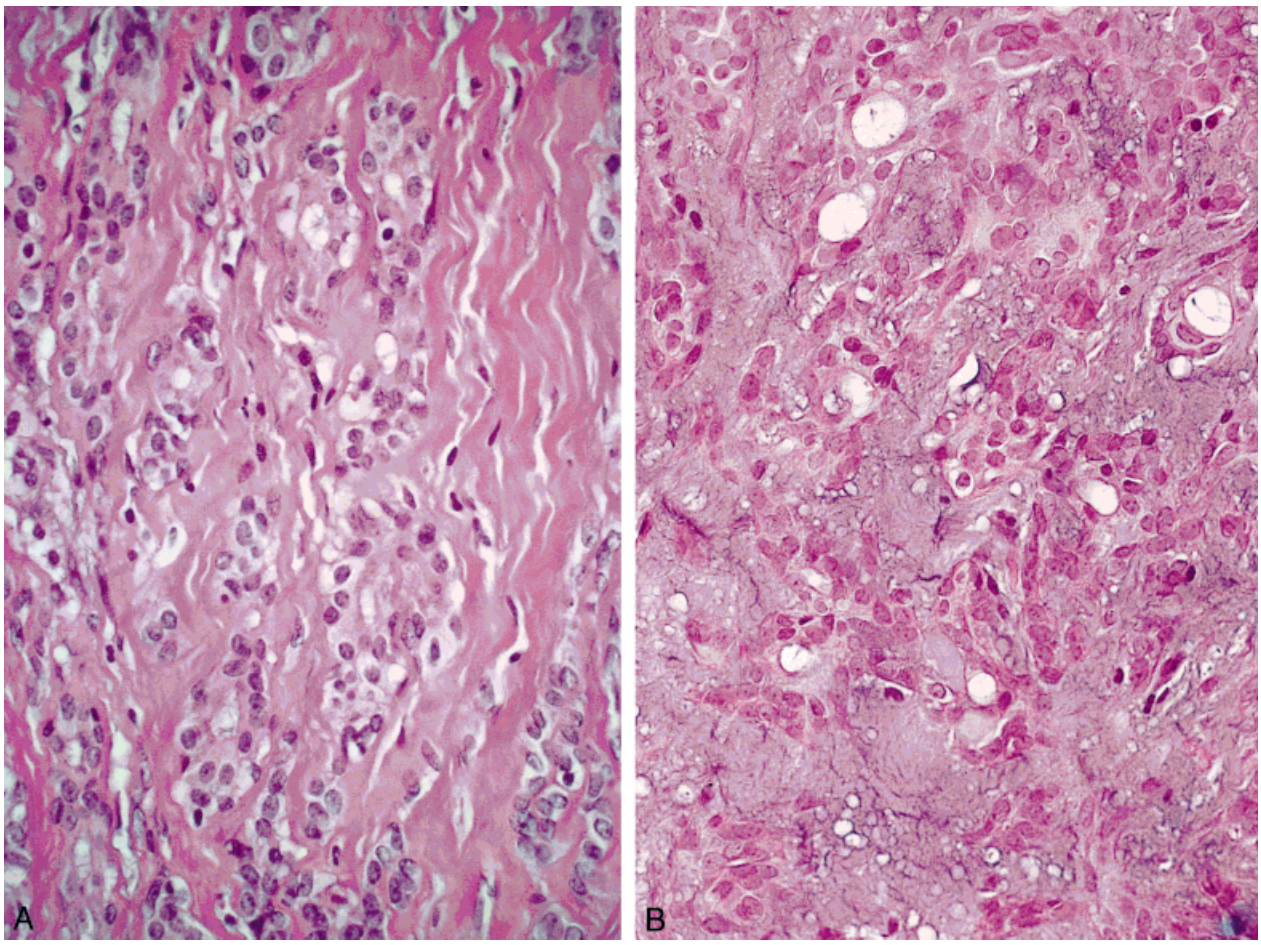

FIGURE 5. (A) Tumor cells are identified within a dense collagenized stroma. (B) A slate gray-blue myxoid change in the background stroma was an almost constant feature for polymorphous low grade adenocarcinoma. Residual entrapped salivary gland acini are observed.

patient developed a local recurrence 4 years after the initial surgery. The recurrence was treated with wide surgical excision and radiation therapy (60 centigrays). He died with locally recurrent disease of the palate with oral cavity extension and lung metastases 131.1 months after the initial excision. The pulmonary material was not available for our review.

The patient outcome enumerated by anatomic site of occurrence is listed in Table 7. Statistically, tumors localized to the hard palate were significantly more likely to be associated with tumor recurrence/persistence or patient death (chi-square = 4.521; P = 0.033). Patients with tumors described as occurring on the "hard palate" or "palate, NOS" were 1.6 times more likely to have evidence of disease at last follow-up compared with all other anatomic locations. The patients with tumors in all of the other anatomic sites of involvement, including the soft palate, were either alive or had died of unrelated causes, without evidence of disease. Patients with mucosal tumors, NOS, had a slightly shorter average follow-up (95.5 months), but this was not statistically different from the rest of the anatomic sites. Overall, there is insufficient evidence to support the conclusion that the overall mean survival is different based on anatomic site alone (F = 0.408; P = 0.873), even though there is a difference in recurrence rate.

There were 17 patients in the study group who had recurrent or persistent tumor. Of the 15 patients who developed recurrent tumors (excluding the 2 patients who refused additional surgery and are therefore considered as having persistent tumor), the range of years to discovery of the recurrent tumor was 2–14 years, with an average of 7.2 years. Women were 1.6 times more likely to develop a recurrence than men (chi-square = 5.00; P = 0.025). There was no difference in age at initial presentation between the patients who developed recurrence (57.5 years) and those who did not (57.7 years) (t = 0.380; P = 0.704). Furthermore, there was no difference in mean age at

presentation between males and females who did and did not develop recurrence (F = 1.606; $P = 0.175$).

Table 8 details the sites of recurrence coupled with patient outcome. Of the 15 patients with recurrent tumor, 9 developed one recurrence each. Of these 9 patients, 8 were alive or had died without evidence of disease after treatment of the recurrence, with an average follow-up of 161.4 months. One patient died with disease despite a complete surgical excision and radiation therapy 131.1 months after initial presentation. Six patients developed two recurrences each. Of these 6 patients, 5 were alive or had died without evidence of disease after successful treatment of the second recurrence, with an average follow-up of 120.2 months. The remaining patient died with disease 63.8 months after initial presentation, without any adjuvant therapy. The type of therapy used for the patients with recurrent disease (Table 9) did not seem to influence the final outcome, as suggested by the treatment regimens discussed above. There is evidence to support the conclusion that the size of the tumor at initial clinical presentation is related to the average survival (i.e., follow-up interval) (F = 4.169; $P = 0.043$). However, an adjusted coefficient of determination ($r^2 = 0.020$) would indicate that the size of the tumor accounts for only 2% of the variance in survival. Therefore, the clinical relevance of this difference is limited. In addition, there is no evidence to support the possibility that there is a difference in mean size of the tumor at initial presentation between those patients with and without evidence of disease at last follow-up (t = 1.131; $P = 0.260$) or between those patients with or without the development of recurrent disease (F = 2.042; $P = 0.11$).

In summary, two patients who underwent incisional biopsy refused any additional surgery and, thus, had inadequate excision resulting in persistent tumor. One of these two patients did undergo radiation and chemotherapy, however, and is still alive but has evidence of tumor 97.1 months after initial presentation. The other patient died with tumor 19.6 months after refusing any supplemental therapy. Death was due to the effects of chronic obstructive pulmonary disease and hypertension. Two additional patients died with disease present. Both had locally recurrent lesions, one with extension into the nasopharynx. In addition to locally recurrent disease, the other patient developed biopsy-proven pulmonary metastases.

DISCUSSION

Since the original description of these tumors, PLGA has been recognized as a distinct salivary gland tumor that has a predilection to occur in the minor salivary glands and is associated with slow growth and indolent biology. The clinicopathologic features of the cases in the present series generally match those reported previously in other series.[1–3,5,17–26] PLGA occurs over a wide age range but does not seem to occur in the first or second decades of life. There is a nearly 2:1 female to male ratio for the patients in our series. PLGA in women tends to occur at a slightly younger age (56.6 years) compared with male patients (59.7 years), but there is no statistically significant difference.

The typical clinical presentation of PLGA is that of an asymptomatic mass located within the oral cavity. Clinical symptomatology ranged in duration from a few days to 40 years, with an average length of symptoms of 27 months. Patients who presented with pain, bleeding, or ulceration did not have more aggressive disease nor were they more prone to develop recurrences (chi-square = 4.446; $P = 0.349$). There was no statistically significant difference in the mean duration of symptoms between each of the anatomic sites of involvement (F = 0.987; $P = 0.437$), even though the "mucosal lesions, not further specified" and "soft palate lesions" tended to have a shorter mean duration of symptoms (6.42 months and 10.65 months, respectively) versus the overall average (27 months).

The tumor size varied from 0.4 cm to 6.0 cm. Tumors located on the lip were statistically significantly smaller than those of the other anatomic sites (F = 3.308; $P = 0.004$). An obvious explanation for this size differential is that lip lesions are identified more readily by the patient and are more accessible to clinical examination.

The overall pathologic features in our cases are similar to those reported in the literature.[1–3,27] The most common patterns of growth included tubular, trabecular, solid, and cribriform patterns, with a focal papillary pattern identified less frequently. Whether or not a tumor with a papillary growth pattern should be included within the spectrum of PLGA or should be classified separately as papillary cystadenocarcinomas has been debated in the literature.[21,25,26,28–30] Perhaps the only contentious issue related to PLGA is whether these tumors can be subclassified into nonpapillary and papillary types. It is our experience that there is not a papillary subtype of PLGA. Although limited foci of papillary growth could occur, no single tumor demonstrated a predominantly papillary pattern. Furthermore, the cytologic features in the foci of papillary growth were similar to the nonpapillary foci. A true papillary cystadenocarcinoma portends a more aggressive clinical course with lymph node metastases and a higher frequency of local recurrence.[3,5,17,21,26,28,31,32] This biologic behavior is distinctly contrary to that of PLGA.

TABLE 4
Immunohistochemical Panel Results

Antibody	No. of cases with positive reactions (%)	No. of cases positive by percentage of cells (%)	No. of cases positive by intensity of reaction[a]
Cytokeratin (AE1/AE3 and CK1)	39 of 39 (100)	39 (>90)	39 (3+)
		5 (51–90)	8 (1+)
CEA	21 of 39 (53.8)	4 (10–50)	11 (2+)
		12 (<10)	2 (3+)
			4 (1+)
SMA	5 of 39 (12.8)	5 (<10)	1 (2+)
		19 (>90)	2 (1+)
		17 (51–90)	
S-100 protein	38 of 39 (97.4)	1 (10–50)	20 (2+)
		1 (<10)	16 (3+)
GFAP	6 of 39 (15.4)	6 (<10)	5 (1+)
		11 (>90)	1 (2+)
		19 (51–90)	9 (1+)
Bcl-2 protein	39 of 39 (100)	6 (10–50)	26 (2+)
		3 (<10)	4 (3+)
		6 (51–90)	31 (1+)
p53	37 of 39 (94.9)	22 (10–50)	6 (2+)
		9 (<10)	11 (1+)
		1 (51–90)	
Ki-67 (MIB-1)	29 of 39 (74.4)	6 (10–50)	13 (2+)
		22 (<10)	5 (3+)

CEA: carcinoembryonic antigen; SMA: smooth muscle actin; GFAP: glial fibrillary acidic protein.
[a] 1+ = weak; 2+ = moderate; 3+ = strong.

TABLE 5
Treatment Regimen and Clinical Outcome

	No. of patients (Average months of follow-up)			
Treatment	DWD (n = 3)	AWD (n = 1)	Dead, NED (n = 36)	Alive, NED (n = 124)
Initial incisional biopsy only (n = 2)	1 (19.6)	0	1 (81.2)	0
Initial wide surgical excision (n = 72)	0	0	3 (30.3)	69 (129.9)
Complete surgical excision (n = 72)[a]	1 (63.8)	0	31 (97.8)	40 (109.0)
Surgery followed by radiation/ chemotherapy (n = 18)	1 (131.1)	1 (97.1)	1 (217.5)	15 (115.0)

DWD: dead with disease; AWD: alive with disease; NED: no evidence of disease.
[a] Patients received incisional or excisional biopsy followed in 4 weeks by wide surgical excision.

TABLE 6
Overall Patient Outcome: Average Months of Follow-Up

Measure	DD	AWD	Dead, NED	Alive, NED
No. of patients	3	1	36	124
Average follow-up	71.5	97.1	95.0	121.4
Follow-up range	19.6–131.1	97.1	3.0–366.1	39.6–428.8

DD: dead with disease; AWD: alive with disease; NED: no evidence of disease.

With PLGA demonstrating a wide variety of growth patterns, the application of immunohistochemical studies to further assist in the differential diagnosis of the tumor has been reported, with variable results.[3–15] In general, our overall results are similar to those found in the literature, including immunoreactivity of the tumor cells with cytokeratin, glial fibrillary acidic protein (GFAP), S100 protein, bcl2, actin, p53, and Ki-67 (MIB1) (Table 4).[3–7,9,13,32] However, the number of cases with reactive cells, the percentage of reactive cells, and the intensity of the reactivity vary by report. The findings of GFAP, S100, and SMA immunoreactivity can lend support to the diagnosis of PLGA, but they are not specific, because these markers frequently are identified in other salivary gland neoplasms, particularly in benign mixed tumor (pleomorphic adenoma).[33–36]

Overall, there was relatively weak staining with the proliferation markers p53 and Ki-67 (MIB1) (Table 4). In general, although nearly all of our cases demonstrated some degree of positive reactivity, usually,

TABLE 7
Site Specific Patient Outcome: Patient Number and Average Months of Follow-Up

Anatomic site	No.	DD	AWD	Dead, NED	Alive, NED
Alveolar ridge	13	0	0	4 (57.1)	9 (115.4)
Buccal mucosa	16	0	0	4 (92.8)	12 (118.3)
Lip	22	0	0	6 (80.2)	16 (130.4)
Mucosa, NOS	7	0	0	1 (13.7)	6 (95.5)
Palate, NOS	52	2 (75.3)	1 (97.1)	9 (91.6)	40 (132.3)
Hard palate	26	1 (63.8)	0	6 (109.6)	19 (115.4)
Soft palate	28	0	0	6 (140.7)	22 (111.1)

DD: dead with disease; AWD: alive with disease; NED: no evidence of disease; NOS: not otherwise specified. Numbers in parentheses indicate average months of follow-up.

TABLE 8
Recurrence and Patient Outcome Based on Anatomic Site

Anatomic site	No.	DWD (n = 3)	AWD (n = 1)	Dead, NED (n = 4)	Alive, NED (n = 9)
Alveolar ridge	1	—	—	—	1 (207.8)
Buccal mucosa	1	—	—	—	1 (250.0)
Hard palate	3	1 (63.8)	—	—	2 (193.6)
Lip	2	—	—	2 (128.3)	—
Mucosa, NOS	2	—	—	1 (13.7)	1 (135.4)
Palate, NOS	4	2 (75.3)	1 (97.1)	—	1 (296.0)
Soft palate	4	—	—	1 (199.4)	3 (187.4)

DD: dead with disease; AWD: alive with disease; NED: no evidence of disease; NOS: not otherwise specified. Numbers in parentheses indicate average months of follow-up.

TABLE 9
Treatment Regimen and Clinical Outcome for the 17 Patients with Recurrent/Persistent Disease

	No. of patients (Average months follow-up)			
Treatment	DWD	AWD	Dead, NED	Alive, NED
Total	3	1	4	9
Initial incisional biopsy only (n = 1)	1 (19.6)	0	0	0
Complete surgical excision (n = 12)	1 (63.8)	0	3 (84.0)	8 (203.9)
Surgery followed by radiation/ chemotherapy (n = 4)	1 (131.1)	1 (97.1)	1 (217.5)	1 (207.8)

DWD: dead with disease; AWD: alive with disease; NED: no evidence of disease.
[a] Patients received incisional or excisional biopsy followed in 4 weeks by wide surgical excision.

<50% of the cells were reactive with a weak intensity (+1). This supports the hypothesis of low overall proliferation activity.

In general, the diagnosis of PLGA is not difficult. However, diagnostic difficulties due to histopathologic overlap may occur with mixed tumor (pleomorphic adenoma) and adenoid cystic carcinoma (ACC). These diagnostic difficulties often occur during frozen section examination or when the biopsy is small. The cytomorphologic features can be quite similar, thus rendering a definitive diagnosis of PLGA of a small biopsy at the time of frozen section virtually impossible. Furthermore, because mixed tumors of minor salivary glands most often are unencapsulated, differentiation from PLGA based on that feature is not reliable. The distinction between PLGA and pleomorphic adenoma usually can be made by identifying the presence of infiltrative growth, especially when combined with the presence of neurotropism.

ACC can mimic the growth patterns identified in PLGA, especially the proclivity for perineural invasion. However, in contrast to PLGA, the cells in ACC tend to be smaller, with hyperchromatic nuclei, less cytoplasm, a higher nuclear-to-cytoplasmic ratio, and coarser nuclear chromatin. The differences in nuclear morphology are particularly striking and are nearly pathognomonic. According to Vargas et al.,[13] the immunohistochemical profile for ACC demonstrated positivity far above that of PLGA for Ki-67, p53, and

BCL-2. In addition, S100 protein was found to be quite weak in ACC relative to PLGA.

This is the first study of a large cohort of patients to analyze patient outcome with a long follow-up period. We demonstrated a recurrence rate of 9.1%, significantly lower than other reports in the literature.[3,5,9,17,20,25,26,29] Our series does not include any patients with evidence of discrete cervical lymph node metastases. However, it is interesting to note that two patients did die from the effects of PLGA (biopsy-proven pulmonary metastases and local extension of the tumor to involve vital structures). There have been previous reports in the literature of distant metastasis of PLGA other than to cervical lymph nodes.[18,38] The long average follow-up in our series supports the previous assertion that patients need to be followed for more than 5 years.[25] Patients in our series developed recurrences from 2–14 years after the initial presentation, with an average 7.2 years to discovery of a recurrence. This finding suggests that yearly patient assessment for recurrent tumor is prudent for the rest of the patient's life. Furthermore, female patients tend to have a higher incidence of recurrence, and, perhaps, should be followed more closely.

In conclusion, in our experience, PLGA occurs only in the minor salivary glands, more frequently in female than in male patients, presenting clinically as a mass lesion, occasionally accompanied by other symptoms, and present for over 2 years on average. The palate is the most frequent anatomic site of occurrence, with an average tumor size of 2.2 cm. The tumors have an infiltrative periphery and usually demonstrate a characteristic perineural targetoid ("onion-skinning") pattern of growth. The tumor cells are arranged in solid to single-file configuration, demonstrating ample cytoplasm surrounding large, ovoid vesicular nuclei. Mitotic figures are inconspicuous. A background myxoid-mucoid matrix exhibits a slate-gray coloration that, when it is present, is characteristic for this neoplasm. Complete surgical excision is appropriate therapy for these patients, with an excellent long term prognosis. Local recurrences may manifest themselves after a long period (average, 7.2 years) but usually are treated adequately with surgical excision. Long term clinical follow-up is suggested.

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