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White Adipose Tissue Inflammation and Cancer Specific Survival in Patients with Squamous Cell Carcinoma of the Oral Tongue

Neil M. Iyengar, MD^{1,2}, Ronald A. Ghossein, MD³, Luc G. Morris, MD, MSc⁴, Xi K. Zhou, PhD⁵, Amit Kochhar, MD², Patrick G. Morris, MD¹, David G. Pfister, MD^{1,2}, Snehal G. Patel, MD⁴, Jay O. Boyle, MD⁴, Clifford A. Hudis, MD^{1,2}, and Andrew J. Dannenberg, MD²

¹Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.

²Department of Medicine, Weill Cornell Medical College, New York, NY.

³Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY.

⁴Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY.

⁵Department of Healthcare Policy and Research, Weill Cornell Medical College, New York, NY.

Abstract

Background—Obesity is associated with increased adipose tissue in the tongue. Chronic white adipose tissue (WAT) inflammation commonly occurs in the obese. We investigated whether WAT inflammation in the tongue impacts survival in patients with squamous cell carcinoma (SCC) of the oral tongue.

Methods—In a retrospective cohort study, patients with T1–T2 oral tongue SCC who underwent curative-intent resection were included. Tongue WAT inflammation was defined by the presence of dead or dying adipocytes surrounded by macrophages forming crown-like structures. The primary and secondary endpoints were disease-specific survival (DSS) and overall survival (OS), respectively. Subgroup analyses were carried out in patients without lymph node involvement for whom adjuvant therapies were not indicated.

Corresponding Author: Andrew J. Dannenberg, Department of Medicine, Weill Cornell Medical College, 525 East 68th St, Room F-206, New York, NY 10065. Phone 212-746-4403; Fax 212-746-4885; ajdannenberg@med.cornell.edu.

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Author Contributions:

N.M. Iyengar: Study conceptualization, methodology development, data collection, data curation, manuscript preparation, project administration

R.A. Ghossein: Methodology development, data collection, manuscript preparation

L.G. Morris: Study conceptualization, data collection, manuscript preparation

X.K. Zhou: Study conceptualization, formal analysis, manuscript preparation

A. Kochhar: Data collection, data curation, manuscript preparation

P.G. Morris: Study conceptualization, data collection, manuscript preparation

D.G. Pfister: Study conceptualization, manuscript preparation

S.G. Patel: Study conceptualization, provision of surgical specimens, manuscript preparation

J.O. Boyle: Study conceptualization, provision of surgical specimens, manuscript preparation

C.A. Hudis: Study conceptualization, methodology development, manuscript preparation

A.J. Dannenberg: Study conceptualization, methodology development, manuscript preparation, research oversight

Results—Archived tissue was available from 125 patients. Median follow-up was 55 (range 3 to 156) months. Overall, 49/125 (39%) patients had tongue WAT inflammation which was associated with higher body mass index (BMI), increased tumor thickness, and vascular invasion ($P<.05$). The 3-year DSS rate for patients with tongue WAT inflammation was 59% (95% CI 46% to 76%) versus 82% (95% CI 73% to 92%) for those without inflammation. For patients without lymph node involvement in whom adjuvant therapy was not indicated ($N=70$), tongue WAT inflammation was associated with shortened DSS and OS ($P<.05$). When adjusted for BMI and potential prognostic covariates, the HRs for DSS and OS were 5.40 (95% CI 1.20 to 24.26) and 2.97 (95% CI 1.02 to 8.65), respectively.

Conclusions—Tongue WAT inflammation is associated with worse cancer specific and overall survival for patients with early stage oral tongue SCC.

Précis

In patients with squamous cell carcinoma of the oral tongue, inflammation of tongue adipose tissue was an independent predictor of worse disease specific survival. In patients without lymph node involvement who are currently classified as having low risk disease, tongue adipose tissue inflammation correlated with shortened disease specific and overall survival.

Keywords

adipose; inflammation; obesity; tongue cancer; body mass index; crown-like structures

INTRODUCTION

Squamous cell carcinoma (SCC) of the oral tongue is the most common oral cavity cancer in the US and Europe.^{1, 2} While lymph node metastasis remains the most important predictor of survival,³ several tumor pathologic features including size, thickness, and vascular invasion are also independent prognostic factors.⁴ Unlike oropharyngeal SCC, oral tongue SCC is rarely associated with infection by carcinogenic strains of the human papilloma virus (HPV).^{5–8} Despite technical improvements in surgical, radiation, and medical management over the past several decades, survival rates have remained unchanged with approximately 50% disease specific survival at 5 years.^{2, 9–11} Identification of patients at increased risk of recurrence and disease specific mortality is a clinical priority because it may facilitate the development of new therapeutic strategies.

We recently reported that obesity is an independent adverse prognostic factor for patients with early stage oral tongue SCC.¹² Specifically, in a retrospective cohort study which included 155 patients, a body mass index (BMI) of 30 kg/m² or greater was associated with a five-fold increase in the risk of death from oral tongue SCC. The development of targeted intervention strategies to improve outcomes in obese patients with cancer has been limited by an incomplete understanding of the biologic mechanisms underlying the obesity-cancer relationship. Elucidating these mechanisms to enable more effective intervention takes on greater urgency given the rising incidence of obesity worldwide.¹³

Chronic inflammation is associated with the development and progression of several common epithelial malignancies.^{14–20} Subclinical adipose tissue inflammation is present in

most obese individuals.^{20, 21} Inflammation of white adipose tissue (WAT) can be detected histologically by the presence of crown-like structures (CLS), which are comprised of a dead or dying adipocyte surrounded by macrophages.²⁰ It is important to distinguish CLS macrophages from tumor-associated macrophages (TAMs), however. This distinction can be made histologically given that CLS macrophages surround an adipocyte. While the presence of TAMs has been associated with later stage disease and tumor invasion in patients with oral cancers,^{22, 23} the impact of CLS macrophages and adipose inflammation on oral tongue SCC prognosis is unknown.

Crown-like structures in visceral and subcutaneous WAT have been associated with increased levels of circulating proinflammatory mediators such as interleukin-6 (IL-6) and others.^{18–20, 24} Importantly, elevated circulating IL-6 levels are associated with increased recurrence and reduced survival in patients with head and neck cancers.²⁵ These findings are particularly relevant to the tongue as obesity is associated with increased adipose tissue in this organ.²⁶ Previously, we reported that WAT inflammation in the breast is present in most obese individuals, is associated with the metabolic syndrome, and is independently associated with shortened distant recurrence free survival in patients with breast cancer.^{24, 27–29} Collectively, these findings led us to hypothesize that WAT inflammation, via local and/or systemic effects, could similarly impact cancer-specific outcomes for patients with oral tongue SCC. To address this hypothesis, we investigated the impact of tongue WAT inflammation on disease specific survival (DSS) and overall survival (OS). In addition, we explored whether tongue WAT inflammation impacted DSS and OS in a subgroup of patients with lymph node negative disease for whom adjuvant therapies are not currently indicated due to a presumed low probability of recurrence and unwarranted treatment-associated toxicity.

PATIENTS AND METHODS

Patients and Study Design

Patients were identified from the prospectively maintained Oral Cancer Clinical Database at Memorial Sloan Kettering Cancer Center (MSKCC), which registers all patients undergoing surgery at MSKCC. We previously reported findings from this cohort which included 155 patients.¹² Eligible cases were adult patients with pathologically confirmed SCC of the oral tongue who underwent curative-intent resection and neck dissection at MSKCC between January 1, 2000 and December 31, 2009. Inclusion was limited to patients with tumor stage (T) T1 or T2 (tumor less than 4 cm in greatest dimension). Cases prior to 2009 were selected to ensure adequate follow-up. From this cohort of 155 patients, archived tongue and neck tissues were available from 125 patients who were included in the current study (Fig. 1). This single-institution study was approved by the Institutional Review Board of MSKCC under a waiver.

Collection of Clinical Data and Outcomes

Demographic and clinicopathologic data were abstracted from the electronic medical record (EMR). Height (m) and weight (kg) recorded on the day of surgery were used to calculate BMI as kg/m². BMI was categorized by standard definitions as under- or normal weight

(BMI < 25.0 kg/m²), overweight (BMI 25.0 to 29.9 kg/m²), or obese (BMI ≥ 30 kg/m²). Tobacco smoking was self-reported and categorized as patients who never smoked (never smokers), quit smoking at any time prior to diagnosis (former smokers), or smoked at the time of diagnosis (current smokers). Similarly, alcohol consumption was self-reported and categorized as never, former, or current users. Tumor (T) and nodal (N) staging were based on the American Joint Committee on Cancer stage of disease classification. Use of adjuvant chemotherapy and/or radiation, date and location of recurrence, and date and cause of death were obtained from the EMR. Vital status data in the EMR is linked with state and national death certificate registries and the Social Security Death Index. If alive, last follow-up date was recorded. The primary endpoint of this study was DSS. Consistent with prior reports, DSS was defined as time from surgery to death as a result of oral tongue SCC.^{12, 30, 31} The secondary endpoint, OS, was defined as time from surgery to all-cause mortality. All data entry was reviewed twice for accuracy independently by 2 physicians (NI, PM) and independent data review was carried out for quality assurance. Specifically, recorded outcomes data were randomly audited for accuracy (NI) and conflicting or ambiguous medical record data were adjudicated by review with a third physician with expertise in head and neck cancer (LM).

Detection of WAT Inflammation

Tongue WAT inflammatory status was categorized as inflamed or non-inflamed according to the presence or absence of CLS in tongue WAT (CLS-T). Representative hematoxylin and eosin (H&E) stained sections were reviewed by the study pathologist (RG), who was blinded to patient outcomes, and one formalin-fixed paraffin-embedded (FFPE) tissue block containing the most WAT from both the glossectomy and neck dissection specimens were selected for analyses. Five sections were generated from each block at 50 µm intervals (5 µm thick and approximately 2 cm in diameter). All sections were immunostained for CD68, a macrophage marker (mouse monoclonal KP1 antibody; Dako; dilution 1:4,000). The stained tongue sections were examined under light microscopy to detect and record the presence or absence of CLS-T. Using the same approach, peri-lymph node neck WAT was also examined for the presence of CLS of the neck (CLS-N). To assess the proliferative state of macrophages within CLS, immunohistochemistry for Ki67 was carried out using MIB-1 antibody (Rabbit monoclonal antibody 30-9; Ventana; dilution 1: 200). Dual immunostaining was then carried out using the diaminobenzidine (DAB) yellow chromogen for MIB-1 and red chromogen (ultra-red) for CD68.

Quantification of Tumor Associated Macrophages

To differentiate tongue WAT inflammation from tumor associated macrophages (TAMs), the anti-CD68 stained tongue sections described above were also examined to quantify the number of TAMs. Macrophages exhibiting cytoplasmic or membranous staining and not forming a CLS were counted as TAMs. The fields with the greatest concentration of TAMs were selected and macrophage density was determined by counting TAMs in 5 contiguous high power fields (HPFs) at 400x objective using an Olympus microscope (U-DO model BX-40; Olympus America Inc., Melville, NY). Average number of TAMs per HPF was recorded.

Biostatistical Design

The primary objective was to examine the association between DSS and tongue WAT inflammation defined by the presence or absence of CLS-T. Data were censored at the date of death from causes not related to oral tongue cancer or at the date of last follow-up as recorded in the EMR. Differences in OS were also examined as a secondary objective. Differences in clinicopathologic features between the CLS-T negative and CLS-T positive groups were examined by the Wilcoxon rank sum test for continuous variables and Fisher's exact or χ^2 test for categorical variables, where appropriate. DSS and OS by CLS-T status were compared using Cox proportional hazards regression and summarized via the Kaplan-Meier method. The multivariate model for DSS was adjusted for the following covariates: age, BMI, race, tumor grade, tumor thickness, perineural and/or vascular invasion, percent of lymph nodes with metastatic disease, smoking status and presence of CLS-N. Hazard ratios between each group and the reference group for categorical variables, and for each unit increase for continuous variables, were reported with 95% confidence intervals (CIs) and two-tailed P-values. A P value < .05 was considered statistically significant. Three year cumulative incidence of recurrence or second primaries stratified by CLS-T status was calculated using competing risk analysis with death from all causes treated as a competing event. Subgroup analyses for survival outcomes stratified by CLS-T status were also conducted in patients without lymph node metastases who did not receive adjuvant therapy. Statistical analyses were conducted using R software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Characteristics & WAT Inflammation

Tongue WAT inflammation (CLS-T, Fig. 2A) was present in 49 of 125 (39%) patients. Notably, macrophages within CLS-T were Ki67 positive indicating a proliferative state (Fig. 2B). Baseline characteristics stratified by tongue WAT inflammation status are presented in Table 1. None of the patients in this study had prior head and neck cancers. Tumors were located at the lateral (95%) or ventral (5%) tongue. We previously reported that primary tumor samples in this cohort were p16 negative.¹² In patients who received adjuvant chemotherapy (N=8), radiation was administered concurrently with a single agent or a combination of agents including platinum, taxane, fluorouracil, or cetuximab. The presence of CLS-T was associated with higher BMI (P=.049), increased tumor thickness (P=.007), and vascular invasion (P<.001). There were no associations detected between CLS-T and TAMs. Additionally, the presence of neck WAT inflammation (CLS-N) was associated with lymph node metastasis (P<.001) and extranodal extension of tumor (P=.01).

Recurrence Patterns and Survival

The median follow-up time was 55 (range 3 to 156) months. During the follow-up period, 77 (62%) patients remained free of disease, 5 (4%) patients were alive with recurrent disease, 5 (4%) patients died of other causes, 4 (3%) patients died of unknown causes, and 33 (26%) deaths related to oral tongue SCC were observed. Postoperative chemotherapy and radiotherapy information was not available for one patient, and this patient was excluded from outcomes analyses. With regard to DSS, 4 patients who died of unknown causes were

censored at the start of the observation period. There were 48 (38%) patients who developed recurrent disease, of whom 12 were rendered free of disease after additional treatment. Of those who developed recurrence, 40 (83%) developed locoregional disease only and 8 (17%) developed distant disease. Additionally, 19 (15%) patients developed second primary tumors of the upper aerodigestive tract. Known prognostic factors were associated with worse DSS including perineural invasion (HR 3.64, 95% CI 1.77 to 7.52) and lymph node involvement (HR 1.09, 95% CI 1.04 to 1.13, Table 2). The 3-year DSS rate for patients with tongue WAT inflammation was 59% (95% CI 46% to 76%) versus 82% (95% CI 73% to 92%) for patients without inflammation. Median DSS was not reached during the observation period. The univariate HR for DSS was 2.06 (95% CI 1.04 to 4.09; Fig. 3A). In the multivariate model, the HR for DSS was 2.56 (95% CI 1.00 to 6.57). The HR for OS was 1.65 (95% CI .90 to 3.04) in patients with tongue WAT inflammation versus those without inflammation, but this result was not statistically significant (Fig. 3B). The DSS and OS patterns are shown in Fig. 3C–D for patients with N0 disease and in Fig. 3E–F for patients with N+ disease.

In the subgroup of patients with lymph node negative disease who did not receive adjuvant treatment with radiation and/or chemotherapy (N=70), 22 (31%) patients had tongue WAT inflammation. The presence of tongue WAT inflammation was associated with worse DSS (HR 3.23, 95% CI 1.11 to 9.39; Fig. 3C) and OS (HR 2.71, 95% CI 1.11 to 6.63; Fig. 3D) compared to no inflammation in this subgroup. The 3 year DSS rate was 58% (95% CI 39% to 88%) for patients with tongue WAT inflammation compared to 85% (95% CI 75% to 97%) for patients without CLS-T. These relationships remained significant in the multivariate models for DSS (HR 5.40, 95% CI 1.20 to 24.26) and OS (HR 2.97, 95% CI 1.02 to 8.65). The recurrence pattern and the frequency of a diagnosis of a second primary malignancy are presented by CLS-T status in Table 3. The majority of recurrences in this subgroup were locoregional.

DISCUSSION

Given the rising incidence of oral tongue SCC and relatively unchanged survival rates, there is an urgent need to identify novel modifiable factors that could lead to improvements in clinical management and survival. In this study, we demonstrate that tongue WAT inflammation in patients with oral tongue SCC is associated with adverse pathologic features including increased tumor thickness and vascular invasion. We also showed it to be independently associated with shortened cancer specific survival in patients with early stage disease. Specifically, in patients who were considered to have low risk disease and thus did not receive adjuvant therapies, tongue WAT inflammation was independently associated with worse cancer specific survival, as well as increased overall mortality.

Obesity is a negative prognostic feature in early stage oral tongue SCC.¹² Although adenocarcinomas are more commonly associated with obesity than SCC, similar obesity-mediated drivers of cancer progression may be active independent of histology.³² These driving mechanisms remain incompletely understood. It is well established that WAT inflammation is present in most overweight and obese individuals and is associated with cardiometabolic disorders including diabetes and atherosclerosis.^{20, 21, 33, 34} We have also reported that WAT inflammation in the breast, which is predominantly comprised of adipose

tissue, is associated with obesity, the metabolic syndrome, and shortened distant recurrence free survival in those who develop breast cancer metastasis.^{18, 27–29} With regard to the tongue, Nashi and colleagues reported that elevated BMI is associated with increased fat content in the tongue.²⁶ Building on these findings, here we show that WAT inflammation, in the form of CLS, occurs in the tongue as it does in the breast and other fat depots, and is associated with worse clinical course.

Our findings are consistent with previously published reports that have described the role of chronic inflammation in the progression of cancer.^{14–17} Several studies have suggested the importance of inflammation, including activation of resident tumor macrophages, in head and neck cancers.^{35–38} In our study, there were no significant interactions between TAM count and WAT inflammation, suggesting the independence of these two processes. Recently, macrophages were reported to proliferate within CLS in the context of obesity.^{39, 40} Here we demonstrate that the same is true within WAT in the context of oral tongue SCC. Future studies are warranted to elucidate the mechanisms underlying macrophage proliferation within CLS and to determine whether targeting this process would be clinically beneficial.

Previously we demonstrated that WAT inflammation in the breast is associated with activation of NF- κ B, a transcription factor that activates expression of proinflammatory mediators, and increased levels of aromatase, the rate-limiting enzyme for estrogen biosynthesis.²⁷ In contrast to breast cancer, a role for steroid hormones in the progression of head and neck cancers is not well described. However, expression of estrogen receptor- α also occurs in head and neck tumors, and women with tumors expressing high levels of estrogen receptor- α have been reported to have shorter progression free survival than those with low levels.⁴¹ Thus, enhanced estrogen signaling may be relevant to the effects of WAT inflammation in the tongue and warrants further investigation.

In addition to local effects in the tongue, related systemic processes may contribute to the negative prognostic impact of tongue WAT inflammation. Deregulated systemic factors could also help to explain why neck WAT inflammation was associated with lymph node metastasis and extranodal extension of tumor. In support of this possibility, WAT inflammation in other adipose depots correlates with increased circulating levels of IL-6, insulin resistance, and altered levels of adipokines.²⁴ Elevated pretreatment levels of serum IL-6 are associated with recurrence and shortened survival for patients with head and neck cancers.²⁵ In addition, alterations in circulating adipokine levels, including low adiponectin and elevated leptin concentrations, occur in association with WAT inflammation and promote cell proliferation and survival.⁴² Elevated levels of insulin promote tumor cell proliferation and survival.^{43, 44} Metformin, an insulin lowering agent, has been shown to prevent malignant transformation of oral premalignant lesions in preclinical models.⁴⁵ Thus, several systemic effects in addition to local effects may help to explain the negative prognostic impact of WAT inflammation in the tongue. Targeting one or more of these factors may represent a therapeutic opportunity if this mechanism is confirmed in tongue cancer.

In our study, the relationship between tongue WAT inflammation and inferior DSS remained significant after adjusting for BMI. This finding suggests that the presence of tongue WAT inflammation provides clinically relevant information beyond that provided by BMI. Similarly, several recent studies have demonstrated that some phenotypically obese individuals, defined by elevated BMI, are metabolically healthy,^{46–48} while metabolic obesity, including insulin resistance, can occur in patients with a normal BMI.^{49, 50} Consistent with these observations, we found tongue WAT inflammation in nearly 20% of individuals with normal or low BMI. Thus, the presence of WAT inflammation provides clinically important and prognostic information which may not have been recognized by assessing BMI alone.

This study is limited by its single-institution, retrospective design and our findings may not be generalizable to patients with bulky disease or other sites of head and neck cancer. Nonetheless, this is the first study demonstrating an independent association between histologic tongue WAT inflammation and reduced survival in patients with early stage SCC of the oral tongue. Limiting the study to one site of oral cavity malignancy represents a design advantage as the heterogeneity of varying prognostic factors by tumor site is minimized. Another strength of our study is the large sample size for a relatively uncommon tumor type. Although our focus was on HPV negative oral tongue SCC, it would be of considerable interest to determine whether similar effects occur in HPV-related tumors.

Our results support further study of the mechanisms by which tongue WAT inflammation worsens prognosis. Elucidating local and systemic changes that occur with tongue WAT inflammation could strengthen the rationale for interventions aimed at reducing WAT inflammation with the goal of improving the outcomes of patients with oral tongue SCC. Possible interventions include weight reduction, exercise, and pharmacological therapies, e.g., anti-inflammatory drugs. These potential interventions may be particularly important for the subgroup of patients who are considered to have low risk disease by traditional parameters (i.e., lack of lymph node involvement) and therefore do not receive adjuvant therapies. In our study, these patients, for whom active surveillance is currently indicated, had worse cancer specific and overall mortality when tongue WAT inflammation was present. Given the locoregional recurrence pattern observed in this subgroup of patients, tongue WAT inflammation as an indication for additional local therapies should be investigated.

In conclusion, we report for the first time that histologic tongue WAT inflammation is an independent predictor of increased risk of death from disease in patients with early stage oral tongue cancer. These findings may help to explain the negative prognostic impact of obesity in patients with oral tongue SCC and support additional study of WAT inflammation as a possible target for intervention. The fact that WAT inflammation has negative prognostic implications for both breast cancer and oral tongue SCC patients strongly suggests that inflammation is important for explaining the relationship between hyperadiposity and poor prognosis for a variety of malignancies.

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Figure 1. Study flow diagram

Abbreviations: SCC; squamous cell carcinoma. CLS-T; crown-like structure of the tongue.

Figure 2. Macrophages within Crown-like Structures of the Tongue (CLS-T) are proliferating
A. Anti-CD68 immunostained tongue section with several crown-like structures of the tongue (200x). Arrow indicates representative CLS-T. Inked margin appears on right. **B.** Dual Ki67 (MIB-1) and CD68 immunohistochemistry (600X). The CLS-T macrophages show both nuclear Ki67 labeling (yellow-brown staining, closed arrows) and cytoplasmic CD68 staining (red staining, open arrows).

Figure 3.

Kaplan-Meier curves for A. Disease specific survival (DSS) and **B.** Overall survival (OS) stratified by CLS-T status in all patients; **C.** DSS and **D.** OS stratified by CLS-T status in patients with N0 disease who did not receive adjuvant therapies; and **E.** DSS and **F.** OS stratified by CLS-T status in patients with N+ disease.

Table 1

Baseline characteristics of patients stratified by CLS-T status.

Variables	CLS-T Negative (n=76)	CLS-T Positive (n=49)	P
Age (years)			
Median (range)	56 (24 to 86)	59 (23 to 96)	.14
Gender, n (%)			
Female	35 (46%)	14 (29%)	.06
Male	41 (54%)	35 (71%)	
Race, n (%)			
White	66 (87%)	42 (86%)	.31
Black	0 (0%)	2 (4%)	
Asian	9 (12%)	5 (10%)	
Other	1 (1%)	0 (0%)	
Smoking, n (%)			
Never	29 (38%)	19 (39%)	.25
Former	31 (41%)	14 (28%)	
Current	16 (21%)	16 (33%)	
Alcohol use, n (%)			
Never	23 (30%)	13 (27%)	.56
Former	4 (5%)	5 (10%)	
Current	49 (65%)	31 (63%)	
BMI (kg/m²)			
Median (range)	27.2 (17.2 to 61.1)	28.0 (18.8 to 43.7)	.049
T stage, n (%)			
T1	53 (70%)	27 (55%)	.13
T2	23 (30%)	22 (45%)	
Tumor thickness, cm			
Median (range)	.62 (.1 to 2.7)	1.00 (.05 to 1.7)	.007
TAMs per HPF			
Median (range)	44 (13 to 82)	49 (7 to 86)	.21
Vascular invasion, n (%)			
No	68 (89%)	31 (63%)	<.001
Yes	8 (11%)	18 (37%)	
Perineural invasion, n (%)			
No	50 (66%)	27 (55%)	.26
Yes	26 (34%)	22 (45%)	
Lymph Node Involvement, n (%)			
No	56 (74%)	28 (57%)	.08
Yes	20 (26%)	21 (43%)	
N Stage, n (%)			
N0	56 (74%)	28 (57%)	
N1	10 (13%)	10 (20%)	

Variables	CLS-T Negative (n=76)	CLS-T Positive (n=49)	<i>P</i>
N2	10 (13%)	11 (23%)	.16
Extracapsular Extension (N+ patients), n (%)			
No	16 (80%)	14 (67%)	
Yes	4 (20%)	7 (33%)	.48
Final Surgical Margins, n (%)			
Negative	60 (79%)	35 (71%)	
Positive	16 (21%)	14 (29%)	.39
Postoperative Chemotherapy, n (%)			
No	73 (97%)	43 (88%)	
Yes	2 (3%)	6 (12%)	.06
Missing	1 (1%)	0 (0%)	1
Postoperative Radiotherapy, n (%)			
No	49 (63%)	28 (57%)	
Yes	26 (37%)	21 (43%)	.45
Missing	1 (1%)	0 (0%)	1

Abbreviations: CLS-T; crown-like structures of the tongue, BMI; body mass index, TAM; tumor-associated macrophage, HPF; high power field

Pathologic features and disease specific survival (DSS) in univariate analysis and multivariate models (adjusted for: age, race, BMI, smoking status, and neck WAT inflammation, tumor grade, tumor thickness, perineural invasion, vascular invasion, and lymph node involvement). Covariates potentially associated with DSS ($P<.25$) and tumor thickness were included in the multivariable model.

Table 2

Pathologic Variables	Univariable			Multivariable		
	HR	95% CI	P	HR	95% CI	P
Tumor Grade						
1	Ref.			Ref.		
2	2.29	.69 to 7.55	.18	1.88	.50 to 7.11	.35
3	2.59	.58 to 11.58	.21	3.02	.56 to 16.36	.20
Perineural Invasion						
No	Ref.			Ref.		
Yes	3.64	1.77 to 7.52	<.001	5.62	2.26 to 13.95	<.001
Vascular Invasion						
No	Ref.			Ref.		
Yes	1.63	.76 to 3.51	.21	.62	.24 to 1.61	.33
Lymph Nodes Involved (per percent increase)	1.09	1.04 to 1.13	<.001	1.08	1.03 to 1.13	.002
Extracapsular Extension						
No	Ref.			Not included		
Yes	.87	.28 to 2.75	.82			
CLS-T						
No	Ref.			Ref.		
Yes	2.06	1.04 to 4.09	.04	2.56	1.00 to 6.57	.05

Abbreviations: WAT, white adipose tissue; Ref, reference (HR = 1.0); CLS-T, crown-like structures of tongue

Table 3

Estimated three year cumulative incidence (95% CI) of recurrence stratified by CLS-T status in patients with N0 disease and no adjuvant therapy.

CLS-T Status	Type of Recurrence		
	Locoregional	Distant or 2 nd Primary	Locoregional and Distant
Negative	30.6% (17.9% to 44.3%)	17.7% (8.2% to 30.2%)	2.2% (.2% to 10.3%)
Positive	40.4% (18.6% to 61.3%)	10.8% (1.6% to 29.8%)	9.8% (1.6% to 27.4%)

Abbreviations: CI, confidence interval; CLS-T; crown-like structures of the tongue