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Tumor Infiltrating Lymphocytes in Patients with Advanced Laryngeal Cancer Undergoing Bioselection

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Abstract

Objective: Bioselection to assess tumor response after induction chemotherapy has been introduced as an alternative treatment strategy to total laryngectomy for patients with advanced larynx squamous cell carcinoma (LSCC). Tumor infiltrating lymphocytes (TILs) have proven to serve as prognostic biomarkers in head and neck cancer, but have not been evaluated as a way to select patients for treatment paradigms. The aim of this study is to evaluate the role of pretreatment TILs in patients with advanced LSCC undergoing the bioselection paradigm.

Study Design: Retrospective study

Setting: Tertiary care hospital

Methods: Patients with advanced LSCC treated with bioselection and with available tissue were included (n=76). Patients were stratified into CD8 low or CD8 high cohorts using the median TIL count. Kaplan-Meier survival analysis and multivariate cox regression was performed using SPSS(v. 26).

Results: After controlling for tobacco use, tumor site and stage, high CD8 TIL count was an independent predictor of improved five-year disease specific survival (HR 0.17, 95% CI 0.03-0.84, p=0.03). CD8 TIL counts did not predict response to induction chemotherapy, however subgroup analysis of patients treated with CRT revealed that CD8 TIL count was significantly associated with degree of response (p=0.012).

Conclusion: These findings support prior data published by our group which shows TILS are predictive of disease specific survival in patients with head and neck cancer. CD8 TIL counts were significantly associated with degree of clinical response after induction chemotherapy. These results suggest that pretreatment assessment of tumor infiltrating CD8 cells could be useful in selecting patients.

Keywords

Larynx cancer; tumor infiltrating lymphocytes; induction selection

Introduction:

Laryngeal squamous cell carcinoma (LSCC) affects around 13,000 people annually and results in approximately 3,700 deaths.[1] The standard of care for patients with advanced stage LSCC was previously laryngectomy followed by adjuvant radiation (RT)[2], however treatment paradigms have shifted towards organ preservation protocols with RT or chemoradiation (CRT)[3]. The Veterans Affairs Laryngeal Cancer Study Group and subsequent Radiation Therapy Oncology Group (RTOG) studies showed equivalent survival for chemoradiation or surgery treatment approaches with a laryngeal preservation rate of 64% for chemoradiation.[4,5] The VA study in particular showed that degree to tumor response to induction chemotherapy was a significant prognostic factor for disease free survival. However, recent epidemiologic studies have suggested a decrease in overall survival for LSCC that corresponded with this treatment shift towards CRT.[6-8]

Bioselection algorithms have been introduced as an alternative treatment strategy for patients with advanced stage disease. These protocols allow for assessment of tumor response after one cycle of induction chemotherapy (typically cisplatin and 5-Fluorouracil). Evaluation during this "window of opportunity" aims to predict response to CRT thereby acting as a bioselection tool. Patients with a greater than 50% response (assessed by direct clinical examination supplemented with imaging) go on to concurrent CRT, and those with a less than 50% response receive surgery followed by post-operative RT. Although these protocols have shown some promise for improved overall survival with high rates of laryngeal preservation[9], there is a need for better predictive tools and standardized assessment of tumor response to further maximize organ preservation rates and decrease rates of late salvage laryngectomy.[10,11] Identification of biomarkers that can predict treatment success during this "window of opportunity" would allow for tailored treatment plans and may improve overall survival and reduce treatment related morbidity.

Recent studies indicate a role for the adaptive immune system in treatment success and suggest immune signatures may serve as clinically useful biomarkers in LSCC.[12–16] Previous work from our group revealed that higher levels of CD4+ and CD8+ tumor infiltrating lymphocytes (TILs) in pretreatment biopsies of recurrent larynx cancer were predictive of disease specific and disease free survival.[12,17,18] This study was undertaken to evaluate the role of TILs in previously untreated advanced stage LSCC patients undergoing bioselection treatment regimens. The role of TILS in predicting survival outcomes, response to induction chemotherapy, and laryngectomy free survival was undertaken. We hypothesize that TIL levels will be associated with improved survival and may serve as predictive biomarkers for response to induction therapy and laryngeal preservation allowing for tailored treatment plans with the goal to reduced treatment related morbidity.

Methods:

Cohort Selection

This study was approved by the University of Michigan IRBMED (HUM00042189). Patients with advanced (stage III-IV) laryngeal squamous cell carcinoma treated with a bioselection regimen at the University of Michigan between 1995 and 2017 were identified and those with available pre-treatment biopsy specimens available for tumor microarray construction were included in the study (n=76). Patient and disease characteristics were collected including age, gender, ethnicity, tobacco use, alcohol use, ACE comorbidity score, body mass index (BMI), tumor overall and TNM stage, tumor subsite, chemotherapeutic agents used for induction, response to induction chemotherapy, treatment regimen (chemoradiation vs. surgery +/ neck dissection), tumor pathology (for surgical arm), recurrence, and survival outcomes.

Tumor Microarray Construction:

Formalin fixed paraffin-embedded (FFPE) tissue blocks were obtained and hematoxylin-eosin stained slides were reviewed by a board certified head and neck pathologist (JBM). Areas of tumor were circled and three 0.7mm diameter cores were obtained from tumor containing areas when sufficient sample was available (n=65). Based on the often small

pre-treatment biopsy samples, some specimens only allowed for 1-2 cores per tumor block (n=45). A tumor microarray was then created by the University of Michigan histology core.

CD8 Tumor Infiltrating Lymphocyte Immunohistochemistry

Staining was performed as previously described using CD8 antibodies (1:40, Nova Castra VP-C320).[12] Tumor infiltrating CD8+ lymphocytes were independently scored by two authors (MEH and JDS). The total number of CD8+ lymphocytes for each core was counted. The percent of each core occupied by tumor was also recorded (0-100%, in increments of 10). CD8 TIL counts for each core was normalized by dividing total CD8+ TIL count by the percent tumor for that core (i.e. if only 50% of the core was occupied by tumor then the count was divided by 0.5). This reduced variation in CD8 TIL count that was due to having varying amounts of tumor in each core. Finally, we standardized final counts to the area of the core; the TIL count was divided by the area of the core ($\pi r2$) so that the cell counts would be represented as number of cells per mm squared. The final CD8 TIL score was calculated for each tumor by averaging TIL counts for each representative core. The median TIL count for the population was calculated and utilized to categorize each tumor as CD8 high (median or above) or CD8 low (below the median) as this has previously been used by our group as a standard cutoff and found to be predictive of survival in other head and neck cohorts.[12,17]

Statistical Analysis

Chi squared analysis was used to compare patient and tumor demographics between CD8 low and CD8 high cohorts and to evaluate the association between CD8 TIL status and response to induction therapy. Kaplan-Meier survival analysis was used to assess the association between clinical variables and CD8 TIL status with five year disease specific survival (DSS), overall survival (OS), and laryngectomy free survival (LFS). Multivariate analysis was performed using a backward selection cox regression analysis. Clinical variables with a p-value <0.2 were included in the multivariate analysis. A p value less than 0.05 was considered significant for all tests. Statistical analysis was performed using SPSS v. 26 (IBM, Armonk, NY).

Results

Patient Characteristics

There were no significant differences in disease or clinical variables between the CD8 low and CD8 high cohorts (Table 1). The majority of patients were male (82%) with a median age of 59 years. The most common subsite was supraglottic (74%) followed by glottic tumors (25%). Both T3 and T4 tumors were equally represented in both CD8 low and CD8 high groups. Included patients underwent induction protocols with either cisplatin/carboplatin and 5-fluorouracil (36%) or cisplatin/carboplatin, Docetaxel and AT-101 (a BCL2 inhibitor) (51%) as part of University of Michigan Rogel Cancer Center AT-101 clinical trial (NCT01633541). Two patients died during induction treatment, one patient was unable to complete induction therapy due to acute renal failure, and one patient did not complete induction due to development of sepsis.

Five year DSS and OS

The median time to follow up was 38 months with a range of 2 months to 15.5 years. The five-year disease specific survival for the cohort was 85% (95% CI 73-92%) and the five-year overall survival was 75% (95% CI62-84%). Five-year disease specific and overall survival were then evaluated stratifying by patient and disease characteristics. Overall stage was the only significant predictor of five-year disease specific survival on univariate analysis with stage III patients showing improved disease specific survival over stage IV patients. (100% 95% CI n/a vs. 81%, 95% CI 68-90%, p=0.04). The same finding was true for OS (96%, 95% CI 75-99% vs. 59%, 95% CI 43-72%, p=0.01).

CD8 TILs predict disease specific survival

Five-year disease specific and overall survival were then evaluated stratifying by CD8 TIL status. On Kaplan-Meier analysis, there was a trend towards improved five-year disease specific survival in the CD8 high group compared to the CD8l ow group (94%, 95% CI 78-98% vs. 78%, 95% CI 58-89%, p=0.12) and five-year overall survival (84%, 95% CI 65-93% vs. 68%, 95% CI 49-71%, p=0.17) (Figure 1a). Multivariate analysis utilizing a backward selection model including variables with a p-value <0.2 on univariate analysis (TIL status as well as overall stage, tobacco use, and tumor subsite for DSS and TIL status, gender, BMI, and overall stage for OS) demonstrated that CD8 TIL status, tobacco use, and overall stage were significant independent predictors of five-year disease specific survival (Figure 1b). The CD8 high TIL cohort showed significantly improved disease specific survival compared to the CD8 low cohort (HR 0.17, 95% CI 0.03-0.84, p=0.03). Of note, never and former smokers showed worse disease specific survival compared to current smokers (HR 0.14, 95% CI 0.03-0.73, p=0.02). No hazard ratio for disease specific survival was available for overall stage, as there were no disease related deaths in the stage III group. Only overall stage was a significant predictor for overall survival (Figure 1c).

CD8 TILs predict degree of response to induction chemotherapy

We evaluated the role of CD8 TIL status in predicting response to induction chemotherapy. Response to induction chemotherapy is evaluated on clinical exam or in the operating room by the treating physician. A reduction in tumor size by 50% or greater is considered a response. Patients were stratified as non-responders (<50%) or responders (≥50%) and by the overall percent response (0-100%). CD8 TIL status did not significantly predict overall tumor response to induction in the cohort (84% vs. 74%, p=0.27), Table 2. Further analysis was performed to determine if there was a correlation between CD8 TIL count and percent response since the degree of tumor regression is one of the strongest overall prognostic indicators in head and neck cancer. Initial sub-group analysis evaluating only the responders (>50% response), revealed that CD8 TIL status was significantly associated with degree of response with patients in the CD8 high cohort being significantly more likely to have an 80% or greater response to induction compared to patients in the CD8 low group, (68% vs. 34%, p=0.012, Figure 2, Table 3). A similar finding was noted for the entire cohort where CD8 high TIL status was associated with ≥80% response, compared to CD8 low TIL status however this did not reach statistical significance (50% vs. 31%, p=0.067), Table 4.

Laryngeal Preservation Rates

Finally, we wished to evaluate the role of CD8 status in predicting larynx preservation. Laryngeal preservation rates were not significantly different between the CD8 low and CD8 High cohorts (71% in both groups, p=0.97), Table 5. We also evaluated the association of CD8 TIL status and larynx preservation in the cohort of patients treated with CRT (responders). Again, we found no significant different in laryngeal preservation rates between CD8 low and high groups (84% vs. 92%, p=0.38), Table 6.

Discussion

Bioselection paradigms with induction chemotherapy are an alternative treatment option to primary total laryngectomy for patients with advanced stage larynx cancer. The goal of these treatment algorithms is to identify tumors that will respond favorably to subsequent radiation and systemic therapy and those that will likely fail CRT and require salvage surgery. Even with bioselection for organ preservation, some patients die from induction chemotherapy and some require salvage surgery with high morbidity and therapeutic redundancy. Thus improved methods of selecting patients for CRT are needed to match definitive treatment to tumor biology that will reduce morbidity and increase rates of organ preservation. Systemic levels of immune reactive cells in pretreatment peripheral blood have been shown to predict response to induction chemotherapy in laryngeal cancer[19], however levels of such cells in the tumor microenvironment have not been assessed. Traditional chemotherapy and radiation treatments have been shown to alter the immune system in head and neck cancer and TILs have been shown to be predictive of response to neo-adjuvant therapy in other tumor types such as breast cancer.[20–22] Given the evidence of immunomodulatory effects of chemotherapy we hypothesized that tumor infiltrating lymphocytes may serves as a predictive biomarker for patients who would ultimately respond to induction chemotherapy. We therefore wished to investigate the role of tumor infiltrating lymphocytes in predicting response to induction chemotherapy and overall survival in a cohort of patients undergoing chemotherapy bioselection at our institution.

Previous studies from our group have demonstrated the role of CD4, CD8, and CD103 tumor infiltrating lymphocytes in predicting survival outcomes in head and neck cancer. [12,13,17] Consistent with these studies, our data demonstrate that CD8 TIL status is a predictor of five year disease specific survival in patients with advanced larynx cancer treated under a bioselection regimen.

In the current study CD8 TIL status did not significantly predict overall response to induction therapy or laryngeal preservation. This is not surprising since the 50% cutoff for characterizing a tumor response as meaningful is based on tradition and not science and as such variations in assessment of response may confound our results. Some patients with such partial responses never become tumor free even after subsequent cycles of chemotherapy or additional radiation and thus are not great candidates for organ preservation. Alternatively, patients showing no response to induction have been shown to have an excellent prognosis after total laryngectomy as compared to patients undergoing salvage laryngectomy after a failure of chemoradiation supporting the concept that matching appropriate treatment to tumor biology can improve results.[3] It is clear from multiple studies

that the greater the response to initial chemotherapy cycles, the better the overall prognosis with the best outcomes in the 15-20% of patients that achieve a complete tumor regression to induction chemotherapy.[23,24] Further, given the complex tumor microenvironment, future studies may need to include additional immune markers to evaluate the role of immune signatures in predicting response to systemic therapy as CD8 TIL status alone may not adequately reflect the tumor immune environment. This is one of the major limitations to this study. Further, the tumor immune microenvironment will not be able to be captured in small biopsy specimen. While we utilized the majority of biopsy specimens in our tumor microarray, it is necessary to keep in mind that these small samples likely oversimplify the picture of the immune microenvironment. However, despite these limitations, CD8 TIL status alone did reflect degree of response in the sub-group analysis of responders suggesting that there is an important role for TILs in predicting degree of response to induction treatment and this could be useful in selecting the most favorable patients for an organ preservation approach.

Tumor infiltrating lymphocytes have been shown to be predictive of response to induction therapy in various other malignancies.[25,26] It is thought that induction therapy induces apoptosis in cancer cells resulting in activation of antigen presentation to the immune environment thereby priming an anti-tumor response.[27–29] This mechanism, in which TILs predict response to systemic therapy, has previously been elucidated in breast tumors. A study by Demaria et al. demonstrated that this initial priming of the anti-tumor response during induction therapy may serve to kill tumor cells that survive initial systemic therapy.[30]

In head and neck cancer, prior in vivo and in vitro studies have demonstrated that cisplatin induces cell death via apoptosis and that this predicts improved survival.[31,32] Further, there is evidence that traditional cytotoxic therapies such as cisplatin increase antigen presentation improving overall anti-tumor response.[20,33,34] Together these studies suggest that cisplatin containing induction therapy may result in tumor cell death via apoptosis resulting in release of tumor neo-antigens and a priming of antigen presenting cells. The increased CD8 TILs in our study may therefore represent an increases anti-tumor response that could potentially act both as a predictive marker as well as a mechanism by which the host immune system aids in additional tumor cell death.

Finally, an additional limitation our study is the use of pre-induction specimens only. Prior studies investigating the role of TILs in response to induction often use post-induction biopsy specimens[22,35,36] which could also explain the lack of significance in our study. Given many of the responders have minimal to no post-induction biopsy specimens due to either complete response or small tumor size, post-induction specimen evaluation remains challenging in this cohort. Future larger prospective studies will be needed to validate our findings and develop standardized and reproducible methodology for determining the density of immune reactive cells in the tumor microenvironment such as what has been published in breast cancer.[37] An important frontier will be understanding what the immune status in the microenvironment can tell us about the potential role for subsequent immunotherapy and not just chemoradiation that could lead to more specific and less morbid treatment modalities.

Conclusion

This study confirms the role of tumor infiltrating lymphocytes in predicting disease specific survival in patients with advanced laryngeal cancer in a cohort of patients undergoing bioselection treatment regimens. CD8 TIL status alone was insufficient in predicting overall response to induction chemotherapy or laryngectomy free survival but was significantly associated with degree of tumor regression in the subgroup analysis. Future studies should include additional immune markers to evaluate overall immune signatures in this cohort.

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References

1. Surveillance E, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2015), National Cancer Institute, DCCPS, Surveillance Research Program, released April 2018, based on the November 2017 submission.
2. Hartl DM, Ferlito A, Brasnu DF, et al. Evidence-based review of treatment options for patients with glottic cancer. Head & neck. 2011;33(11):1638–1648. [PubMed: 21990228]
3. Wolf GT. Integrating surgery into treatment paradigms for organ preservation: tailoring treatment to biology improves outcomes. Int J Radiat Oncol Biol Phys. 2007;69(2 Suppl):S4–7. [PubMed: 17848290]
4. Forastiere AA, Goepfert H, Maor M, et al. Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. The New England journal of medicine. 2003;349(22):2091–2098. [PubMed: 14645636]
5. Wolf GT, Fisher SG, Hong WK, et al. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. The New England journal of medicine. 1991;324(24):1685–1690. [PubMed: 2034244]
6. Megwalu UC, Sikora AG. Survival outcomes in advanced laryngeal cancer. JAMA otolaryngology--head & neck surgery. 2014;140(9):855–860. [PubMed: 25144163]
7. Hoffman HT, Porter K, Karnell LH, et al. Laryngeal cancer in the United States: changes in demographics, patterns of care, and survival. The Laryngoscope. 2006;116(9 Pt 2 Suppl 111):1–13.
8. Wolf GT, Bellile E, Eisbruch A, et al. Survival Rates Using Individualized Bioselection Treatment Methods in Patients With Advanced Laryngeal Cancer. JAMA otolaryngology-- head & neck surgery. 2017;143(4):355–366. [PubMed: 28152117]
9. Urba S, Wolf G, Eisbruch A, et al. Single-cycle induction chemotherapy selects patients with advanced laryngeal cancer for combined chemoradiation: a new treatment paradigm. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(4):593–598. [PubMed: 16380415]
10. Bradford CR, Wolf GT, Carey TE, et al. Predictive markers for response to chemotherapy, organ preservation, and survival in patients with advanced laryngeal carcinoma. Otolaryngology–Head and Neck Surgery. 1999;121(5):534–538. [PubMed: 10547465]
11. de Bree R, Wolf GT, de Keizer B, et al. Response assessment after induction chemotherapy for head and neck squamous cell carcinoma: From physical examination to modern imaging techniques and beyond. Head & neck. 2017;39(11):2329–2349. [PubMed: 28815841]
12. Hoesli R, Birkeland AC, Rosko AJ, et al. Proportion of CD4 and CD8 tumor infiltrating lymphocytes predicts survival in persistent/recurrent laryngeal squamous cell carcinoma. Oral oncology. 2018;77:83–89. [PubMed: 29362129]

13. Mann JE, Smith JD, Birkeland AC, et al. Analysis of tumor-infiltrating CD103 resident memory T-cell content in recurrent laryngeal squamous cell carcinoma. Cancer immunology, immunotherapy : CII. 2018.
14. Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. Immunologic research. 2005;33(2):113–133. [PubMed: 16234579]
15. Concha-Benavente F, Srivastava R, Ferrone S, Ferris RL. Immunological and clinical significance of HLA class I antigen processing machinery component defects in malignant cells. Oral oncology. 2016;58:52–58. [PubMed: 27264839]
16. Meissner M, Reichert TE, Kunkel M, et al. Defects in the human leukocyte antigen class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(7):2552–2560. [PubMed: 15814633]
17. Spector ME, Bellile E, Amlani L, et al. Prognostic Value of Tumor-Infiltrating Lymphocytes in Head and Neck Squamous Cell Carcinoma. JAMA otolaryngology-- head & neck surgery. 2019;145(11):1012–1019. [PubMed: 31486841]
18. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. Head & neck. 2016;38(7):1074–1084. [PubMed: 26879675]
19. Bradford CR, Kumar B, Bellile E, et al. Biomarkers in advanced larynx cancer. The Laryngoscope. 2014;124(1):179–187. [PubMed: 23775802]
20. Heft Neal ME, Haring CT, Mann JE, Brenner JC, Spector ME, Swiecicki PL. Novel Immunotherapeutic Approaches in Head and Neck Cancer. J Cancer Metastasis Treat. 2019;5.
21. Grandal B, Evrevin C, Laas E, et al. Impact of BRCA Mutation Status on Tumor Infiltrating Lymphocytes (TILs), Response to Treatment, and Prognosis in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. Cancers (Basel). 2020;12(12).
22. Park YH, Lal S, Lee JE, et al. Chemotherapy induces dynamic immune responses in breast cancers that impact treatment outcome. Nat Commun. 2020;11(1):6175. [PubMed: 33268821]
23. Ensley JF, Jacobs JR, Weaver A, et al. Correlation between response to cisplatinum-combination chemotherapy and subsequent radiotherapy in previously untreated patients with advanced squamous cell cancers of the head and neck. Cancer. 1984;54(5):811–814. [PubMed: 6204738]
24. Spaulding MB, Fischer SG, Wolf GT. Tumor response, toxicity, and survival after neoadjuvant organ-preserving chemotherapy for advanced laryngeal carcinoma. The Department of Veterans Affairs Cooperative Laryngeal Cancer Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1994;12(8):1592–1599. [PubMed: 8040671]
25. Qian D, Wang Y, Zhao G, et al. Tumor Remission and Tumor-Infiltrating Lymphocytes During Chemoradiation Therapy: Predictive and Prognostic Markers in Locally Advanced Esophageal Squamous Cell Carcinoma. Int J Radiat Oncol Biol Phys. 2019;105(2):319–328. [PubMed: 31228553]
26. West NR, Milne K, Truong PT, Macpherson N, Nelson BH, Watson PH. Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer. Breast Cancer Res. 2011;13(6):R126. [PubMed: 22151962]
27. Baxevanis CN, Dedoussis GV, Papadopoulos NG, Missitzis I, Stathopoulos GP, Papamichail M. Tumor specific cytolysis by tumor infiltrating lymphocytes in breast cancer. Cancer. 1994;74(4):1275–1282. [PubMed: 7914469]
28. Rabinowich H, Cohen R, Bruderman I, Steiner Z, Klajman A. Functional analysis of mononuclear cells infiltrating into tumors: lysis of autologous human tumor cells by cultured infiltrating lymphocytes. Cancer research. 1987;47(1):173–177. [PubMed: 3491673]
29. Topalian SL, Solomon D, Rosenberg SA. Tumor-specific cytolysis by lymphocytes infiltrating human melanomas. J Immunol. 1989;142(10):3714–3725. [PubMed: 2785562]
30. Demaria S, Volm MD, Shapiro RL, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2001;7(10):3025–3030. [PubMed: 11595690]

31. Bauer JA, Kumar B, Cordell KG, et al. Targeting apoptosis to overcome cisplatin resistance: a translational study in head and neck cancer. Int J Radiat Oncol Biol Phys. 2007;69(2 Suppl):S106–108. [PubMed: 17848273]

32. Yip HT, Chopra R, Chakrabarti R, et al. Cisplatin-induced growth arrest of head and neck cancer cells correlates with increased expression of p16 and p53. Arch Otolaryngol Head Neck Surg. 2006;132(3):317–326. [PubMed: 16549753]

33. de Biasi AR, Villena-Vargas J, Adusumilli PS. Cisplatin-induced antitumor immunomodulation: a review of preclinical and clinical evidence. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(21):5384–5391. [PubMed: 25204552]

34. Tran L, Allen CT, Xiao R, et al. Cisplatin Alters Antitumor Immunity and Synergizes with PD-1/PD-L1 Inhibition in Head and Neck Squamous Cell Carcinoma. Cancer Immunol Res. 2017;5(12):1141–1151. [PubMed: 29097421]

35. Waks AG, Stover DG, Guerriero JL, et al. The Immune Microenvironment in Hormone Receptor-Positive Breast Cancer Before and After Preoperative Chemotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2019;25(15):4644–4655. [PubMed: 31061067]

36. Pelekanou V, Carvajal-Hausdorf DE, Altan M, et al. Effect of neoadjuvant chemotherapy on tumor-infiltrating lymphocytes and PD-L1 expression in breast cancer and its clinical significance. Breast Cancer Res. 2017;19(1):91. [PubMed: 28784153]

37. Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of oncology : official journal of the European Society for Medical Oncology. 2015;26(2):259–271. [PubMed: 25214542]

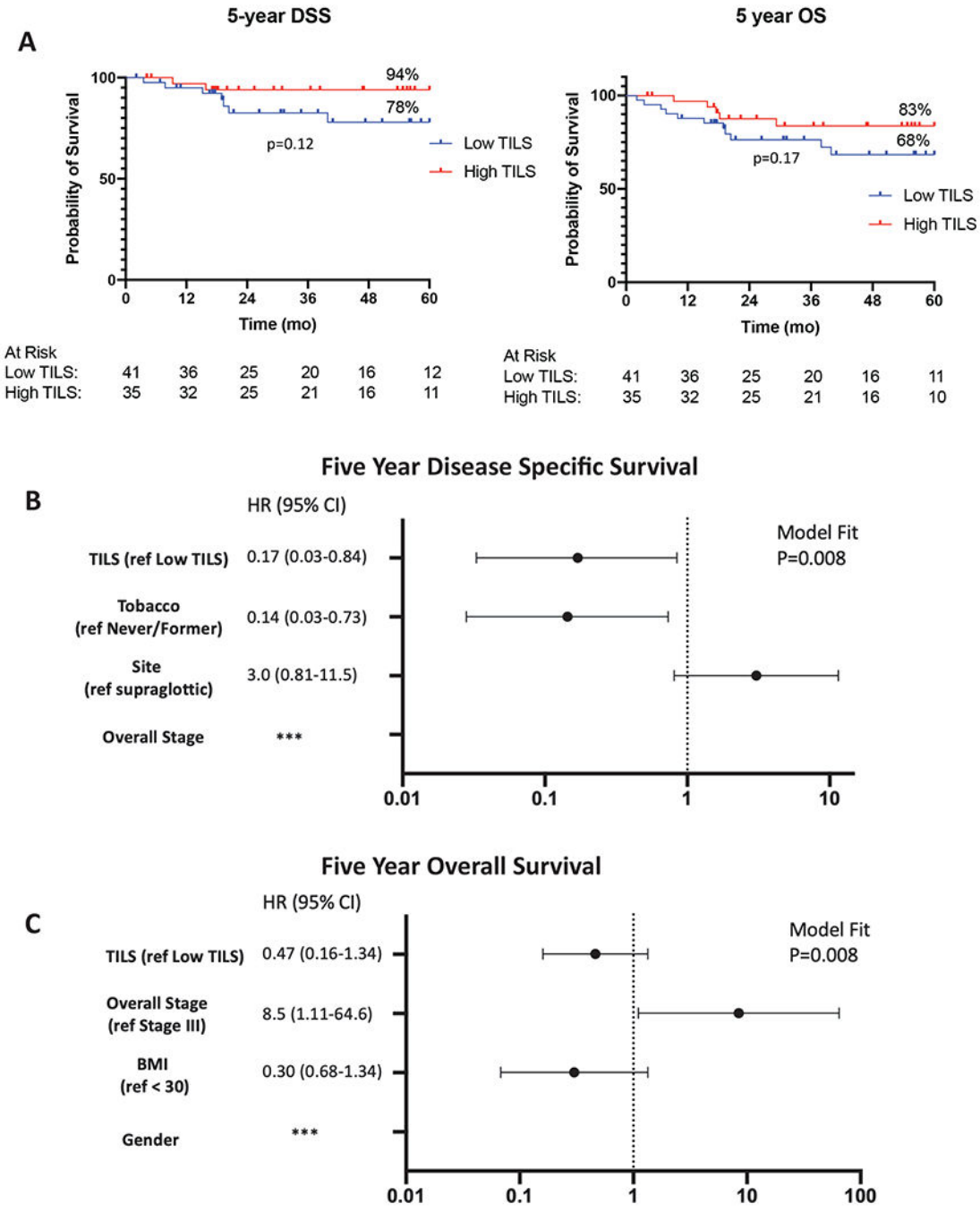

Figure 1:

CD8 TIL status is an independent predictor of disease specific survival. A) Kaplan-Meier curves stratifying by CD8 TIL status. B-C) Forrest plots depicting hazard ratios and 95% confidence intervals for DSS and OS.

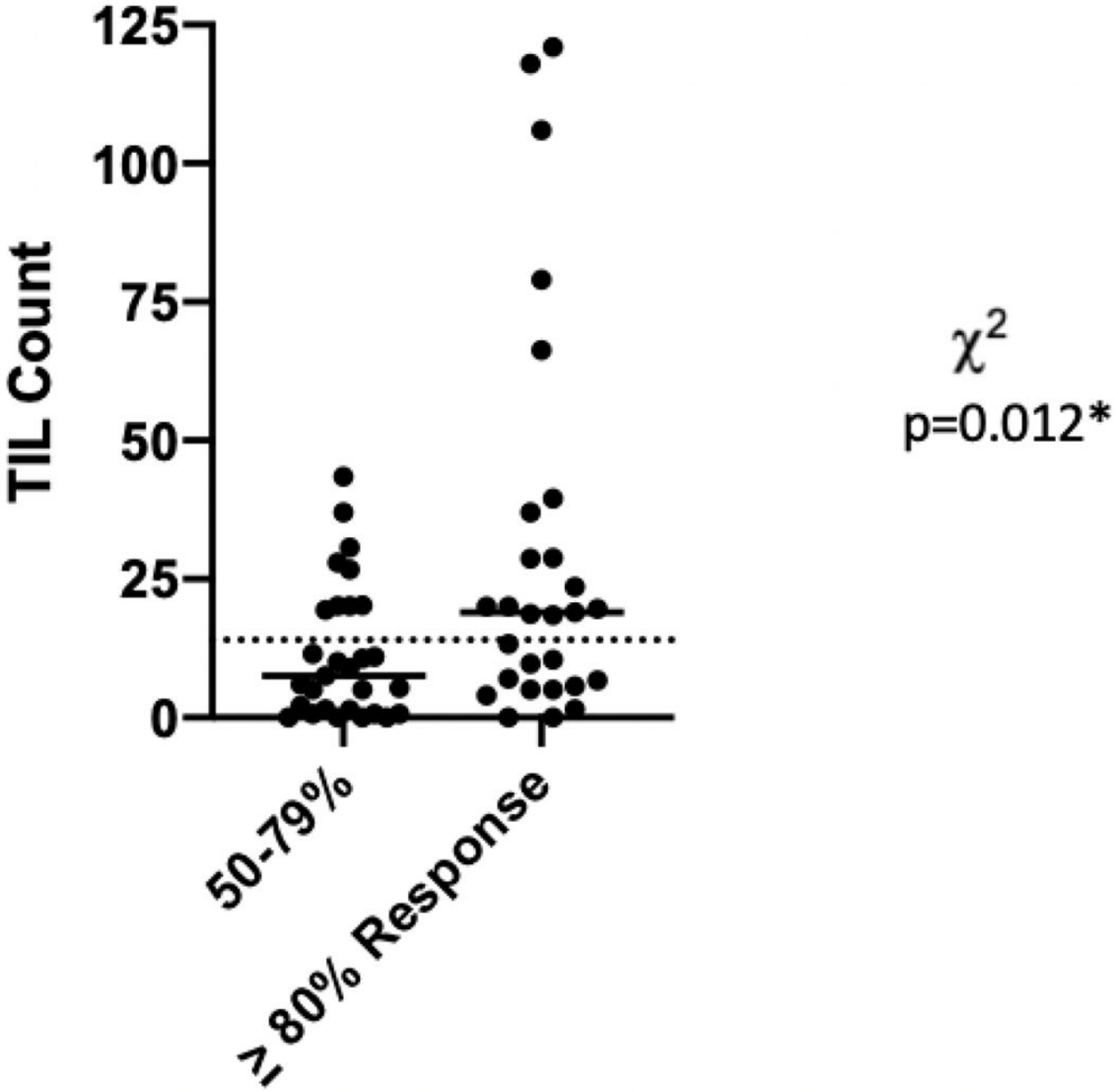

Figure 2:

CD8 TIL status predicts degree of response in sub-group analysis. Chi-squared analysis of the responder sub-group reveals a significant association between CD8 high TIL status and ≥ 80% response to induction therapy.

Table 1:

Patient and Disease Characteristics by TIL Status

		Total Count (%), n=76	Low TILs Count (%), n=41	High TILS Count (%), n=35	p-value
Age (mean yrs)		59	59	59	
Gender	Male Female	62 (82) 14 (18)	35 (85) 6 (15)	27 (77) 8 (23)	0.39
Tobacco	Never Former Current	4 (5) 40 (53) 31 (41)	2 (5) 21 (51) 17 (41)	2 (6) 19 (54) 14 (40)	0.99
ACE Comorbidity Score	None/Mild Moderate/Severe	47 (62) 29 (38)	25 (61) 16 (39)	22 (63) 13 (37)	0.79
BMI	<30 >30	60 (79) 16 (21)	30 (73) 11 (27)	30 (86) 5 (14)	0.28
Site	Supraglottic Glottic Subglottic	56 (74) 19 (25) 1 (1)	30 (73) 11 (27) 0 (0)	26 (74) 8 (23) 1 (3)	0.52
Overall Stage	III IV	22 (29) 54 (71)	12 (29) 29 (71)	10 (29) 25 (71)	0.89
T Stage	T2 T3 T4	2 (3) 35 (46) 39 (51)	0 (0) 21 (51) 20 (51)	2 (6) 14 (40) 19 (54)	0.23
N Stage	N0 N1 N2 N3	21 (28) 12 (16) 42 (55) 1 (1)	13 (32) 6 (15) 22 (54) 0 (0)	8 (23) 6 (17) 20 (57) 1 (3)	0.28
Trial	9520-like AT101	37 (36) 39 (51)	19 (46) 22 (54)	18 (51) 17 (49)	0.67

Table 2:

Binary Response by Tumor-Infiltrating Lymphocyte Status

	Non-Responders (<50%)	Responder (≥ 50%)	p=.27
CD8 Low	6	32	
CD8 High	9	25	

Table 3:

Percent Response by Tumor-Infiltrating Lymphocyte Status

Responders Only	50-80%	≥ 80%	p=.012
CD8 Low	21	11	
CD8 High	8	17	

Table 4:

Binary Response (Using 80% Cutoff) by Tumor-Infiltrating Lymphocyte Status

All Patients	<80%	≥ 80%	p=.067
CD8 Low	27	11	
CD8 High	17	17	

Table 5:

Total Laryngectomy by Tumor-Infiltrating Lymphocyte Status (All Patients)

All Patients	No TL	Yes TL	p=0.97
CD8 Low	27	11	
CD8 High	24	10	

Table 6:

Total Laryngectomy by Tumor-Infiltrating Lymphocyte Status (Responders Only)

Responders Only	No TL	Yes TL	p=0.38
CD8 Low	27	5	
CD8 High	23	2	