Impact of Neoadjuvant Durvalumab with or without Tremelimumab on CD8$^+$ Tumor Lymphocyte Density, Safety, and Efficacy in Patients with Oropharynx Cancer: CIAO Trial Results

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ABSTRACT

Purpose: In oropharyngeal squamous cell carcinoma (OPC), high CD8$^+$ tumor-infiltrating lymphocyte (CD8$^+$TIL) density confers improved prognosis. We compared neoadjuvant durvalumab (PD-L1 inhibitor) with durvalumab + tremelimumab (CTLA-4 inhibitor) in terms of impact on CD8$^+$TIL density, safety, and efficacy in patients with OPC.

Patients and Methods: Patients with newly diagnosed stage II–IVA OPC or locoregionally recurrent OPC amenable to resection were included. Patients were randomized to two cycles of durvalumab or durvalumab + tremelimumab before surgery. The primary endpoint was change between baseline and resection specimen in CD8$^+$TIL density between arms. Secondary endpoints included safety, response rate per RECIST, major pathologic response (MPR; ≤10% viable tumor cells) rate, and patient-reported outcomes.

Results: Of 28 eligible patients (14/arm), 20 (71%) had newly diagnosed OPC, and 24 (86%) were p16-positive. The posttreatment to pretreatment median CD8$^+$TIL density ratio was 1.31 for durvalumab and 1.15 for combination treatment ($P = 0.97$; 95% CI: −1.07–2.28). In each group, 6 patients (43%, 95% CI: 17.66–71.14) had a response. Eight patients (29%) had a MPR at the primary tumor and/or nodal metastases. Neither baseline CD8$^+$TIL density nor PD-L1 expression level correlated with overall response, but a trend toward greater CD8$^+$TIL change in patients with a MPR was seen ($P = 0.059$; 95% CI: −0.33–3.46). Four patients (14%) had grade ≥3 adverse events. At median follow-up time of 15.79 months, all patients were alive, and one had an additional recurrence.

Conclusions: Durvalumab + tremelimumab did not increase CD8$^+$TIL density more than durvalumab alone did. The observed safety and activity support further investigation of neoadjuvant checkpoint inhibitor for OPC.

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Introduction

The incidence of oropharyngeal squamous cell carcinoma (OPC) has risen in recent decades, driven largely by a steep increase in the number of human papillomavirus (HPV)-positive cancers. Despite favorable survival outcomes with current standard-of-care therapies, approximately 25% of patients with locally advanced HPV$^+$ OPC will experience recurrence (1). Moreover, patients with HPV$^-$ OPC and those with locoregional recurrence of OPC, irrespective of HPV status, have dismal outcomes (1–3). Novel therapeutic approaches are urgently needed for these patients.

In patients with OPC, high density of CD8$^+$ tumor-infiltrating lymphocytes (CD8$^+$TIL) is a favorable prognostic biomarker (4–7). PD-1 and other checkpoint molecules are strongly expressed on intratumoral CD8$^+$TIL reactive to mutational neoantigens (8). In preclinical models, combination therapy targeting PD-1 and CTLA-4 increased CD8$^+$TIL density more than either treatment alone (9, 10).

Antibodies targeting the PD-1 checkpoint axis improve overall survival in patients with recurrent/metastatic head and neck squamous cell carcinoma (HNSCC) and have an attractive safety profile (11). In melanoma, the combination of PD-1 and CTLA-4 checkpoint inhibitors (CPI) has led to better progression-free survival than that observed with either agent alone (12). These findings suggest that dual checkpoint inhibition is a potential strategy for increasing the proportion of patients with OPC who benefit from immunotherapy.

Translational Relevance

In patients with oropharyngeal cancer (OPC), high density of $CD8^+$ tumor-infiltrating lymphocytes ($CD8^+$TIL) is a favorable prognostic biomarker. In preclinical models, combination therapy targeting PD-1 and CTLA-4 increased $CD8^+$TIL density more than either treatment alone. This randomized study represents the first reported clinical assessment of a PD-L1 inhibitor (durvalumab) with or without a CTLA-4 inhibitor (tremelimumab) administered in the neoadjuvant setting for OPC. While combination treatment did not increase $CD8^+$TIL density compared with pretreatment levels more than single-agent durvalumab, 12 patients (43%, 6/arm) had a response per RECIST and eight patients (29%) had a major pathologic response at the primary tumor and/or nodal metastases. Furthermore, 45% of newly diagnosed patients with OPC were treated with checkpoint inhibitor (CPI) therapy and surgery without radiotherapy. The efficacy and safety observed support further investigation of neoadjuvant CPI for patients with OPC and suggests this strategy could be used for treatment deescalation.

Durvalumab is a mAb that inhibits PD-L1. Tremelimumab is a mAb that inhibits CTLA-4. We hypothesized that a short course of tremelimumab combined with durvalumab before standard-of-care surgery would increase $CD8^+$TIL density compared with pretreatment levels more than durvalumab alone would. We designed the Checkpoint Inhibitors Assessment in Oropharynx Cancer (CIAO) clinical trial to test this hypothesis and to gain a more comprehensive understanding of immunologic mechanisms active in the tumor immune microenvironment during treatment with durvalumab as a single agent or combined with tremelimumab. Here, we report the impact of neoadjuvant durvalumab and durvalumab + tremelimumab on $CD8^+$TIL density in OPC, along with the safety, efficacy, and tolerability of these regimens.

Patients and Methods

Study design and patient population

CIAO was a randomized, investigator-initiated, single-institution clinical trial (Clinicaltrials.gov identifier: NCT03144778). Eligibility criteria included either newly diagnosed stage II–IVA OPC per the seventh edition of the *AJCC Cancer Staging Manual* (13) amenable to transoral robotic surgery or locally recurrent OPC (with or without regional recurrence) amenable to salvage surgery after a disease-free interval of ≥6 months; age ≥18 years; and ECOG performance status of 0–1. Major exclusion criteria included prior systemic therapy for the same OPC and active autoimmune disease. This study followed the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. Patients were enrolled with approval from the ethics committees and institutional review boards of the MD Anderson Cancer Center (Houston, TX), and all patients provided written informed consent before starting study-specific procedures. The trial protocol is available in the Appendix.

Procedures

The study schema is outlined in **Fig. 1**. Patients were stratified by tumor p16 status and randomized 1:1 to receive two cycles of intravenous durvalumab 1,500 mg with or without tremelimumab 75 mg on day 1 of a 28-day cycle before surgery. The ideal window for surgery was prespecified as 8 weeks (day 52 to 72) after initiation of neoadjuvant CPI therapy. Adjuvant radiotherapy with or without concurrent cisplatin was administered as indicated by pathologic findings (14). Because most patients with locoregionally recurrent OPC will recur after salvage surgery (15), those who achieved at least a partial pathologic response to neoadjuvant CPI (defined as ≤50% viable tumor in the surgical specimen) were eligible to receive up to 1 year of adjuvant CPI therapy.

Computed tomography of the head and neck and chest was performed at baseline and after completion of neoadjuvant CPI therapy. Radiographic response was defined per RECIST v1.1 (16).

Toxicity was monitored from treatment start until 30 days after the last dose of neoadjuvant CPI and graded according to the National Cancer Institute Common Terminology Criteria v.4.03.

Patient-reported outcomes were prospectively collected at day 1, day 29, and before surgery using the MD Anderson Symptom Inventory Head and Neck Module (MDASI-HN; ref. 17). The MDASI-HN is a brief patient-reported outcomes questionnaire developed to measure severity or burden of systemic and HNSCC-specific symptoms and their interference with patients' daily functioning. MDASI-HN includes items that map to the most commonly reported clinician-graded toxic effects of immune CPIs in published trials, including fatigue, rash, nausea, lack of appetite, and shortness of breath. A questionnaire sample is available in the Appendix.

CD8 and PD-L1 staining and quantification

A formalin-fixed and paraffin-embedded tumor specimen was mandatory for enrollment. HPV status was determined using p16 expression by IHC and RNA-scope as described previously (18).

CD8 and PD-L1 expression were evaluated using IHC (CD8: clone C8/144B, Thermo Fisher Scientific MS-457-S; PD-L1: clone 22C3, DAKO DK006). Slides were digitally scanned using the Aperio ScanScope Turbo Slide Scanner (Leica Microsystems) and analyzed using the Aperio Image Toolbox. CD8 expression was scored using a quantitative Nuclear-Staining Algorithm (Aperio Technologies). Areas of interest included the intratumoral region (analyzed before and after treatment) and the invasive tumor front (analyzed before treatment). These two regions were considered to be different layers of annotation, and on each layer, five areas of 400 μm^2 (200-μm squares) were selected corresponding to areas with the highest density of IHC-positive cells. For the PD-L1 analysis, the tumor proportion score and combined proportion score were calculated as described previously (19).

Statistical analysis

The primary objective was to compare the change in $CD8^+$TIL density between baseline and the posttreatment surgical specimen between patients treated with durvalumab and those treated with durvalumab + tremelimumab. Secondary objectives included assessing safety, efficacy [response per RECIST 1.1 and major pathologic response (MPR), defined as ≤10% viable tumor cells in the surgical specimen], and patient-reported outcomes.

The study was designed to investigate whether addition of tremelimumab to durvalumab would increase the ratio of posttreatment to pretreatment $CD8^+$TIL density (in cells/mm^2) by 0.5. Under this assumption, a sample size of 14 patients per arm would achieve 82% power to detect a difference of 0.5 in the ratio at a significance level of 0.05 using a two-sided, two-sample *t* test. Because of the skewed distribution of $CD8^+$TIL density and small sample size, two-sided Wilcoxon rank-sum test was used for the primary endpoint analysis. Response was assessed in all eligible patients. Safety was evaluated in

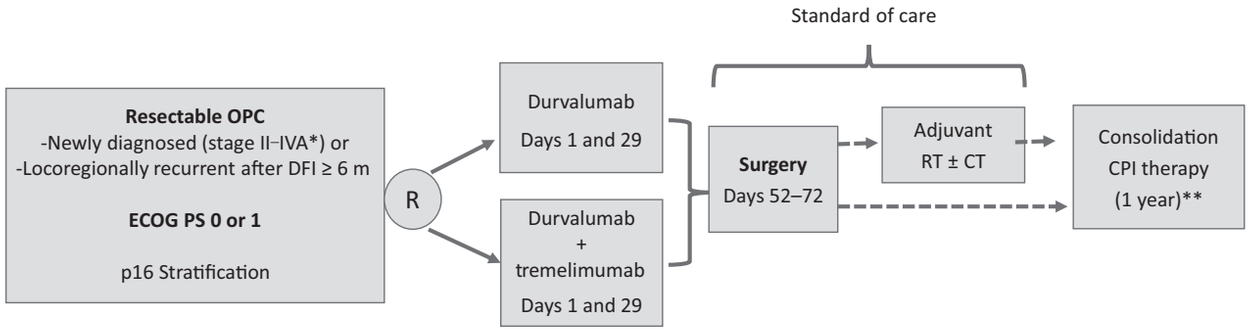

Figure 1.

Study schema. *Per the 7th edition of the *AJCC Cancer Staging Manual*. **Only an option for patients with recurrent disease and ≤50% viable tumor in the surgical specimen. Patients randomized to receive durvalumab + tremelimumab as neoadjuvant therapy would receive two more cycles of the combination followed by durvalumab as a single agent for up to 1 year; those randomized to receive single-agent durvalumab would receive durvalumab for up to 1 year. CT, chemotherapy; DFI, disease-free interval; PS, performance status; RT, radiotherapy.

patients who received at least one dose of a CPI. Ordinal logistic regression and two-sided Fisher exact test were used to study possible differences between treatment arms in adverse event severity and incidence, respectively.

Association between response and treatment arm and response by RECIST and pathologic response was assessed using two-sided Fisher exact test and Wilcoxon rank-sum test, respectively.

Correlative analysis included the association of $CD8^+$ T lymphocytes (at the invasive tumor margin or intratumorally) and PD-L1 expression (tumor proportion score or combined proportion score) at baseline with response to therapy. These associations were assessed using Wilcoxon rank-sum test.

For the patient-reported outcomes, a population-averaged model for repeated measures was used to analyze the global MDASI-HN score. Global MDASI-HN score was the average of MDASI-HN scale items. Scores were transformed using Box-Cox to meet the assumption of normality for outcomes in the repeated measures models. Guided by model fit statistics (−2Log likelihood, Akaike Information Criteria), we chose the first-order autoregressive moving average covariance structure. Treatment arm, time, and the interaction between these two were used as fixed independent variables to predict MDASI-HN global scores. Restricted maximum likelihood was used as the estimation method. Tukey–Kramer adjusted *P* values were inspected for *post hoc* least squares means comparisons.

Analyses were conducted using SAS 9.4 (SAS Institute). All tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patients and treatment

From July 2017 through February 2019, 29 patients enrolled in the study, 20 with newly diagnosed and nine with locoregionally recurrent OPC. All patients with recurrent disease had prior radiation. Twenty-four patients (83%) had p16 expression by IHC, and 23 (79%) had HPV confirmed by RNA-scope. Twenty-eight patients (97%) were male, and 22 (76%) had a smoking history of ≤10 pack-years. One patient randomly allocated to durvalumab was found to be ineligible after enrollment because of nodal recurrence only and no baseline tumor specimen available. This patient was included in the analysis of treatment-related adverse events (TRAE) but not the efficacy analysis. Patient characteristics are summarized in **Table 1**. A patient flow diagram is provided in Supplementary Fig. S1.

Change in $CD8^+$TIL density

$CD8^+$TIL measurements for the overall study group and each study arm are summarized in Supplementary Table S1. Five patients were not evaluable for the primary endpoint due to exposure to chemotherapy prior to surgery ($n = 3$), or lack of tissue at baseline ($n = 1$) or posttreatment ($n = 1$). Tremelimumab + durvalumab did not increase $CD8^+$TIL density more than durvalumab alone (median ratio of posttreatment to pretreatment $CD8^+$TIL density, 1.31 for durvalumab and 1.15 for durvalumab + tremelimumab, $P = 0.97$; 95% CI: −1.07–2.28; **Fig. 2**). However, some patients had a higher than expected increase in $CD8^+$TIL density (the largest $CD8^+$TIL density ratios were 26.2, 4.27, and 4.21 in the durvalumab arm and 50 and 4.2 in the durvalumab + tremelimumab, Supplementary Fig. S2)

Safety

Twenty-six patients (90%) experienced a TRAE. The most common and all grade ≥3 TRAEs are shown in **Table 2**. The incidence and severity of TRAEs were similar in the two treatment arms [for incidence of grade ≥3, OR (durvalumab + tremelimumab vs. durvalumab) = 0.32; $P = 0.6$; 95% CI: 0.01–3.32; and for severity, OR (more severe vs. less severe, durvalumab + tremelimumab vs. durvalumab) = 0.76; $P = 0.7$; 95% CI: 0.19–3.04]. Four patients (13.8%) experienced serious TRAEs, all grade 3; two patients required steroid treatment (for transaminitis and diarrhea, respectively) and two patients (one with transaminitis and one with increase in lipase) received only one of the two planned doses of CPI. All TRAEs resolved fully.

As an additional safety measure, we evaluated the effect of neoadjuvant CPI therapy on the timing of surgery and surgery-associated adverse events. All patients underwent the proposed surgery, and 21 (75%) underwent surgery within the prespecified window (day 52 to 72). Four patients underwent surgery earlier than day 52, two that received only one dose of CPI due to an adverse event and two due to scheduling issues (surgery done on day 47 and 49). Three (10%) patients that received chemotherapy after CPI had surgery after day 72.

Twenty-six patients (93%) had transoral robotic surgery, and two (both with recurrent disease) had open surgery. Simultaneous free-tissue-transfer reconstruction was performed in seven patients (25%), six with recurrent disease and one with newly diagnosed OPC of the soft palate. Four patients (14%) had acute surgical complications; three of these were related to free-tissue-transfer reconstruction (flap failure, donor site cellulitis, and fistula), and one was minor bleeding from the surgical site on postoperative day 8 that resolved without intervention.

Table 1. Patient demographics and pretreatment characteristics.

Characteristic	Total ($N = 29$[a])	Durva ($N = 15$)	Durva + Treme ($N = 14$)
Disease status			
Newly diagnosed	20 (69)	10 (67)	10 (71)
Recurrent	9 (31)	5 (33)	4 (29)
p16 status			
Negative	4 (14)	2 (13)	2 (14)
Positive	25 (86)	13 (87)	12 (86)
Sex			
Female	1 (3)	1 (7)	0 (0)
Male	28 (97)	14 (93)	14 (100)
Smoking			
>10 pack-years	7 (24)	4 (27)	3 (21)
1–10 pack-years	12 (41)	5 (33)	7 (50)
Nonsmoker	10 (34)	6 (40)	4 (29)
Alcohol use			
Excessive[b]	5 (17)	3 (20)	2 (14)
Social or moderate	24 (83)	12 (80)	12 (86)
Pretreatment T category			
0	1 (3)	1 (7)	0 (0)
1	10 (34)	7 (47)	3 (21)
2	14 (48)	4 (27)	10 (71)
3	3 (10)	2 (13)	1 (7)
4	1 (3)	1 (7)	0 (0)
Pretreatment N category			
0	8 (28)	3 (20)	5 (36)
1	8 (28)	6 (40)	2 (14)
2a	1 (3)	1 (7)	0 (0)
2b	12 (41)	5 (33)	7 (50)
Stage			
II	1 (3)	0 (0)	1 (7)
III	7 (24)	5 (33)	2 (14)
IVA	12 (41)	5 (33)	7 (50)
Recurrent disease	9 (31)	5 (33)	4 (29)
TPS			
<1%	2 (7)	1 (7)	1 (7)
≥1%	25 (86)	13 (87)	12 (86)
Not evaluable	2 (7)	1 (7)	1 (7)
CPS			
≥1%	27 (93)	14 (100)	13 (100)
Not evaluable	2 (7)	1	1

Note: Data are provided as No. (%).

Abbreviations: CPS, combined proportion score; durva, durvalumab; TPS, tumor proportion score; treme, tremelimumab.

[a]One patient with recurrence in the neck node only and no available baseline tissue was not included in the efficacy analysis.

[b]Defined as four or more drinks on any day or eight or more drinks per week for women and five or more drinks on any day or 15 or more drinks per week for men, per the US Department of Health and Human Services definition.

Efficacy

Twenty-eight patients were evaluable for response per RECIST after at least one dose of CPI. Twelve patients (43%, 95% CI: 17%–71%) had a partial response. The response rate was the same in the two study arms (Supplementary Table S2; **Fig. 3A**). Three patients (11%) received chemotherapy before surgery because of clinical progression ($n = 2$) or physician preference ($n = 1$).

Twenty-five patients were evaluable for pathologic response to CPI therapy at the primary tumor; 19 of whom were also evaluable for pathologic response at involved lymph nodes. Two (7%) of 25 patients achieved an overall MPR. Two of 25 patients had a MPR at the primary tumor, and both had a pathologic complete response (pCR); eight of 19 patients had a nodal MPR, including both patients with a primary-tumor MPR.

Overall, seven (25%) of 28 patients had their disease downstaged by neoadjuvant CPI therapy (six with newly diagnosed and one with recurrent disease). The percentages of viable tumor cells in the primary-tumor and lymph node surgical specimens in each treatment arm are plotted in **Fig. 3B**. Of the three patients who received chemotherapy before surgery, two had a pCR in the primary tumor and nodes and one had a pCR at the primary tumor site but remnant viable tumor in the nodes.

At surgery, one patient (4%) had a microscopically positive surgical margin, and 10 (36%) had extranodal extension. Of the 20 patients with newly diagnosed OPC, nine (45%) were treated with surgery alone (MPR at the primary and node $n = 3$, MPR at the node $n = 4$, and patient refusal $n = 2$), six (30%) received adjuvant radiotherapy, and five (25%) received postoperative chemoradiation. None of the eight patients with recurrent disease were reirradiated following salvage

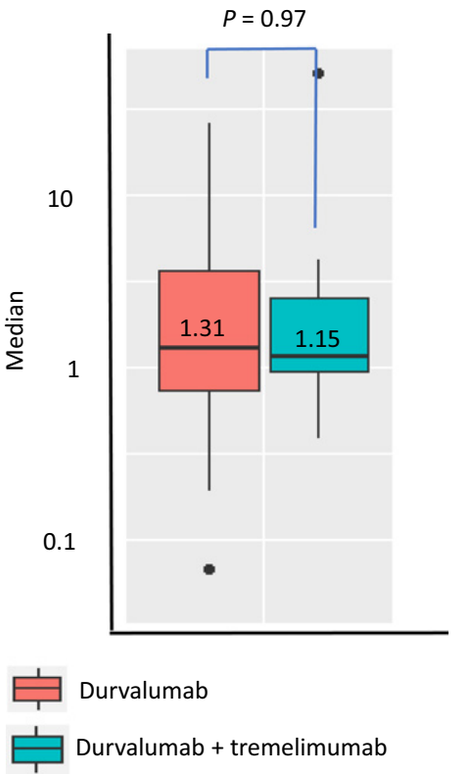

Figure 2.
Impact of CPI therapy on CD8$^+$TIL. Ratio of posttreatment to pretreatment CD8$^+$TIL density by treatment arm (n = 23).

surgery. Three received adjuvant CPI therapy, and one is still receiving CPI therapy at this writing.

Biomarker analysis

CD8$^+$ lymphocytes density at the leading tumor edge or intratumorally (CD8$^+$TIL) at baseline was not significantly different between responders and nonresponders by RECIST (median CD8$^+$ lymphocytes at the leading tumor edge = 1,962 in responders vs. 1,965 in nonresponders, P = 0.65; 95% CI: −696–1,391 and median CD8$^+$TIL = 1,353 in responders vs. 986 in nonresponders, P = 0.46; 95% CI: −526–1,043) or pathologic assessment (MPR vs. non-MPR, median CD8$^+$ lymphocytes at the leading tumor edge = 1,873 in MPR vs. 1,902 in non-MPR, P = 0.76; 95% CI: −1,563–836, and median CD8$^+$TIL = 762.5 in MPR vs. 1,178 in non-MPR, P = 0.2, respectively). However, the ratio of posttreatment to pretreatment CD8$^+$TIL density was higher in patients with a MPR at the primary tumor and/or nodal metastases, although this difference did not reach statistical significance (median CD8$^+$TIL density ratio = 2.47 in MPR vs. 0.88 in non-MPR groups, P = 0.059; 95% CI: −0.33–3.46; **Fig. 4**).

PD-L1 expression was assessed at baseline in 27 specimens, 25 of which (86%) had a tumor proportion score ≥1% and all of which had a combined proportion score ≥1%. There was a nonsignificant trend toward an association between higher tumor proportion score or combined proportion score and partial response by RECIST or MPR (Supplementary Fig. S3).

Correlation between imaging and pathologic response

We explored whether response by imaging predicted pathologic response to CPI therapy. In the 25 patients with a partial response or stable disease per RECIST, imaging response was significantly associated with the median percentage of viable tumor cells in the primary-tumor surgical specimen (37.5% for partial responders vs. 80% for patients with stable disease; significant differences between the two

Table 2. Summary of adverse events possibly, probably, or definitely related to the study drug(s) that occurred in at least two patients or in any patient if grade ≥3.

		Durva				Durva + Treme			
Adverse event[a]	Total N = 29	G1	G2	G3	Total N = 15	G1	G2	G3	Total N = 14
Fatigue	10 (34%)	5	0	0	5 (33%)	5	0	0	5 (36%)
Anemia	8 (28%)	4	0	0	4 (27%)	4	0	0	4 (29%)
Transaminitis	8 (28%)	3	0	1	4 (27%)	4	0	0	4 (29%)
Rash	7 (24%)	3	0	0	3 (20%)	4	0	0	4 (29%)
Thyroid dysfunction	7 (24%)	3	0	0	3 (20%)	2	2	0	4 (29%)
Lymphopenia	5 (17%)	0	2	0	2 (13%)	3	0	0	3 (21%)
Lipase increase	5 (17%)	1	0	2	3 (20%)	1	1	0	2 (14%)
Nausea	4 (14%)	3	0	0	3 (20%)	1	0	0	1 (7%)
Creatinine increase	3 (10%)	2	0	0	2 (13%)	1	0	0	1 (7%)
Diarrhea	3 (10%)	1	0	0	1 (7%)	0	1	1	2 (14%)
Myalgia	3 (14%)	2	0	0	2 (13%)	1	0	0	1 (7%)
Amylase increase	3 (14%)	1	1	0	2 (13%)	1	0	0	1 (7%)
Pruritus	2 (7%)	0	0	0	0 (0%)	2	0	0	2 (14%)
Any adverse event[b]	29 (100%)	9	2	3	15 (100%)	7	4	1	14 (100%)

Note. Data are provided as No. (%).
Abbreviations: durva, durvalumab; G1, grade 1; G2, grade 2; G3, grade 3; treme, tremelimumab.
[a]For patients with more than one adverse event of the same type, the worst outcome (i.e., highest grade) is recorded.
[b]One patient in the durva arm and two patients in the durva + treme arm experienced TEAE that were not deemed related to the study drug(s). The distribution of the AE toxicity grade is not significantly different between treatment groups (P = 0.56, two-sided Fisher exact test; P = 0.70, ordinal logistic regression). The proportion of AE toxicity grade 3 is not significantly higher in the Durva + Treme group (P = 0.6, two-sided Fisher exact test).

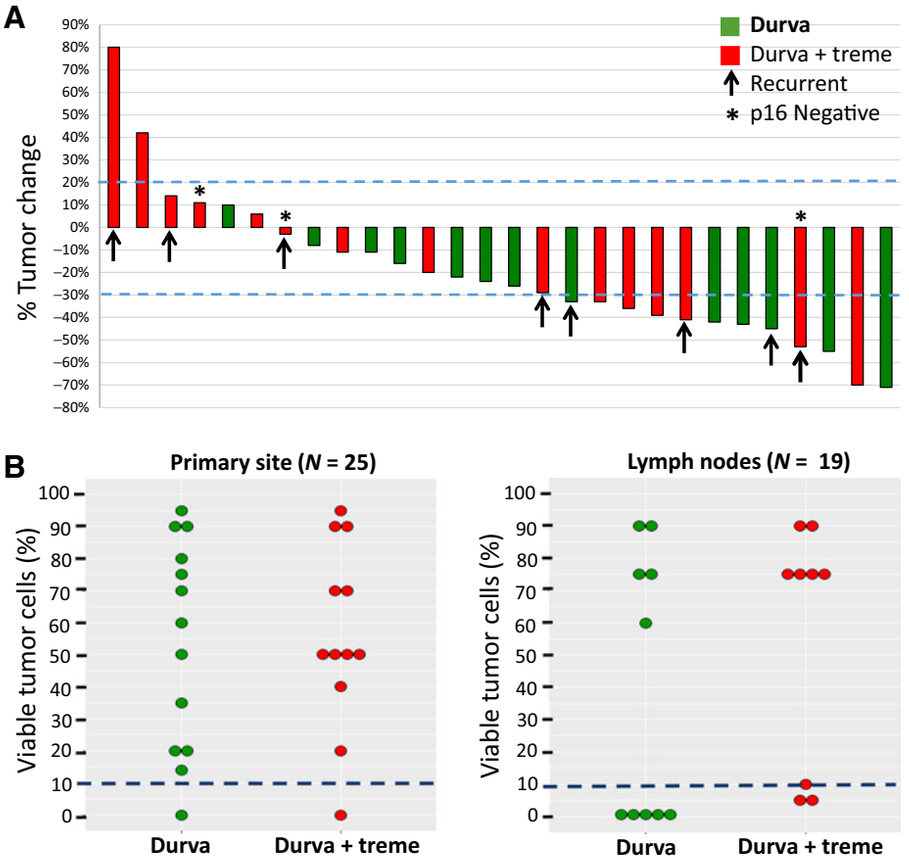

Figure 3.
Efficacy of neoadjuvant durvalumab (durva) ± tremelimumab (treme). **A,** Percentage change from baseline in target lesions per RECIST 1.1 ($n = 28$). **B,** Percentage of viable tumor cells in the surgical specimen at the primary tumor site ($n = 25$) and in the nodal metastases ($n = 19$).

groups, $P < 0.001$; 95% CI: −60 to −20). When the analysis was performed using the percentage of viable tumor cells in the lymph nodes, the association did not reach statistical significance (Supplementary Tables S3 and S4).

Disease progression

At the cut-off date of November 8, 2019, the median follow-up time was 15.79 months (range, 8.38–23.83). At date, all patients were alive, and only one patient, with recurrent disease at baseline, had experienced an additional locoregional recurrence, 9 months after initiation of neoadjuvant CPI therapy.

Quality of life

Twenty-four patients (12 in each arm) completed at least one MDASI-HN questionnaire, and 14 patients completed questionnaires at all three time points (day 1, day 29, and presurgery; Supplementary

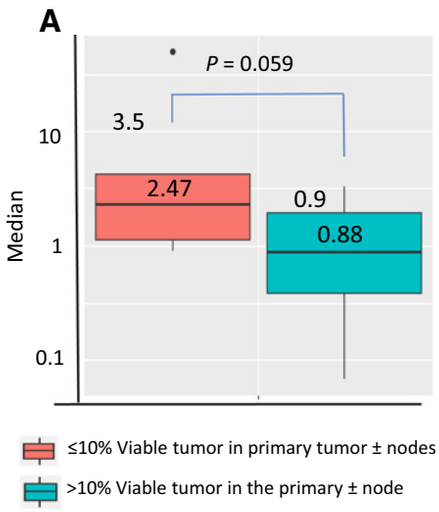

Figure 4.
$CD8^+$TIL changes by pathologic response. **A,** Ratio of posttreatment to pretreatment $CD8^+$TIL density by pathologic response ($n = 8$). **B,** Representative images showing increase in $CD8^+$TIL density between before (Pre) and after (Post) CPI administration.

Table S5). The median global MDASI scores were all less than 1, indicating mild symptoms (Supplementary Table S6). *Post hoc* tests did not detect MDASI-HN score differences between treatment arms at any time point or differences within each treatment arm in global MDASI scores across time points (Supplementary Tables S7 and S8).

Discussion

There has been a resurgence of interest in neoadjuvant systemic therapy across many cancer types given the encouraging activity and tolerability of CPIs. To our knowledge, this is the first study to report $CD8^+$TIL changes, safety, and efficacy of CPI therapy before surgery in OPC.

Interestingly, our hypothesis that tremelimumab + durvalumab would increase $CD8^+$TIL density more than durvalumab alone was not confirmed, which is consistent with our efficacy results and the previously reported lack of benefit of adding tremelimumab to durvalumab for recurrent/metastatic HNSCC (20, 21). Whether our results are due to the tremelimumab dose (1 mg/kg equivalent), the choice of anti-CTLA-4 (tremelimumab instead of ipilimumab), or lack of responsiveness of OPC to anti-CTLA-4 remains to be determined.

Two-thirds of the patients enrolled in this trial were newly diagnosed. In such patients with highly curable disease, demonstration of safety of neoadjuvant treatment is imperative. In our study, all patients underwent the proposed surgery, and the rate of surgical complications, the majority of which (3/4, 75%) occurred in patients undergoing salvage surgery, was not noticeably higher than the rate previously reported in patients with OPC who did not receive neoadjuvant therapy (22). The adverse events observed were as expected for the agents used, and patient-reported outcomes revealed no significant quality-of-life impact of neoadjuvant CPI therapy in either treatment arm (21).

The response rate by RECIST of 43% compares favorably with response rates reported with anti-PD1 for recurrent/metastatic disease (13%–16%; refs. 23, 24). In a phase II study of durvalumab for recurrent/metastatic HNSCC, the overall response rate was higher in patients with HPV-related OPC (29.4%), the disease type for most of the patients (86%) enrolled in CIAO (11). In general, rates of response to systemic therapy are higher in the neoadjuvant than in the palliative setting (25, 26). Reasons for that may include less genetic heterogeneity, fewer resistant clones as a result of previous therapy, and a more robust immune response.

Overall, 2 patients (7%) in CIAO had a MPR, and an additional 6 patients (21%) had a MPR in lymph nodes but not at the primary tumor site. In breast cancer, different response to neoadjuvant chemotherapy at the primary tumor and nodal sites has been well documented; however, responses to cytotoxic drugs are more common at the primary tumor site. Nevertheless, regardless of whether viable tumor cells remain at the primary tumor site, pCR in the nodes is more strongly associated with improved long-term outcomes than pCR at the primary tumor site is (27). Our observation of a higher MPR rate at the nodes than at the primary tumor site is intriguing. Characterization of the immune environment of the primary tumor and matched nodal metastases is planned.

In patients with OPC, survival is not the only outcome of interest; cosmesis and function, which are essential for good quality of life, are also important. Radiotherapy, particularly when combined with chemotherapy, can negatively impact swallowing outcomes; thus, the possibility of omitting adjuvant radiotherapy with or without concurrent chemotherapy in patients with a MPR detected at surgery is intriguing. While CIAO was not intended to be a therapeutic deintensification study, 45% of newly diagnosed patients with OPC were treated with CPI therapy and surgery without radiotherapy. This compares favorably with rates of avoidance of radiotherapy in two previously published series of patients with OPC treated with up-front transoral robotic surgery, in which only 29% of patients did not receive adjuvant radiotherapy, particularly given that the patients in the two earlier studies had earlier-stage disease (57% and 65%, respectively, had stage I–III and none had stage IVB disease) than the patients enrolled in CIAO (28, 29).

It is well established that MPR to chemotherapy predicts better outcomes in HNSCC; however, whether this is due to a direct effect of treatment or to "chemotherapy selection" of patients with improved prognosis for other reasons is not known (30, 31). Treatment deescalation for patients with a MPR to CPI therapy, whether due to a direct effect of treatment or to "immunotherapy selection," seems reasonable considering the favorable toxicity profile of CPIs and the potential for immunologic memory leading to long-term tumor surveillance.

Our finding of higher $CD8^+$TIL density in patients with MPR, although not statistically significant, agrees with the findings of many previously published studies of neoadjuvant CPI therapy and confirms the central role of $CD8^+$TIL in the mechanism of response to CPIs. On the other hand, in our study, neither $CD8^+$ T cells at the invasive margin or intratumorally nor PD-L1 expression at baseline correlated with MPR. The correlation between $CD8^+$ T cells and PD-L1 expression at baseline and response to CPI therapy has been inconsistent in studies across many cancer types, including HNSCC (23, 32–34).

While our median follow-up time was short, it was longer than the median time to progression previously reported for newly diagnosed and recurrent OPC, approximately 8 months (35). Preclinical and clinical studies evaluating PD-1 inhibitors with or without anti-CTLA-4 as neoadjuvant versus adjuvant therapy have demonstrated better outcomes when these drugs are administered before local treatment, possibly because of more robust expansion of tumor-specific T cells in the primary tumor and peripheral blood (36–38).

Although this was a randomized clinical study, it was powered for correlative analyses. As such, interpretation of efficacy outcomes is limited by the small sample size, the mixed population (newly diagnosed and recurrent cases), and the limited follow-up period. Taken together with other studies accessing neoadjuvant CPI therapy in HNSCC, most of which examined oral-cavity primary tumors, CIAO confirms the feasibility of this strategy (39, 40).

In conclusion, neoadjuvant durvalumab with or without tremelimumab was safely administered to patients with OPC and well tolerated. Compared with single-agent durvalumab, combination treatment did not lead to a greater increase in $CD8^+$TIL density or better responses. The activity of CPIs was encouraging, but these results require validation in a larger cohort. Interrogation of biological specimens is ongoing and should provide insight into the biological effects of neoadjuvant CPI therapy in OPC, guide biomarker discovery, and provide a rationale for testing novel combinations in future trials. The use of MPR to CPI therapy to select patients for local treatment deintensification could significantly improve patient's quality of life and merits further investigation.

Disclosure of Potential Conflicts of Interest

R. Ferrarotto is an employee/paid consultant for Regeneron-Sanofi, Ayala Pharma, Klus Pharma, Medscape, Cellestia Biotech, Carevive, and Prelude. K.A. Hutcheson is an employee/paid consultant for The University of Texas MD Anderson Cancer Center, reports receiving speakers bureau honoraria from MedBridge, and is an advisory board member/unpaid consultant for

American Speech Language Hearing Association. J.M. Johnson is an employee/paid consultant for Kura Oncology, and reports receiving commercial research grants from Blue Earth Diagnostics. F.M. Johnson reports receiving commercial research grants from PIQUR Therapeutics and Trovagene. A. Sikora reports receiving commercial research grants from Tessa Therapeutics, SQZ and Heat/Pelican. M.L. Gillison is an employee/paid consultant for EMD Serono, AstraZeneca, Amgen, TRM Oncology, NewLink, Shattuck Labs, Merck, BMS, Roche, Bayer, Genocea, and Aspyrian, and reports receiving commercial research grants from Genocea, BMS, and Cullinan. N.D. Gross is an employee/paid consultant for PDS Biotechnology, Shattuck Labs, Genzyme, and Intuitive Surgical, and reports receiving commercial research grants from Regeneron. No potential conflicts of interest were disclosed by the other authors.

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