Title

Phase II Clinical Trial of Neoadjuvant and Adjuvant Pembrolizumab in Resectable Local–Regionally Advanced Head and Neck Squamous Cell CarcinomaNeoadjuvant and Adjuvant Pembrolizumab in Resected HNSCC

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Phase II clinical trial of neoadjuvant and adjuvant pembrolizumab in resectable local-regionally advanced head and neck squamous cell carcinoma

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Abstract

Purpose: Patients with resected, local-regionally advanced, head and neck squamous cell carcinoma (HNSCC) have a 1-year, disease-free survival (DFS) of 65-69% despite adjuvant

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(chemo)radiotherapy. Neoadjuvant PD-1 immune checkpoint blockade (ICB) has demonstrated clinical activity, but biomarkers of response and effect on survival remain unclear.

Experimental Design: Eligible patients had resectable squamous cell carcinoma of the oral cavity, larynx, hypopharynx or oropharynx (p16-negative) and clinical stage T3-T4 and/or two or more nodal metastases or clinical extracapsular nodal extension (ENE). Patients received neoadjuvant pembrolizumab 200mg 1-3 weeks prior to surgery, were stratified by absence (intermediate-risk) or presence (high-risk) of positive margins and/or ENE, and received adjuvant radiotherapy (60-66Gy) and concurrent pembrolizumab (q3 wks x 6 doses). Patients with high-risk HNSCC also received weekly, concurrent cisplatin (40mg/m2). Primary outcome was 1-year DFS. Secondary endpoints were 1-year OS and pathological response (PR). Safety was evaluated with CTCAE v5.0.

Results: From February 2016 to October 2020, 92 patients enrolled. Median age was 59 years (range, 27 – 80), 30% were female, 86% had stage T3-T4 and 69% had ≥N2. At a median follow-up of 28 months, 1-year DFS was 97% (95% CI 71-90%) in the intermediate-risk group and 66% (95% CI 55-84%) in the high-risk group. Patients with a PR had significantly improved 1-year DFS relative to patients without response (93% vs 72%, HR 0.29, 95% CI 11-77%). No new safety signals were identified.

Conclusions: Neoadjuvant and adjuvant pembrolizumab increased 1-year DFS rate in intermediate-risk, but not high-risk, HNSCC relative to historical control. PR to neoadjuvant ICB is a promising surrogate for DFS.

BACKGROUND

Approximately 745,000 new cases and 364,340 deaths from head and neck squamous cell carcinoma (HNSCC) occurred in 2020[1]. A majority of patients present with local-regionally advanced HNSCC for which the current standard of care is surgical resection followed by adjuvant (chemo)radiation (RT). Two randomized control trials demonstrated increased disease-free survival (DFS) in resectable HNSCC when cisplatin was administered concurrent with adjuvant RT versus RT alone, albeit with higher toxicity[2,3]. A comparative analysis showed the survival benefit was restricted to patients with high-risk pathological features of positive margins and/or extracapsular nodal extension (ENE)[4]. NCCN guidelines therefore recommend chemoRT for the high-risk group and RT alone in those with intermediate-risk features (e.g. multiple lymph node involvement, perineural invasion, lymphovascular invasion, T3 or T4 stage). Unfortunately, risk of progression remains high in both groups: in RTOG 9501, one-year DFS was 65% for the high-risk group with chemoRT and 69% in the intermediate-risk group with RT alone (provided by Jonathan Harris [ACR]). Novel treatments are necessary to improve outcomes in these patient populations.

A majority of HNSCCs express the inhibitory immune checkpoint programmed death ligand -1 (PD-L1), which binds to the PD-1 receptor expressed on cytotoxic T cells to suppress their function. PD-1 immune checkpoint blockade (ICB) with pembrolizumab or nivolumab increased overall survival (OS) in patients with recurrent/metastatic or platinum-refractory HNSCC, respectively[5,6]. In murine HNSCC xenograft models, upregulation of PD-L1 was observed in response to RT, and the addition of PD-L1 ICB to RT led to

superior survival [7]. Therefore, we hypothesized that the addition of pembrolizumab to adjuvant RT could improve survival in HNSCC patients after primary surgical resection and that neoadjuvant pembrolizumab would allow for interrogation of potential correlative biomarkers of response.

We performed a multi-center phase II window of opportunity clinical trial (NCT02641093) to estimate 1-year DFS in patients with local-regionally advanced, resectable, HNSCC when neoadjuvant and adjuvant pembrolizumab was added to standard of care (chemo)RT. Secondary outcomes included OS, toxicity, pathological response (PR) and tumor immune microenvironmental changes after neoadjuvant pembrolizumab.

METHODS

Study Design and Treatment

The study was designed as a multi-center, open-label, non-randomized, two-arm phase II trial of the addition of neoadjuvant and adjuvant pembrolizumab with or without concurrent cisplatin in patients with surgically resectable previously untreated, local-regionally advanced HNSCC. Patients and investigators were not masked. The trial was registered on clinicaltrials.gov (NCT02641093). The study was approved by the institutional review boards of all participating sites and was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients were required to sign written informed consent. The study was monitored by the University of Cincinnati Cancer Center Data Safety Monitoring Board.

Eligibility criteria included: age ≥ 18 years; newly diagnosed histologically or cytologically confirmed HNSCC; local-regionally advanced stage III/ IV AJCC 8th edition T3 or T4 or ≥N2 disease or clinical evidence of ENE on diagnostic imaging; Disease was determined resectable by the treating head and neck surgeon with no involvement of skull base or T4b stage; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1; and adequate organ function. Key exclusion criteria included: human papillomavirus (HPV+) oropharyngeal cancer (HPV positive disease outside of oropharynx was allowed, but testing was not required); nasopharyngeal cancer; metastatic disease determined by chest CT and/or PET/CT; autoimmune disease; active intercurrent illness (e.g. significant cardiovascular disease, viral infections or major psychiatric illness); and steroid use (> prednisone 10mg daily). Please refer to full eligibility criteria in the supplement. Full clinical trial protocol is available upon request.

All patients received one dose of pembrolizumab (200mg IV) 7-21 days prior to surgery (schema, Suppl. Fig. 1A). After surgery, patients were stratified into two arms based on presence or absence of high-risk pathological features (e.g. positive margins and/or ENE). Patients received adjuvant pembrolizumab 200mg IV every three weeks for a total of up to six doses starting 1 week prior to intensity modulated RT to 60-66Gy in 30-33 fractions over six weeks. High-risk patients received concurrent weekly cisplatin (40mg/m^2) for up to six doses during radiation. Dose reductions, modifications and/or interruptions were performed per standard of care for cisplatin and investigational brochure for pembrolizumab.

The first 8 patients in the intermediate and high-risk groups were enrolled in a safety lead-in designed to investigate dose limiting toxicity (DLT), defined as an adverse event attributable to pembrolizumab that resulted in a delay in initiation of standard of care treatment (e.g. >7 days for surgery, >3 days for RT, >7 days for cisplatin). The study arm was to be discontinued if more than two of eight patients developed a DLT. The primary endpoint was 1-yr DFS and secondary endpoints included overall survival (OS) and change in the tumor immune microenvironment after neoadjuvant pembrolizumab.

Pathological analyses

The resected tumor specimen was evaluated by pathologists as part of the standard of care for the presence of pathological risk features including ENE, margin status, lymphovascular invasion (LVI), perineural invasion (PNI), and number of lymph involved nodes.

Pathological treatment effect (TE) and PD-L1 status were determined on central review by a board-certified pathologist at the University of Cincinnati (BH). TE was defined as tumor showing necrosis with associated histiocytic inflammation and/or giant cell reaction to keratinaceous debris. TE percentage was determined by dividing estimates of total area showing the latter features by the total area showing residual viable tumor and TE. Based on the percentage of TE seen, pathological response (PR) was divided into no (NPR, <20%), partial (PPR, ≥20% and <90%) or major (MPR, ≥90%) PR. PR was also determined for those patients with positive lymph nodes and tissue available for analysis.

PD-L1 expression was evaluated using the 22C3 antibody clone (Agilent, Dako) and pharmDx IHC assay at either Caris Life Technologies or Neogenomics and confirmed by BH. PD-L1 expression on both tumor and tumor infiltrating immune cells were evaluated. For tumor cells, membranous staining of any intensity was considered positive. Only inflammatory cells infiltrating invasive tumor and in adjacent intra- and peri-tumoral stroma were scored. Combined positivity score (CPS) was calculated by summing the numbers of PD-L1 positive tumor cells and immune cells and dividing by the total number of viable tumor cells [8].

mRNA expression analysis

Paired formalin-fixed and paraffin-embedded tumor specimens from before and after neoadjuvant pembrolizumab were selected from the first 23 available patients with PPR/MPR (n=11) vs NPR (n=12). Purified total RNA was evaluated by a hybridization-based digital counting assay (Nanostring® nCounter platform) using the PanCancer IO 360™ panel (for research use only) that measures 770 immune related genes and controls. Raw data counts were normalized using the geometric mean of the housekeeping genes, and each gene was adjusted based on IO360 panel standards and normalized data was $\log_2$ transformed. Gene expression signature scores for 48 signatures measuring immune cell abundance, immune signaling, tumor and stromal biology [9] were calculated as weighted averages of the signature genes with a signature-specific constant added to express values in a similar range [10]. Signature scores were compared between pre- and post-pembrolizumab treatment and between those with PPR/MPR and NPR.

Statistical analysis

The All-Patients-as-Treated (APat) population including any patient receiving at least one dose of pembrolizumab was used for safety analysis. Safety endpoints included all adverse events (AEs) graded 1 to 5 per CTCAE v5.0. The proportion of AEs among patients treated with pembrolizumab and RT and pembrolizumab and chemoRT were compared to historical control patients treated with RT only and chemoRT, respectively. Descriptive analysis of grade ≥3 AEs were compared to data from RTOG 9501 (e.g. 46% for RT and 78% for chemoRT)[3].

All patients who received ≥1 dose of adjuvant pembrolizumab were considered evaluable for efficacy per protocol and served as the primary analysis population in this study. Patient attrition is included in the consort diagram in Supplemental Figure 1. The primary efficacy endpoint was 1-year DFS, defined as time from treatment allocation to documented relapse or death. Key secondary endpoints include 1-year and 2-year overall survival (OS), defined as time from treatment allocation to death due to any cause. With an expected sample size of approximately N=40 in both risk groups, we expected to reach over 80% power to detect an increase of 19-21% in DFS at year 1 with the addition of pembrolizumab. Kaplan-Meier method was used to estimate survival rates for 1-year and 2-year DFS and OS. The survival rates were compared to historical censored data (RTOG 9501) using the log-rank test. We used estimates of the survival probabilities at specific time points of the RTOG 9501 trial for DFS in order to compare our results. All data and events up to 4 years of follow-up in RTOG 9501 were used to be consistent with our study. We repeated these analyses by limiting the follow-up to one year and censoring all patients who recurred after that time. The hazards were compared using Wald test in a Cox proportional hazards model.

Statistical analysis of gene expression signature scores was performed using the Empirical Bayes Linear Model as implemented in the *limma* R package[11]. Signature scores at baseline and difference in score before and after neoadjuvant pembrolizumab were compared in patients with PPR/MPR versus NPR. For the baseline comparison, the signatures scores were normalized by subtracting the median score for each sample, and were then compared using Empirical Bayes two-sample t-test. For the comparison of differences, the patient level differences were calculated by subtracting the before scores from after scores for each patient separately, and differences were compared using the Empirical Bayes two-sample t-test. Gene signatures differences with FDR (false discovery rate) adjusted[12] p-values less than 0.1 were considered statistically significant.

The PD-L1 expression was categorized as 0, 1-19, and ≥20 for CPS and 0, 1-49, and ≥50 for TPS, and associations with PR, DFS, and OS were evaluated. Fisher's exact test was used to compare associations between PD-L1 expression and PR. Kaplan-Meier estimates and log-rank test were used to determine difference in survival probabilities for DFS and OS.

Raw data for mRNA signatures were generated in a core facility (Nanostring®). Patient level data for biosignatures are provided in supplemental data (Suppl Table 9). All other data was generated by the authors and included in the article or supplemental data.

RESULTS

Patient population

Between January 2016 and October 2020, 92 patients were enrolled (Table 1). Median age was 59 years (27 – 80). The majority were male (70%), white (95%), diagnosed with oral cavity cancer (86%), had T3-T4 stage (86%) and cervical nodal metastases (81%). No patients were enrolled based on clinical ENE alone. Four patients did not proceed with surgical resection: two due to rapid progression of disease, one due to presence of unresectable disease at the skull base which was not identified on pre-operative scans, and one patient due to withdrawal of consent (Suppl. Fig. 1B). Of the 92 All-Patients-as-Treated (APaT) patients evaluable for toxicity and who received at least a single dose of neoadjuvant pembrolizumab, 42 patients were in the intermediate-risk and 50 were in the high-risk group (including the 4 patients that did not proceed with surgery). Of the resected patients, 75 (31 intermediate and 44 high-risk) patients received adjuvant treatment and therefore were evaluable for efficacy per protocol. The most common reasons for not proceeding with adjuvant treatment per protocol included identification of secondary malignancy at time of surgery (thyroid cancer), and withdrawal of consent (Suppl. Fig. 1B).

Survival outcomes

At a median follow-up of 28 months among all evaluable patients (N=75), the 1-year DFS rate was 80% (95% CI, 71-90%, Fig. 1A) with a hazard ratio of 0.60 (95% CI, 0.39-0.93; p=0.0233) which was significantly higher when compared to the entire cohort from RTOG 9501. Forty-four (58%) had high-risk pathological features: 39 (52%) had ENE and 17 (23%) had positive margins (Suppl Table 1). One-year DFS was 96% (95% CI, 90-100%) for the intermediate-risk group (N=31). This was significantly higher than the 1-year DFS of 69% (95% CI, 59-78%) observed in the intermediate-risk group treated with RT alone in RTOG 9501 (p=0.0007, Fig. 1B) with a hazard ratio of 0.23 (95% CI, 0.09-0.58; p=0.0018). 1-year OS was also higher relative to historical control in the intermediate-risk group (Fig. 1C). Similar results were seen when comparing the intent to treat (ITT) population (N=96) (Suppl. Fig. 2).

In contrast, 1-year DFS rate in the high-risk group was 66% (95% CI, 55-84%), which was similar to that observed for this population in RTOG 9501 treated with chemoRT (65%; 95% CI, 57-64%, Fig 1B) with a hazard ratio of 0.86 (95% CI, 0.52-1.44; p=0.5736). Among the high-risk group, DFS was not significantly different among patients stratified by presence of ENE and/or positive margins (Suppl. Fig. 3).

Analysis of pathological response (PR)

Representative images of primary tumors from each PR group are shown in Fig. 2A. PR (PPR/MPR) was observed in 39% of evaluable patients, and MPR in 7% (Fig. 2B). Rates of PR were higher in the intermediate-risk group (55%) than in the high-risk group (28%) (Fig. 2B and 2C). Of those patients with primary site and lymph node (LN) resection tissue available for evaluation, 23 out of 28 (82%) patients had concordance of PR between both primary site and LNs (Suppl Table 2). The other five patients had a PR in primary site but not LNs. In general, PR was lower in LNs compared to primary site.

When compared to the clinical stage at enrollment, pathological downstaging after neoadjuvant pembrolizumab was more frequent in patients with intermediate-risk than high-risk features (55% versus 7%, P<0.0001, Suppl Table 3). This difference was less pronounced when rates of pathological downstaging were compared in patients with (PPR/MPR, 34%) and without a PR (NPR, 22%, Suppl Table 4).

We investigated associations between PR to neoadjuvant pembrolizumab and DFS and OS. Importantly, those that had a PPR/MPR had significantly increased 1-year DFS rate compared to those with NPR (93% [95% CI, 84-100%] versus 72% [95% CI, 59-87%], p=0.0086, Fig. 2D), respectively. OS was also significantly different in the two groups (100% [95% CI, 100-100%] vs 93% [95% CI, 85-100%], p=0.004, Fig. 2E).

Toxicity

No DLTs were observed during the eight-patient safety lead-in in either group. Treatment-related adverse events (TRAEs), defined as attributed to pembrolizumab, radiation and/or cisplatin, of any grade occurred in 78 (85%) patients (Suppl Table 5). Grade ≥3 TRAEs occurred in 15 of 42 (36%) patients in the intermediate-risk group and in 32 of 50 (64%) patients in the high-risk group (Table 2). Comparable rates in RTOG 9501 were 46% and 78%, respectively. Surgical wound complications including dehiscence, fistulas, and/or infections were reported in 33 (36%) patients. All grade pembrolizumab related AEs were reported in 27 patients in the intermediate-risk group and 35 patients in the high-risk group. No immune related AE associated deaths were noted. One patient in each group discontinued immunotherapy due to gastrointestinal toxicity attributable to pembrolizumab.

Association between PD-L1 expression and PR

Among 72 evaluable patients, PD-L1 CPS in the baseline tumor tissue was 0 in 20 (28%), and ≥1 in 52 (72%) patients. Higher PD-L1 (CPS ≥ 1 and ≥20) was associated with PPR/MPR (p=0.0183) (Table 3). TPS was also calculated and showed similar trends with significant differences between TPS and PR (p=0.0074). Supplemental Table 6 includes all patients for which CPS and TPS were available for the intent to treat group. However, PD-L1 expression was not associated with DFS or OS in univariate analysis (Suppl. Fig. 4). No difference in PD-L1 CPS was detected between high and intermediate-risk groups (Fig. 3A).

Association between PR and the tumor immune microenvironment

In order to better understand and characterize patients who develop PR compared to those who do not, gene expression levels were analyzed in a subset of patient tissues pre- and post-pembrolizumab. Measured gene expression signatures (GES) were grouped into 48 pathways representing immune cell phenotypes, and tumor and stromal cell characteristics. The heat map in Suppl. Fig. 5 shows the GES scores for all 48 pathways in all patient samples. Upon further analysis, the most significantly (FDR <0.1) differentially expressed gene/GES between PPR/MPR and NPR patients at baseline (pre-treatment tissue) are shown in Fig. 3B. Single gene expression of IDO-1, PD-L1 and PD-L2 and interferon gamma signaling GES were all significantly higher in those with PPR/MPR versus NPR at baseline using the Empirical Bayes Linear Model. When comparing changes in expression from

post-treatment (surgical tissue after pembrolizumab) to pre-treatment, the signatures with the most significant difference (FDR<0.1) in pre/post scores between NPR and PPR/MPR are shown in Fig. 3C. GES for macrophages, major histocompatibility complex −2 (MHC-2) and mast cells were significantly higher post-treatment whereas proliferation was decreased post-treatment in patients with PPR/MPR. The values for biosignatures at baseline and the change from baseline are included in Supplementary Tables 7 and 8. Patient level data for biosignatures are also provided in supplemental data (Suppl Table 9).

Discussion:

When neoadjuvant and adjuvant pembrolizumab is added to SOC (chemo)RT, 1-year DFS is estimated as 97% and 66% in patients with intermediate-risk and high-risk, resected HNSCC, respectively. These estimates are significantly higher in comparison to historical controls from RTOG 9501 for the entire cohort, and when stratified by pathological risk features, the intermediate-risk group only. This is true even when time of enrollment is adjusted to RT completion for more direct comparison to RTOG 9501 as well as when the intent to treat population is compared (Suppl Fig. 2). Importantly, PR to neoadjuvant pembrolizumab is associated with a significant improvement in DFS relative to NPR patients. Additionally, rates of PPR/MPR increase significantly with increased PD-L1 CPS. Our data indicate that PD-L1 CPS may be a predictive biomarker of PPR/MPR and that PPR/MPR is a surrogate marker of long-term disease control. These data confirm and extend results from similar window of opportunity studies in HNSCC and other tumor types[13–20].

Uppaluri and colleagues[14] demonstrated previously that patients with high-risk resected HNSCC who received neoadjuvant and adjuvant pembrolizumab had a lower rate of 1-year distant or local relapse rate (16.7%) when compared to historical controls (35%). In contrast, the 1-year relapse rate of the high-risk group in our study (32%) was similar to historical controls. Several factors may contribute to this difference in outcomes. A higher proportion of patients in our study had oral cavity primary cancers, which have a worse outcome than other anatomic sites. In our study, pembrolizumab was administered concurrently with chemoRT whereas in the Uppaluri trial pembrolizumab was administered after completion of chemoRT. Immunosuppression associated with cytotoxic chemotherapy may impair immune responses associated with PD-1 ICB. In support of this hypothesis is the lack of improvement of OS when the PD-L1 inhibitor avelumab was administered concurrent with chemoRT in local-regionally advanced HNSCC[21]. This is in contrast to combined chemotherapy and PD-1 blockade in recurrent and metastatic HNSCC which did result in an improvement in OS for those with PD-L1 CPS ≥1[22], which may suggest inherent differences in treatment-naïve and refractory patients.

Patients in the intermediate-risk group had an improvement in 1-year DFS and OS with the addition of pembrolizumab when compared to historical controls. This intermediate-risk group also had relatively high rates of downstaging when pathological stage was compared with clinical stage at enrollment (Suppl Table 2). In addition, the rate of high-risk pathology (59%) in this current study was somewhat lower compared to historical studies RTOG 9501 and EORTC 22931 for which rates were 60-70%[4]. Therefore, it is possible that response to neoadjuvant pembrolizumab is associated with resolution of high-risk pathological features.

Given that intermediate-risk patients are known to have favorable outcomes compared to high-risk patients and that current trials are investigating additional treatments in this intermediate-risk group (cetuximab in RTOG 0920), further interrogation to understand the population of patients that convert to a more favorable risk status is imperative.

Given the evidence of downstaging, it is also important to consider that the high-risk group survival endpoints were underestimated due to previously high-risk patients being assigned to the intermediate-risk group after neoadjuvant pembrolizumab. In addition, our high-risk group had increased poor prognostic risk factors in general with a higher proportion of oral cavity (86%) compared to historical controls (30%), and oropharyngeal cancers were all HPV negative.

The impact of PR to neoadjuvant immunotherapy on long-term disease control remains an active area of investigation for several tumor types. Complete (cPR) and MPR are considered the most clinically significant outcome measures. In lung cancer, MPR rates as high as 45% have been reported after neoadjuvant PD-1 ICB [23]. In contrast, MPR rates are consistently <10% in HNSCC after PD-1 ICB[19] (Fig. 2B). The findings from this study suggest that a PPR, defined by a TE as low as 20%, may be a clinically meaningful measure of benefit from neoadjuvant PD-1 ICB and a surrogate for long-term disease control. DFS and OS at 1-year were 93% and 100%, respectively, among the 39% of patients who experienced a PPR, regardless of pathological risk features or PD-L1 status. These data suggest that pembrolizumab mediated PR may be a stronger predictor of survival than pathological risk features or tumor PD-L1 status.

Patient selection for neoadjuvant PD-1 ICB as well as those most likely to benefit from adjuvant treatment would be improved should a predictive biomarker of PR be identified at diagnosis. As mentioned, PD-L1 was strongly correlated with PR (Fig. 3A) but not directly related to survival. Therefore, PD-L1 expression alone, is not sufficient to predict long-term disease control. Our gene expression data also suggest IFN-γ, IDO-1, PD-L1 and PD-L2 expression at baseline was predictive of PR. An IFN-γ gene expression profile appears necessary but insufficient to predict pembrolizumab response in metastatic/recurrent HNSCC and is currently being validated in clinical trials as a predictor of PD-1 ICB response[24]. Macrophages and mast cells also appeared to be important mediators of PPR/MPR as they increased substantially upon treatment with pembrolizumab. Our data indicate that the additional activation of the myeloid compartment may enhance the response and survival achieved with PD-1 ICB alone.

Hyperprogression upon ICB treatment has been described in the literature and has been associated with *EGFR* amplification[25]. Three patients out of 92 were unable to proceed with curative intent surgery due to either involvement of the skull base not initially identified on pre-operative scans, or disease that progressed quickly during the 7-21 day window prior to surgery. Therefore, it is prudent to consider that a small portion of patients may experience more aggressive growth of disease upon ICB treatment in the neoadjuvant setting, necessitating the development of biomarkers to predict potential hyperprogression in these patients. Given the rare occurrence on this study, it is unclear if these patients truly experienced hyperprogression or had more aggressive biology at baseline.

This study has several important limitations. Use of historical controls, prior to improvement in standard and supportive therapies, is a poor substitute for prospective randomization and comparison to placebo control, and therefore our findings should be interpreted with caution. Our median follow-up time and outcomes were shorter by design as a signal-seeking phase II trial. Ongoing randomized clinical trials will clarify the effect of the addition of PD-1 ICB on longer-term survival outcomes and potential ICB related toxicities when combined with surgery. These include a randomized, placebo-controlled trial of neoadjuvant and adjuvant PD-1 ICB in patients with high-risk HNSCC (NCT03765918) and RTOG 1216 (NCT01810913). In the latter, patients with high-risk resected HNSCC are randomized 2:1:1 to receive adjuvant atezolizumab and cisplatin vs docetaxel and cetuximab vs. SOC cisplatin RT. However, there is no current randomized study restricted to patients in the intermediate risk group. Randomized studies are needed to determine the validity of using PR, PD-L1 CPS and macrophage infiltration as reliable biomarkers for survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest:

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Translational Relevance:

Patients with resected, local-regionally advanced, head and neck squamous cell carcinoma (HNSCC) have a poor disease-free survival (DFS) despite adjuvant (chemo)radiotherapy. Neoadjuvant PD-1 immune checkpoint blockade (ICB) has demonstrated clinical activity in this patient population, but biomarkers of response and effect on survival remain unclear. Compared to historical controls, the addition of pembrolizumab improved the 1-year DFS rate in intermediate-risk, but not high-risk, resected HNSCC. Importantly, pathological response (PR) to neoadjuvant pembrolizumab was associated with PD-L1 status and significantly higher DFS. Therefore, PR to neoadjuvant PD-1 ICB is a likely surrogate for DFS in resectable HNSCC. The effect of neoadjuvant and adjuvant PD-1 ICB on survival of local-regionally advanced, resectable, HNSCC and associated biomarkers of PR warrant investigation in a randomized clinical trial.

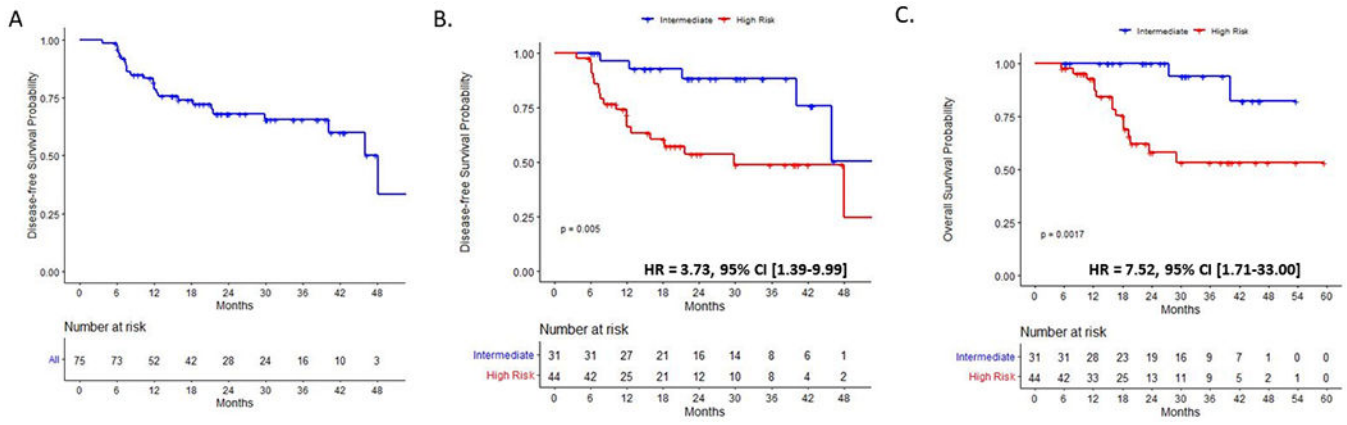

Figure 1. Survival Stratified by Pathological Adverse Features.

KM curves representing all patients DFS (A), as well as DFS (B) and OS (C) stratified by high and intermediate risk disease. P value by KM method provided. Hazard ratios (HR) were calculated by comparing high-risk to intermediate-risk.

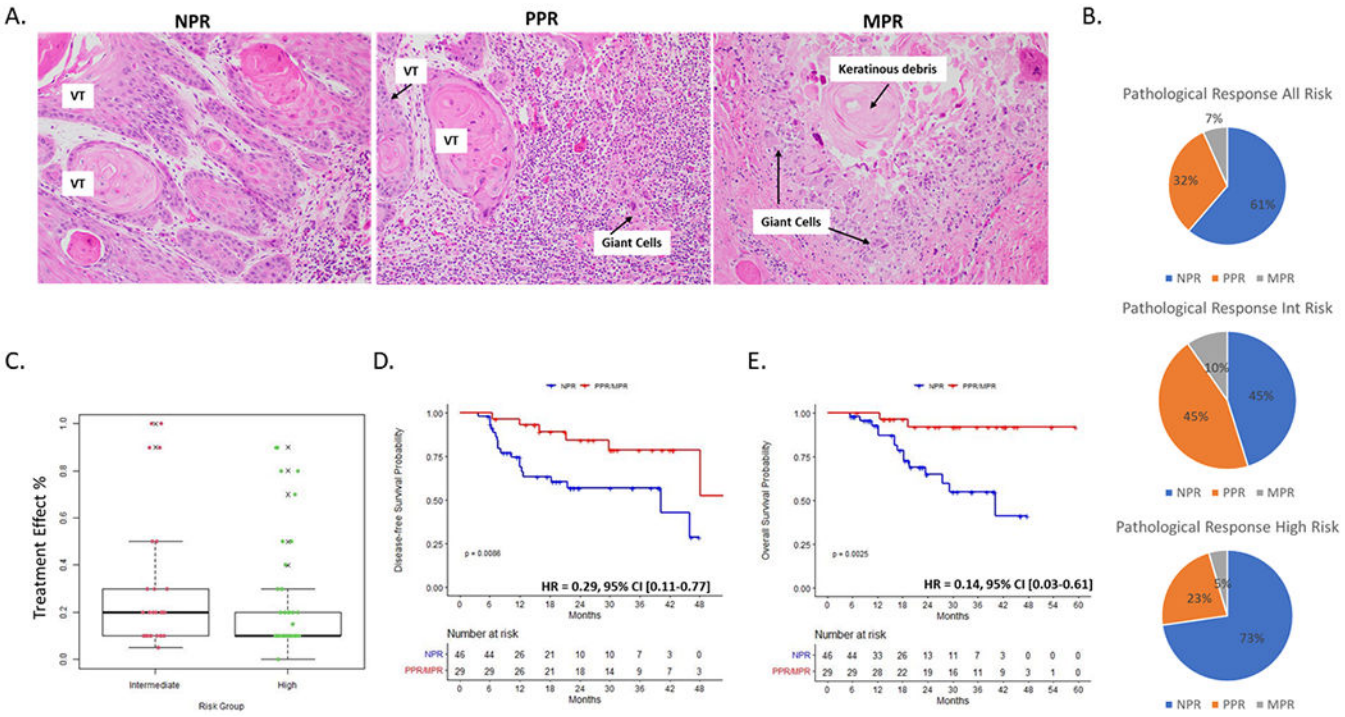

Figure 2. Pathological responders have increased survival.

A. Representative H&E pictures of patients with no (NPR), partial (PPR) and major (MPR) pathological response characterized by <20%, 20-90% and ≥ 90% treatment effect (TE) respectively. Images were all at 200x. TE was defined as tumor necrosis with associated histiocytic inflammation and/or giant cell reaction to keratinaceous debris. TE percentage was determined by dividing estimates of area showing these features by the total area showing residual viable (VT) and TE. B. Proportion of patients with NPR, PPR or MPR. C. Percent treatment effect in intermediate and high-risk patients. Survival curves comparing NPR and PPR/MPR for DFS (D) and OS (E).

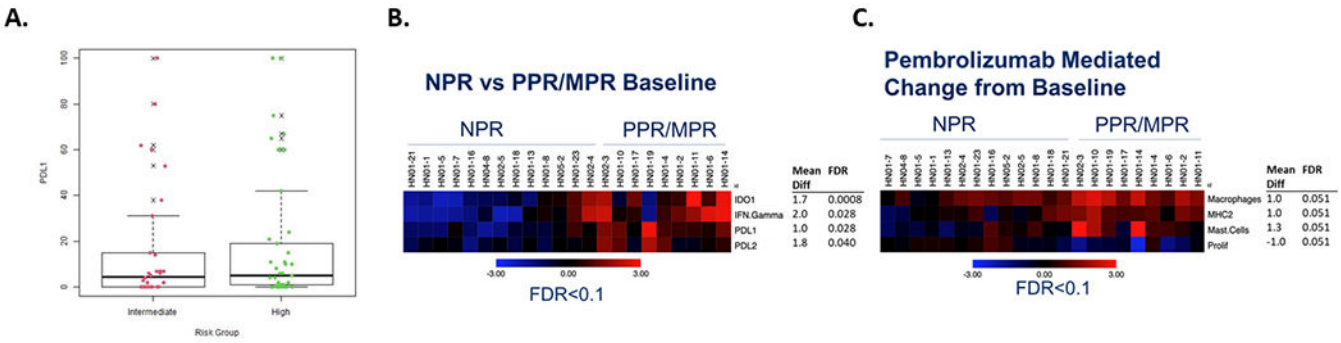

Figure 3. PD-L1 expression and immune gene expression signature compared to pathological response.

A. PD-L1 was measured using the 22c3 antibody and CPS was determined. Comparison of PD-L1 CPS in intermediate and high-risk groups. There was no statistical difference between groups. B. Comparison of most differentially expressed gene signatures in NPR versus PPR/MPR tissues at baseline prior to treatment. The values are row-centered by subtracting average scores for each signature. C. Comparison of the gene signatures most changed between pre and post treatment tissues in NPR versus PPR/MPR patients. Red designates an increase in expression while blue designates a decrease in gene expression. Changes in B and C were highly significant at FDR <0.1.

Table 1.

Baseline Patient and Disease Characteristics (n = 92)

Patient Characteristics	No. (%)
Median Age y, (range)	59 (27 – 80)
Sex	
Male	64 (70)
Female	28 (30)
Race	
White	87 (95)
African American	2 (2)
Unknown/Other	3 (3)
Smoking History (> 10pk per year)	
Yes	59 (64)
Alcohol History (> 5 drinks per week)	
Yes	41 (45)
Primary Disease Site	
Larynx	10 (11)
Oral Cavity	79 (86)
Oropharynx	2 (2)
Hypopharynx	1 (1)
Tumor Classification	
Tx	1 (1)
T1	3 (3)
T2	9 (10)
T3	18 (20)
T4	61 (66)
Lymph Node Classification	
Nx	1 (1)
N0	16 (17)
N1	11 (12)
N2	60 (65)
N3	4 (4)
ECOG Performance Status	
0	55 (60)
1	37 (40)

Table 2.

Adverse Events by Pathological Risk

	Intermediate risk group (RT+ Pembrolizumab) n=42 * No Grade 4 events	High risk group (RT+ Cisplatin+ Pembrolizumab) **n= 50 **Includes patients that did not proceed with surgery	
	Grade 3	Grade 3	Grade 4
Any Adverse Event: Number of patients (%)	15 (36)	31 (62)	5 (10)
Acute Kidney Injury	0	0	1
Adrenal Insufficiency	1	0	0
Anemia	0	6	0
Anorexia	2	5	0
Aspiration or Lung Infection	0	5	0
Autoimmune Hepatitis	0	1	0
Bone Infection	0	1	0
Colitis/Duodenal Ulcer	1	0	0
Dehydration	0	1	0
Dental Caries	1	0	0
Dermatitis Radiation	1	0	0
Dysphagia	4	8	0
Dyspnea	0	1	0
Fatigue	1	1	0
Febrile Neutropenia/ Neutrophil Count Decreased	0	14	3
Hearing Impaired	0	2	0
Hypokalemia	0	2	0
Hyponatremia	0	1	0
Hypophosphatemia	2	0	0
Lymphocyte Count Decreased	2	6	0
Failure to Thrive	2	1	0
Mucositis Oral	6	6	0
Nausea/vomiting	0	1	0
Pancreatitis	0	1	0
Platelet Count Decreased	0	3	0
Salivary Duct Inflammation	1	1	0
Sinusitis	0	1	0
Skin Infection	1	1	0
Tracheal Obstruction	0	0	1
Weight Loss	5	9	0
Wound Complication	1	0	0

Table 3.

CPS and TPS by PR

CPS	**NPR**	**PPR/MPR**
PD-L1=0	16/20 (80%)	4/20 (20%)
PD-L1 = 1-19	23/35 (66%)	12/35 (34%)
PD-L1 ≥ 20	6/17 (35%)	11/17 (65%)
TPS	**NPR**	**PPR/MPR**
PD-L1=0	27/34 (79%)	7/34 (21%)
PD-L1 = 1-49	10/23 (43%)	13/23 (57%)
PD-L1 ≥ 50	4/10 (40%)	6/10 (60%)