

# A Phase II Trial of Pembrolizumab and Vorinostat in Recurrent Metastatic Head and Neck Squamous Cell Carcinomas and Salivary Gland Cancer



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## ABSTRACT

**Purpose:** This clinical trial combined pembrolizumab and vorinostat in recurrent/metastatic squamous cell carcinomas of the head and neck (HN), and salivary gland cancer (SGC).

**Patients and Methods:** Patients with progressing incurable HN and SGC, Eastern Cooperative Oncology Group (ECOG)  $\leq 1$ , no prior immunotherapy, RECIST1.1 measurable disease, and normal organ function were eligible. Pembrolizumab 200 mg was given intravenous every 21 days, and vorinostat 400 mg given orally 5 days on and 2 days off during each 21-day cycle. Primary endpoints were safety and objective response rates.

**Results:** From November 2015 to August 2017, 25 patients with HN and 25 SGC were enrolled. Median age was 61 (range, 33–86) years, 39 (78%) were male, 21 (62%) were never smokers, and 27 (54%) had ECOG 0. In HN, 13 (52%) were p16+ oropharynx. Most common SGC histologies were adenoid cystic 12 (48%), acinic cell 3 (12%), and mucoepidermoid 3 (12%). Adverse events

(AEs) in all patients were: 27 (54%) with grade  $\geq 1$  and 18 (36%) with grade  $\geq 3$ . The most common AEs in all patients were renal insufficiency in seven, (14%), fatigue in six, (12%), and nausea in three (6%). There were three (12%) deaths on study. Responses in HN were complete response (CR) 0, partial response (PR) eight (32%), and stable disease (SD) five (20%). Efficacy in SGCs was CR 0, PR four (16%) in one lymphoepithelioma-like carcinoma, two acinic cell, one adenoid cystic, and SD 14 (56%). In the HN group, median follow-up (mFUP) was 12.6 months, median overall survival (mOS) was 12.6 months, and median progression-free survival (mPFS) was 4.5 months. In SGC, mFUP was 13.1 months, mOS was 14.0 months, and mPFS was 6.9 months.

**Conclusions:** This combination demonstrated activity in HN, with fewer responses in SGC. Toxicities were higher than reported with pembrolizumab alone.

## Introduction

The anti-PD-1 mAbs have well-demonstrated activity in recurrent metastatic squamous cell carcinomas of the head and neck (HN), and limited efficacy in salivary gland cancers. Two phase III randomized trials in the previously cisplatin-treated recurrent metastatic squamous cell carcinomas demonstrated a survival advantage among patients receiving an PD-1 inhibitor compared with investigators choice second-line chemotherapy (1, 2). More recently, a randomized phase III trial of pembrolizumab versus the EXTREME regimen in first-line recurrent metastatic HN revealed superior survival outcomes in patients receiving pembrolizumab with a combined proportion score (CPS)  $>1$  (3, 4). Among recurrent metastatic salivary gland cancers (SGCs), an uncommon and biologically diverse group of malignancies

with no therapeutic standards of care in the recurrent metastatic setting, a more limited experience with pembrolizumab has been described. Keynote-028 enrolled patients with SGCs and PD-L1 expression  $>1\%$  and observed three objective responses among the 26 study patients (5).

Various therapeutic strategies are under investigation aiming to increase objective response rates (ORRs) and improve clinical outcomes in patients receiving these agents. One intriguing avenue is the concurrent administration of epigenetic modifiers to anti-PD-1 inhibition. DNA methylation and histone hypoacetylation lead to silencing of key genes involved in immune recognition. Preclinical observations support the synergistic activity of epigenetic modification and PD-1 inhibitors through various proposed mechanisms that relate to altering gene expression in malignant cells, lymphocytes, and cells within the tumor microenvironment. Histone deacetylase (HDAC) inhibitors have been shown to upregulate CD40 expression, as well as MHC class I and II tumor antigen expression, and prostate and neuroblastoma cell lines (6, 7), result in increased expression of immune checkpoints in melanoma cell lines and tumor-infiltrating lymphocytes (8), and induce myeloid suppressor cell apoptosis within the tumor microenvironment of mouse breast cancer models (9). Using murine models of breast, prostate, renal, and colorectal carcinomas, Christiansen and colleagues observed augmentation of tumor responses in mice receiving the combination of vorinostat and a mAb against CD40 and CD147 (10). Similarly, Gameiro and colleagues reported increased sensitivity to CTL-mediated cell lysis of prostate and breast carcinoma cells exposed to vorinostat (11).

There are limited clinical data with the use of vorinostat in both HN and SGCs. Among HN, Blumenschein and colleagues observed one unconfirmed partial response (PR) among 12 patients with heavily

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### Translational Relevance

The synergy of epigenetic modification and PD-1 inhibition in preclinical models provide a rationale for combining these two therapeutic approaches. Combinations of epigenetic modifiers such as histone deacetylase inhibitors and hypomethylating agents with anti-PD-1 mAbs are actively undergoing clinical investigation for various malignancies. Our experience represents the first reported prospective clinical experience of this novel therapeutic regimen in two groups of head and neck malignancies (recurrent metastatic squamous cell carcinoma and salivary gland cancers), where the activity of single-agent PD-1 inhibitors is low. In this article we describe an overall response rate that is higher than reported with anti-PD-1 agents alone, indicating avenue for improving outcomes in these two diseases and meriting further study.

pretreated recurrent metastatic HN (12). In a subset of SGCs, adenoid cystic carcinomas, Goncalvez and colleagues published the results of phase II trial of vorinostat in 30 patients with recurrent/metastatic adenoid cystic carcinomas, and observed two objective responses and a clinical benefit rate of 97% (13).

This article describes a phase II clinical trial that explored the combination of the orally administered panHDAC inhibitor vorinostat with the anti-PD-1 mAb pembrolizumab among two biologically distinct patient populations: HN and SGC. Both groups were studied in separate cohorts due to the desire, of both the investigator and the sponsor, to explore the combination in these two disease entities, and to streamline clinical trial startup and resource use.

## Patients and Methods

This trial was an open-label, single-institution, phase II study with a safety lead-in cohort that enrolled patients in two groups, HN and SGCs. The primary study objectives for both groups were (i) safety according to Common Terminology Criteria for Adverse Events (CTCAE) v. 4 and (ii) ORR using RECIST 1.1. Secondary objectives included progression-free survival (PFS) and overall survival (OS). Exploratory endpoints included tissue and peripheral blood correlative studies. This study was reviewed and approved by the Fred Hutchinson Cancer Research Institutional Review Board and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients enrolled.

### Patients

For both groups, trial eligibility required an age  $\geq 18$  years at enrollment, Eastern Cooperative Oncology Group performance status  $\leq 1$ , and adequate bone marrow and hepatic and renal function. RECIST 1.1 evidence of measurable disease was required for participation. Fresh or archival tissue submission for correlative studies was required prior to study entry. Key ineligibility criteria included inability to swallow the vorinostat pills and/or gastrostomy tube dependence, uncontrolled central nervous system involvement, prior treatment with an anti-PD-1 inhibitor and/or vorinostat, and autoimmune disease requiring systemic immunosuppression in the 3 years leading up to trial enrollment.

HN permitted squamous cell carcinomas of the oral cavity, oropharynx, larynx, hypopharynx, paranasal, skin, and nasopharynx primary sites. Patients were eligible for SGC if they had confirmed

major or minor salivary gland cancer as defined in the World Health Organization 2005 classification. Patients in both the HN and SGC groups were required to have RECIST 1.1 evidence of disease progression determined by the treating physician in the past 3 months prior to trial participation.

### Treatment

Patients received pembrolizumab at a fixed dose of 200 mg intravenous every 21 days. Vorinostat was administered orally at 400 mg daily, 5 days on, 2 days off during each week of the 21-day cycle, at least 2 hours prior to the pembrolizumab infusion. This dosing schedule was recommended by the study sponsor based on previously completed studies of intermittently dosed vorinostat indicating good tolerability (14–16).

Patients were assessed prior to the initiation of each cycle and evaluated for adverse events (AEs), which were graded and recorded according to CTCAE v4. Radiographic imaging was repeated every 9 weeks, RECIST 1.1 assessments of response were made by an independent radiologist. Patients who had RECIST 1.1 progressive disease on the first radiographic imaging assessment after starting investigational therapy were permitted to continue treatment if there was no evidence of performance status decline. Treatment interruptions for both pembrolizumab and vorinostat were instituted in the event of treatment-related toxicities, dose modifications were required once treatment-related toxicities returned to baseline. Treatment was permanently discontinued in patients who withdrew consent, had evidence of confirmed disease progression, who completed 2 years of trial therapy, who required two vorinostat dose modifications, or who experienced grade 2 or higher toxicities that did not resolve within 12 weeks of the last pembrolizumab infusion.

### Correlative sample collection

Submission of archived tissue was required prior to study entry. In patient with no available archived tissue, fresh tissue biopsies were recommended. Biopsies were attempted after three cycles of therapy were completed, in patients who consented. PD-L1 membrane expression was centrally assessed at Qualtek Laboratories using the Merck 22C3 antibody and reported through a modified percent score (MPS) ranging from 0 to 100. At the time of study design and patient accrual, data on the clinical relevance of CPS was not yet available and therefore CPS not utilized for correlative analysis. The MPS includes PD-L1 expression among tumor cells, as well as macrophages and lymphocytes within tumor nests. The MPS differs from CPS, in that it excludes PD-L1 expression in stromal cells  $>$ half a high-power field from tumor nests (17), because of this, based on the amount of stromal cells present in the tumor, MPS either approximates or underestimates the CPS score. For instance, a sample with an MPS of 20 would be predicted to have a CPS of 20 or higher. In addition, tumor slice culture was created from patients' tumors when feasible, and described in an accompanying supplement (18). Correlative blood samples were also collected from patients at baseline and after three cycles of therapy, with the intent of examining circulating immune cell phenotypes. Modified LSRII flow cytometry (BD Biosciences) was performed on these samples using Woodlist 3.1 software. Both, tissue and blood correlative analysis were exploratory and had no prespecified statistical plan for analysis.

### Study design

At the time of study design, pembrolizumab and vorinostat were FDA approved for other indications and individual drug toxicities were well-described. However, because the safety profile of these drugs in combination was previously unexplored, it was necessary to conduct

a safety lead-in consisting of six patients, prior to expanding enrollment. Enrollment was halted after the first six patients were enrolled and assessed for dose-limiting toxicities (DLTs) during the first 21-day cycle. In general, a DLT was defined as any grade 3–5 toxicity judged by the investigator as possibly, probably, or definitely related to study drug administration and/or resulting in a >2 week delay in the initiation of the subsequent cycle of therapy. If  $\geq 2$  DLTs were observed in this six-patient cohort, dose reductions and modifications were planned for subsequent patients enrolled in the study. After completion of DLT evaluation of the phase I run-in, enrollment was reopened to the two study cohorts. Patients enrolled in the lead-in cohort were included in the efficacy analysis.

At the time of study design, reported response rates to single-agent pembrolizumab in HN was approximately 18%. A sample size of 25 patients in the HN cohort would have 85% power (exact) to rule out an ORR of 20% if the true rate is 40% using a one-sided 0.10 level test (exact). The observation of at least eight of 25 (32%) responses will be considered evident to rule out a response rate of 20% and support further study of this combination in this patient population.

The SGC cohort was conducted in a two-stage design. If no objective response was seen in the first 15 patients enrolled, the cohort will be closed to further enrollment. If at least one patient response was observed, a total of 25 patients would be enrolled. Using a one-sided alpha of 0.05 level test, the design has 85% power to rule out an ORR of 5% or less if the true ORR is 25% or greater. The observation of at least four of 25 (16%) responses would be considered evident to rule out an ORR of 5%.

**Statistical analysis**

Summary statistics for continuous measures included sample size, median, and range for both baseline and postbaseline measurements (if applicable). Summary statistics for categorical measures included sample size, frequency, and percentages. Missing values for both continuous and categorical measures were excluded from calculation, and the count and percentage of missing values were reported if any. For instance, toxicities were summarized as the number and percentage of patients with each type of toxicity. Responses were summarized as frequencies and percentages. Kaplan–Meier estimates were used to produce OS and PFS estimates, confidence intervals (CIs), the median survival time, and a plot of the survival curve.

Correlative data were analyzed using the nonparametric Wilcoxon rank-sum test, which is more robust than two-sample *t* test for small sample size, to identify a potential prognostic biomarker associated with clinical benefit [PR + stable disease (SD)], PR, or progressive disease (PD). Because this was an exploratory analysis, correction for multiple testing was not considered. CIs will be at the unadjusted 95% level unless otherwise specified.

**Results**

**Patient and tumor characteristics**

Between November 2015 and August 2017, 25 patients were enrolled in HN and 25 patients were enrolled in SGC. Of the first six patients (three in HN and three in SGC), comprising the safety lead-in cohort, only one patient developed DLT, the patient with recurrent/metastatic oropharynx cancer who developed grade 3 fatigue after initiation of the first cycle of treatment. The dosing of both, vorinostat and pembrolizumab, were unchanged upon reopening of enrollment to the rest of the patients on study. **Table 1** displays the patient and tumor characteristics according to group.

**Table 1.** Patient and tumor characteristics.

Patient/tumor Characteristics	HN N = 25 (%)	SGC N = 25 (%)
Median age (range)	64 (44–78)	57 (33–86)
Female gender	2 (8)	9 (36)
Race		
White	20 (80)	21 (84)
Asian	3 (12)	4 (16)
Native American	2 (8)	
ECOG PS		
0	12 (48)	15 (60)
1	13 (52)	10 (40)
Tobacco use		
Never	15 (60)	16 (64)
Former	9 (36)	9 (36)
Current	1 (4)	
Primary site	Oropharynx 17 (68) Nasopharynx 4 (16) Oral cavity 1 (4) Skin 2 (8) Unknown 1 (4)	Parotid 11 (44) Oral cavity 8 (32) Submandibular 4 (16) Oropharynx 1 (4) Nasopharynx 1 (4)
Histology	P16+ oropharynx 13 (52) P16 unknown OP 3 (12) P16- oropharynx 1 (4) EBER + nasopharyngeal carcinoma 2 (8)	Adenoid cystic 12 (48) Acinic cell 3 (12) Mucoepidermoid 3 (12) Adenocarcinoma 1 (4) Carcinoma ex pl. ad 2 (8) Salivary duct 1 (4) Myoepithelial 1 (4) Lymphoepithelioma 1 (4) Clear cell 1 (4)
Prior lines of therapy		
0	12 (48)	13 (52)
1	9 (36)	7 (28)
2	2 (8)	4 (16)
3	1 (4)	1 (4)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

**Toxicities**

AEs observed during treatment are detailed in **Tables 2** and **3**. An AE of any cause and grade was observed in 14 (56%) patients with HN and 13 (52%) patients with SGC. Grade  $\geq 3$  AEs of any cause were observed in nine (36%) patients with HN and nine (36%) patients with SGC. Nine patients required vorinostat dose reductions due to vorinostat-related toxicity: one with fatigue and weight loss, one with malaise, two with severe fatigue, and five because of increased serum creatinine. We observed 10 immune-related AEs in nine (18%)

**Table 2.** AEs.

Patients with AE of any cause	All patients N = 50 (%)	HN n = 25 (%)	SGC n = 25 (%)
Any grade	27 (54%)	14 (56%)	13 (52%)
Grade $\geq 3$	18 (36%)	9 (36%)	9 (36%)

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**Table 3.** Drug-related events observed.

Drug-related AE	All grades N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4	Grade 5
Creatinine increased	7 (14)	5 (10)	1 (2)		
Fatigue	6 (12)	2 (4)	4 (8)		
Nausea	3 (6)	2 (4)	1 (2)		
Adrenal insufficiency (immune related)	2 (4)		2 (4)		
Hyponatremia	2 (4)		2 (4)		
Vomiting	2 (4)	1 (2)			
Alanine aminotransferase increased	1 (2)		1 (2)		
Anorexia	1 (2)		1 (2)		
Arthritis	1 (2)	1 (2)			
Aspartate aminotransferase increased	1 (2)		1 (2)		
Cough	1 (2)		1 (2)		
Dermatitis	1 (2)		1 (2)		
Diarrhea	1 (2)	1 (2)			
Fever with leukocytosis	1 (2)		1 (2)		
Hypothyroidism	1 (2)	1 (2)			
Malaise	1 (2)	1 (2)			
Nephritis	1 (2)	1 (2)			
Neuritis	1 (2)		1 (2)		
Pneumonitis	1 (2)				1 (2)
Colitis and ileitis	1 (2)		1 (2)		
Tracheitis/epiglottitis	1 (2)		1 (2)		

patients, six (12%) of these were grade  $\geq 3$ . One patient had a grade 5 pneumonitis and two patients had a grade 3 adrenal insufficiency. Biopsy proven grade 3 colitis, a grade 3 dermatitis, and grade 3 tracheitis/epiglottitis observed in one patient each.

There were three grade 5 events observed, which occurred within 100 days of last dose of study drug. One patient developed worsening shortness of breath and was found to have CT imaging consistent with pneumonitis, this did not respond to high dose oral corticosteroids and patient declined further workup and hospital admission, electing instead for enrollment in hospice care. Another patient had an

**Table 4.** Treatment and responses.

Treatment and responses	HN N = 25 (%)	SGC N = 25 (%)
Median No. of cycles initiated (range)	6 (1-33)	9 (1-34)
Median duration of therapy (range)	14 weeks (1-101)	24 weeks (1-98)
RECIST 1.1 response		
CR	0	0
PR	8 (32)	4 (16)
SD	4 (16)	14 (56)
PD	10 (40)	7 (28)
Median		
Follow-up (survivors only)	12.6 mo	13.1 mo
Overall survival	12.6 mo (95% CI, 8.1-NR)	14.0 mo (95% CI, 8.5-NR)
Progression-free survival	4.5 mo (95% CI, 4.1-8.4)	6.9 mo (95% CI, 4.1-NR)

Abbreviation: mo, months.

aspiration event leading to aspiration pneumonia, respiratory distress, and need for mechanical ventilation; the patient did not improve despite broad spectrum antimicrobial therapy leading to withdrawal of care. The third patient had a syncopal episode during strenuous activity and shortly afterwards died in the emergency department, autopsy was performed revealing a massive myocardial infarction.

### Treatment and responses

**Table 4** summaries the treatment response and outcomes observed according to group. In HN, the median number of cycles initiated was six (range, 1-33), and the median duration of therapy was 14 weeks (range, 1-101). Among the 25 patients enrolled in HN, we observed eight PRs (32%) with a median duration of response of 6.2 months (range, 3-20), the ORR was 32% (95% CI, 15%-55%). In this group, six patients were treated beyond initial RECIST 1.1 PD, all six patients had confirmed PD on their subsequent imaging studies. **Figures 1** and **2** detail these responses in waterfall and swimmers plots, respectively. With a median follow-up among survivors of 12.6 months, median OS in this group was 12.6 [95% CI, 8.1-not reached (NR)] months, and median PFS was 4.5 (95% CI, 4.1-8.4) months.

Three patients were not evaluable for RECIST responses due to rapid progression leading to early discontinuation of therapy. The first patient was unable to complete cycle No. 1 due to fatigue and weakness leading to hospitalization for clinical deterioration. He was taken off study 30 days after enrollment and subsequently died in hospice care 60 days after enrollment. The second patient was in the midst of cycle No. 1 when he developed tracheitis/epiglottitis and shortly afterward withdrew consent. During follow-up, 3 months after study enrollment and 1 month after discontinuation of all study drugs, he died in hospice care. The third patient withdrew consent shortly after initiation of cycle No. 1 after reporting symptoms related to locoregional disease progression (dysphagia and odynophagia); he discontinued follow-up at our center and died 51 days after the last dose of vorinostat.

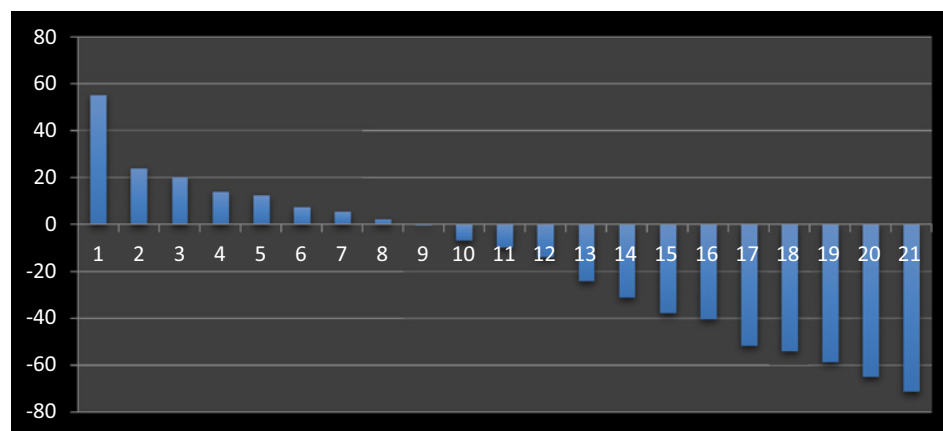
In the SGC group, the median number of cycles initiated was nine (range, 1-34), and median duration of therapy was 24 weeks (1-98), we observed four (16%) PRs in the following histologies: one patient with lymphoepithelioma-like carcinoma of the parotid, two patients with acinic cell carcinoma, and one patient with adenoid cystic carcinoma. SD > 6 months was observed in 10 patients. The median duration of response was 10.5 months (range, 8.7-21). The ORR was 16% (95% CI, 5%-37%). Six patients were treated beyond initial RECIST 1.1 PD, four had confirmed PD on subsequent scan, one achieved a PR, and one had SD > 6 months. **Figures 3** and **4** detail these responses in waterfall and swimmers plots, respectively. With a median follow-up among survivors of 13.1 months, median OS was 14 (95% CI, 8.5-NR) months, and PFS was 6.9 (95% CI, 4.1-NR) months. At the time of data analysis, five patients had completed 2 years of study therapy, and treatment was ongoing in four patients.

### Correlative data

#### Tissue

In HN, a baseline tissue sample was obtained in 24 patients and a subsequent tissue sample was obtained in six. The one patient who did not have an evaluable sample had fine-needle aspirate tissue block as the only biopsy material, which was inadequate for testing. Among the 24 pretreatment HN samples, three contained no evaluable tumor tissue. Of the 21 evaluable pretreatment samples, the median MPS was 12 (0-100). Among the eight partial responders, seven had available pretreatment tissue, the median MPS among the responders was 67 (range, 0-95). There were 14 evaluable samples in patients who did not achieve an objective response (PR and SD), the median MPS was 1

**Figure 1.**  
Waterfall plot of evaluable patients with HN ( $N = 21$ ).



(range, 0–100). Among patients with PD, 11 evaluable samples were available, the median MPS was 0 (range, 0–45).

In the six HN samples obtained after treatment initiation, three were in patients with PRs. Among these three, two had an increase in MPS on the subsequent tumor sample (from 0 to 75, and 15 to 60), the third patient had an unevaluable posttreatment sample. Three samples belonged to patients with PD, one patient had an increase in MPS from 5 to 35, another from 0 to 1, and the last 0 to 0.

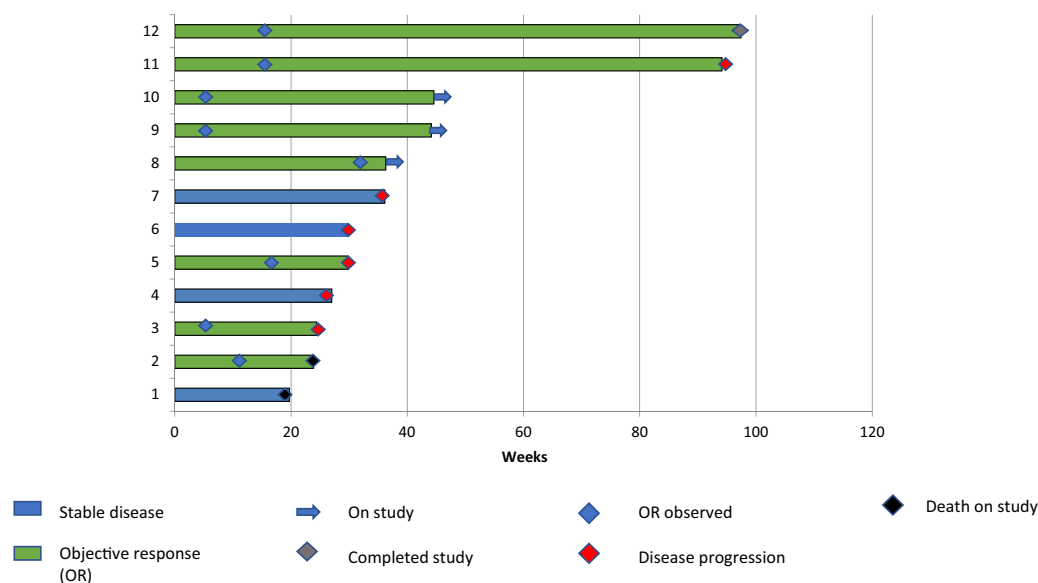
In the SGC group, four patients did not have a baseline tissue sample, all of these patients had older pathology samples (consistent with the longer natural history of some SGC subtypes) that were located out of state, which we were unable to obtain for testing. Among 21 pretreatment samples, the median MPS was 0 (range, 0–95), 17 had an MPS of 0, two with an MPS of 1, one with an MPS of 3, and one with an MPS of 95. Pretreatment samples were available for three of the four patients with PRs, MPS scores in these were 0, 1, and 95. Five SGC samples were obtained after treatment initiation, one sample had no evaluable tumor, which was from a patient

with objective response. In one patient with adenocarcinoma and PD, MPS increased from 0 to 10. The three remaining samples were obtained in patients with stable disease, two had MPS 0 on both samples, one had an MPS of 0 on the pretreatment sample, and two on subsequent sample.

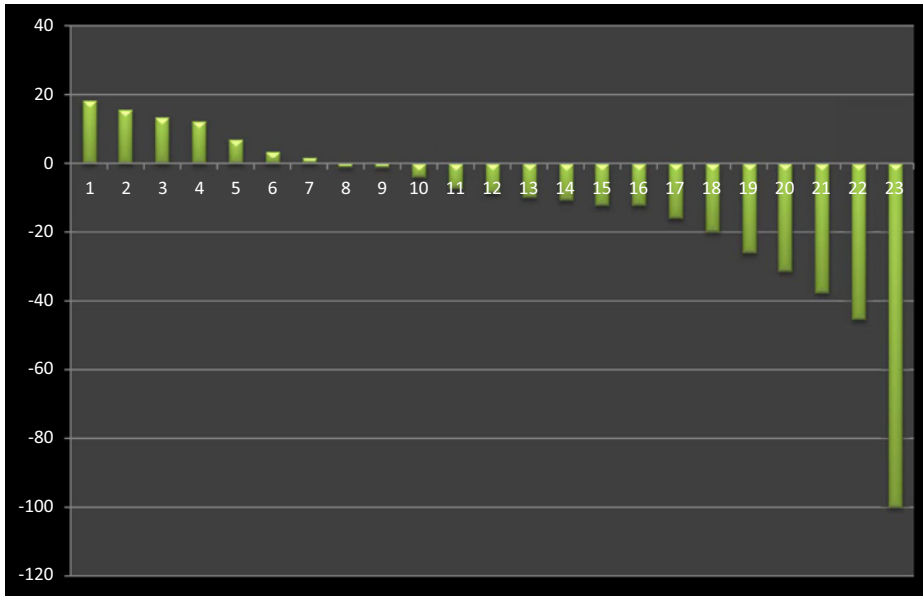
Adequate fresh tumor samples for slice culture were obtained in only one patient with HN, and the results are detailed in an accompanying supplement (see Supplementary Materials and Methods; Supplementary Fig. S1).

**Peripheral blood**

In the HN group, we obtained 24 pretreatment, and 14 on-treatment peripheral blood samples for immune cell phenotyping. We observed a higher percentage of pretreatment circulating PDL2<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> peripheral mononuclear cells among patients with objective responses compared with patients with PD ( $P = 0.013$ ; Fig. 5A). In the SGC group, 24 pretreatment and 13 on-treatment blood samples were obtained. We observed a higher percentage of pretreatment PDL2<sup>+</sup>CD4<sup>+</sup> T cells among objective responders compared with



**Figure 2.**  
Swimmers plot for patients with HN with SD or objective response ( $N = 12$ ).



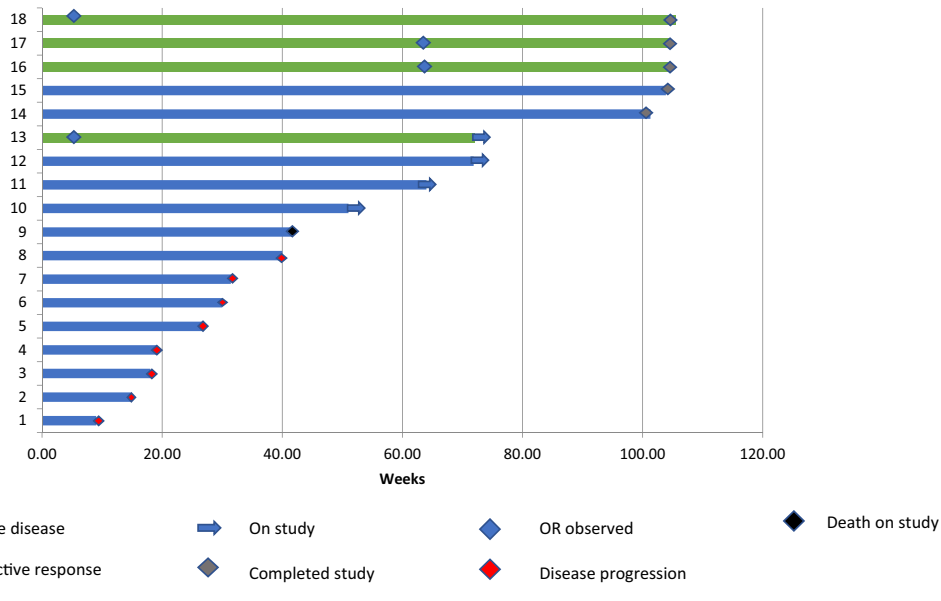
**Figure 3.**  
Waterfall plot of evaluable patients with SGC ( $N = 23$ ).

patients with PD ( $P = 0.047$ ; **Fig. 5B**). In both HN and SGC groups, we did not observe differences in pretreatment percentage of circulating mononuclear cell populations in patients with  $MPS \geq 1$  versus  $MPS \leq 0$ .

In the HN group, no differences in the percentages of pre- and on-treatment peripheral mononuclear cell populations were noted among patients with clinical benefit (PR and SD) compared with those with PD. Among the patients with SGC, we observed an increase in the percentage of circulating  $PDL1^+CD8^+$  T cell and  $PDL1^+CD4^+$  T-cell populations in the on-treatment samples in patients with clinical benefit ( $N = 11$ ) compared with those with PD ( $N = 2$ ; both  $P = 0.038$ ).

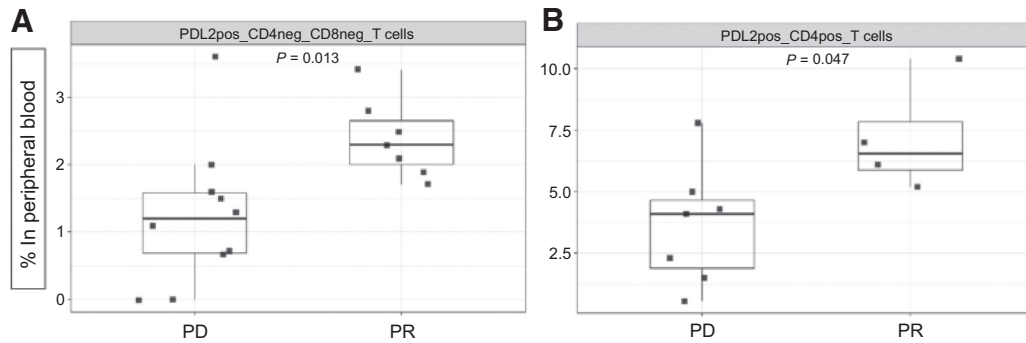
### Discussion

Immunotherapy has transformed the landscape of systemic therapy for various malignancies, and although a minority of patients appears to benefit from this approach, combination strategies provide potential avenues for improving response rates. To our knowledge, our study is the first reported experience using the combination of vorinostat and pembrolizumab among two different groups of head and neck malignancies. From a safety and feasibility standpoint, we observed 36% grade  $\geq 3$  toxicities related to the combination, which is higher than the reported treatment-related high-grade toxicities with the use of pembrolizumab alone, (12% in Keynote-28 salivary gland and 13% in



**Figure 4.**  
Swimmers plot for patients with SGC with SD or objective response ( $N = 18$ ).

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**Figure 5.** Pretreatment peripheral mononuclear cell percentages among patients with PD and PR in HN (A) and SGC (B).

Keynote-40). We observed a 12% grade  $\geq 3$  immune-related AE rate, which is also higher than previously reported with pembrolizumab alone (4% in Keynote-40). These observations are in keeping with the growing body of literature exploring combination immunotherapy strategies in various tumor types reporting, not unexpectedly, higher toxicity rates when the anti-PD-1 agents are administered with anti-CTLA-4 antibodies (19), chemotherapy (3), and tyrosine kinase inhibitors (20). There is emerging data on the safety profile of epigenetic modifiers given in combination with PD-1 inhibitors both in solid tumors and hematologic malignancies. Preliminary reports of the oral HDAC inhibitor, entinostat, combined with pembrolizumab in the multicohort phase II study ENCORE 601 have been presented in abstract form. Johnson and colleagues reported five treatment-related AEs, and one patient with grade 3 and four AEs among 13 enrolled patients with melanoma (21). The same group also reported preliminary safety data in non-small cell lung cancer (NSCLC), and observing only one grade 3 toxicity among nine patients enrolled (22). Nie and colleagues published the results of a three cohort study in relapsed refractory Hodgkin lymphoma, observed a 20% grade  $\geq 3$  immune-related AEs in 67 patients with relapsed refractory Hodgkin lymphoma receiving low-dose decitabine and the anti-PD-1 antibody camrelizumab, and a 26% grade  $\geq 3$  immune-related AEs in 19 patients receiving camrelizumab monotherapy (23). Among patients with relapsed/refractory acute myeloid leukemia treated with azacitidine and nivolumab, Daver and colleagues reported a phase IB/II experience in 70 patients and observed eight (11%) grade 3/4 immune-related AEs (24). Wang and colleagues reported in abstract form, a site agnostic phase I trial of low-dose decitabine and an anti-PD-1 mAb, showing 45% rate of grade 3 or higher toxicities in eight patients with advanced lymphomas and solid tumors, with one patient death due to cytokine release syndrome (25). As a multitude of trials combining epigenetic modifiers with anti-PD-1 therapies in solid tumors are completed and reported, we anticipate gaining additional insight into the nature of toxicities in this novel combination.

We observed a higher ORR in HN than published response rates with single agent anti-PD-1 mAbs. The observation of eight (32%) responses is encouraging, and based on our study design, likely to exclude a true response rate of 20%. However, it must be emphasized that inherent limitations in this small study may confound this observation. First, the HN group was heterogeneous, and included biologically distinct squamous cell carcinomas such as those from cutaneous primaries, nasopharyngeal carcinomas. Although PD-1 inhibition seems to have activity in these subsets, cutaneous squamous cell carcinomas may have a higher response rate based on a small phase

I experience using cemiplimab that has led to FDA approval (26). In addition, data have since emerged showing higher rates of response to single-agent pembrolizumab in patients with CPS 1 or higher (3, 4). PD-L1 expression was not an eligibility criterion in both groups of this trial, and the higher response rate may be attributable to inadvertent overexpression of high PD-L1-expressing tumors in this group, rather than a true synergy between vorinostat and pembrolizumab. Caution in overinterpretation of these results is warranted, especially in the wake of combination strategies that appeared encouraging in small early-phase trials that ultimately failed to show benefit in expanded populations (27, 28).

Although we observed only four responses in SGC, these responses are ongoing, with all four patients without evidence of disease progression. Our experience augments the limited experience with the use of immunotherapy in this rare disease. A previously published article by Cohen and colleagues describes three responders among 26 with SGC with PD-L1 expression  $>1\%$  (5). We only had MPS scoring for three of our responses, one patient had an MPS of 0 and another had an MPS of 1, questioning whether PD-L1 expression is a reliable biomarker for response in this heterogeneous population. Although the prospective studies with targeted and cytotoxic agents in SGC are limited by small numbers, it is interesting to note that targeted therapy strategies alone have been largely unsuccessful. However, combination cytotoxic therapy with targeted biologic agents, such as the combination of docetaxel and trastuzumab in Her2<sup>+</sup> SGCs (29) have resulted in encouraging rates of response, and may very well be a direction for further study of these tumors. Similar to HN, our observations are limited by a small population with significant variability in histology and disease biology that is well recognized in salivary gland carcinomas.

Among solid tumors, there is limited data on response rates with epigenetic modifiers in combination with checkpoint inhibitors. Johnson and colleagues have reported preliminary efficacy results from the multicohort ENCORE 601 study. Among the NSCLC cohort (22), three patients among the nine enrolled have SD. In the melanoma cohort, one confirmed response was observed in 13 enrolled patients (21). The disparity between the observed responses among the patients with HN and SGC in our study is likely attributable to the divergent disease biology of these two sets of malignancies. Overall, SGC have lower rates of PD-L1 expression, lower tumor mutational burden, and are generally unrelated to prior tobacco, alcohol, or viral exposure, and these are likely responsible for the lower rate of response observed (5, 30, 31).

Our experience with obtaining posttreatment biopsies in both cohorts was limited, mostly by the clinical status of the patients



precluding collection. Among the patients who we were able to obtain biopsy specimens, PD-L1 testing was limited by paucity of tissue samples collected, which were mostly core biopsies from lung metastases. Interestingly, two of these patients who were responding to therapy had no viable tumor and fibrotic tissue in the biopsy specimen despite radiographically detectable metastases. Although differences in circulating mononuclear cell phenotypes were noted among the H and SGC cohorts, we view these findings as hypothesis generating, and limited by the small number of samples, the small number of patients with responses, and the absence of a comparator arm receiving pembrolizumab alone. Similar correlative samples have been obtained in ongoing immunotherapy trials at our institution, and future studies with a larger sample size are planned.

In conclusion, our prospectively collected data provide insight into the activity and tolerability of this novel combination in two disease entities. We observed encouraging responses in HN and SGC using the combination of pembrolizumab and vorinostat, with both treatment groups meeting their prespecified efficacy endpoints. We also observed higher than reported rates of high grade toxicities compared with single agent anti-PD-1 mAbs. It is evident that for a small proportion of SGCs, the anti-PD-1s are effective, continued work toward exploring the activity of these agents in ongoing.

### Disclosure of Potential Conflicts of Interest

C.P. Rodriguez is a paid consultant for Cue Pharmaceuticals, reports receiving commercial research grants from Merck, AstraZeneca, Bristol-Myers Squibb, Ignyta, Ayala, and is an unpaid consultant for Ayala. Dr. Rodriguez's significant other is a paid consultant for Astra Zeneca and Merck, and has received commercial research funding Acerta Pharma, AstraZeneca, Genentech, Incyte,

Merck, Pharmacycics, Portola, and Seattle Genetics. J.R. Fromm reports receiving other commercial research support from Merck. V.G. Pillarisetty is an employee/paid consultant for Merck, Invax, and GlaxoSmithKline, and reports receiving commercial research grants from Merck and AstraZeneca. S.M. Lee reports receiving commercial research grants from Juno. L.Q.M. Chow is an employee/paid consultant for Merck, Novartis, AstraZeneca, Pfizer, Takeda, Dynavax, Genentech, Synthorx, and Alkermes. K. Eaton reports receiving other commercial research support from Mirati Therapeutics. R. Martins reports receiving speakers bureau honoraria from Roche Brazil Webinar. No potential conflicts of interest were disclosed by the other authors.

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