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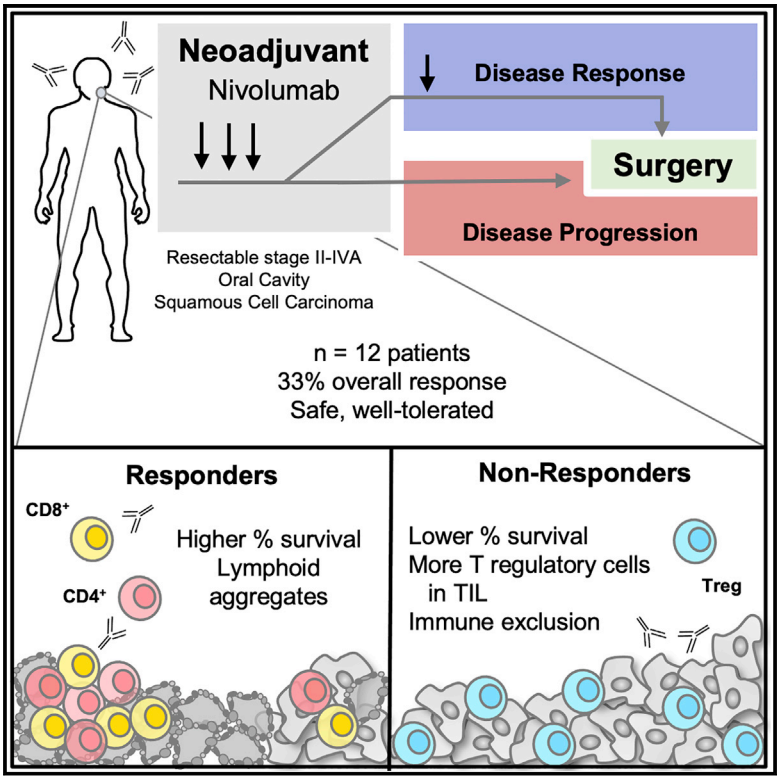
Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma

Graphical abstract



Highlights

- Presurgical nivolumab in patients with resectable oral cancer has an ORR of 33%
- 10 out of 12 treated patients remain alive with a median follow up of 2.23 years
- Neoadjuvant nivolumab is safe, well-tolerated, and not associated with treatment delays
- This shows feasibility of nivolumab in the neoadjuvant setting for OCSCC (NCT03021993)

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In brief

Oral cavity squamous cell carcinoma is a prevalent subset of head and neck cancer with high recurrence rates and poor survival outcomes despite complex multimodality therapy. The current study by Knochelmann et al. demonstrates that presurgical nivolumab therapy has an overall response rate of 33% in this patient population.

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Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma

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SUMMARY

Oral cavity squamous cell carcinoma (OCSCC) is a prevalent surgically treated subset of head and neck cancer with frequent recurrence and poor survival. Immunotherapy has demonstrated efficacy in recurrent/metastatic head and neck cancer. However, whether antitumor responses could be fostered by neoadjuvant presurgical immunotherapy remains unclear. Using a Simon's two-stage design, we present results of a single-arm phase-II trial where 12 patients with stage II-IVA OCSCC received 3 to 4 biweekly doses of 3 mg/kg nivolumab followed by definitive surgical resection with curative intent. Presurgical nivolumab therapy in this cohort shows an overall response rate of 33% (n = 4 patients; 95% CI: 12%–53%). With a median follow up of 2.23 years, 10 out of 12 treated patients remain alive. Neoadjuvant nivolumab is safe, well-tolerated, and is not associated with delays in definitive surgical treatment in this study. This work demonstrates feasibility and safety for incorporation of nivolumab in the neoadjuvant setting for OCSCC (ClinicalTrials.gov: NCT03021993).

INTRODUCTION

Oral cavity squamous cell carcinoma (OCSCC) is a tobacco-related head and neck cancer that accounts for 350,000 new cancer diagnoses and 170,000 fatalities worldwide.[1] Treatment of OCSCC often requires complex, multimodality therapy of surgical resection followed by post-operative radiation with the addition of platinum-based chemotherapy for patients at high risk of failure.[2,3] Despite these comprehensive strategies (and in contrast to less aggressive human papillomavirus [HPV]-related oropharyngeal cancers), OCSCC outcomes are poor, and the disease recurs in 25%–50% of patients.[4,5] Due to these poor outcomes, different treatment regimens have been considered, including neoadjuvant chemotherapy. Unfortunately, meta-analysis of induction chemotherapy prior to definitive surgical or radiation therapy revealed only a modest reduction in

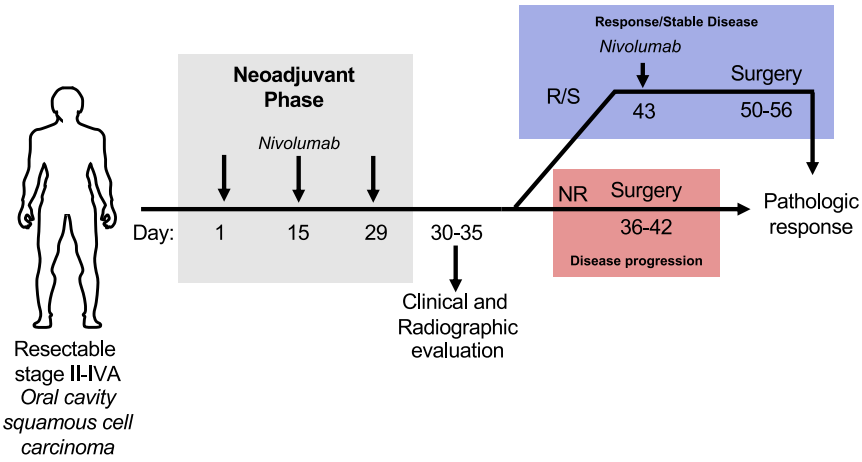

Figure 1. Trial schema

Patients with surgically resectable, locoregionally advanced oral cavity squamous cell carcinoma (OCSCC) stage II-IVA underwent baseline clinical and radiographic imaging and then received 3 mg/kg nivolumab every 2 weeks for 3 doses (green). Repeat post-nivolumab clinical and radiographic assessment between days 30 and 35 was then performed. If there was disease progression (red) by RECIST 1.1 criteria, patients proceeded directly to surgery between days 36 and 42. Conversely, if there was a response or stable disease (blue), patients received a single 4th dose of nivolumab 3 mg/kg on day 43 before proceeding to surgery on day 50 to 56. Pathologic response was determined by comparison of tumor size on final pathologic evaluation compared to tumor size on baseline radiographic imaging.

distant metastases and no improvement in locoregional control or overall survival—therefore, it is not considered to be the standard of care.[6]

Recent advances have revealed that immunotherapies, like programmed death 1 (PD-1) blockade, demonstrate benefits in some cohorts of patients over traditional chemotherapy in recurrent and metastatic head and neck cancer.[7,8] Therefore, while neoadjuvant chemotherapies prior to surgery have not demonstrated significant improvement in outcomes, perhaps neoadjuvant immunotherapy could be beneficial for some patients. Head and neck squamous cell carcinoma (HNSCC) demonstrates among the highest immune infiltration of all solid tumors,[9,10] has a high tumor mutational burden,[11,12] and expresses elevated levels of PD-1 and its primary ligand PD-L1 within the malignancy.[13] It is therefore appealing to expand the clinical indications for PD-1 blockade into larger subsets of head and neck cancer patients, including patients with resectable disease, at an earlier point in disease progression.

Neoadjuvant immunotherapy for surgically resectable OCSCC is expected to reduce tumor burden and generate anti-tumor immunity, thereby improving long-term clinical outcomes.[14] Success of this strategy is predicted based on the emerging results of presurgical PD-1 inhibition in similarly immunogenic tumors such as melanoma[15] and lung cancer.[16] A single pre-operative dose and adjuvant pembrolizumab for high-risk HPV-unrelated HNSCC is associated with a pathologic tumor response (pTR) >10% in 44% of patients, with pTR defined as the proportion of the resection bed with tumor necrosis, keratinous debris, and giant cells/histocytes.[17] A subsequent study investigating neoadjuvant nivolumab or nivolumab plus ipilimumab reports volumetric response rates of 50% or 53%, respectively, with volumetric response defined as any reduction in tumor size (which may increase the response rate).[18] Neither trial compares clinical or radiographic tumor response to pathologic response.[17,18] Other preliminary trials show evidence of pathological response to neoadjuvant pembrolizumab,[19] with additional cycles of immunotherapy prior to surgery associated with better responses.[20] Therefore, these early reports concur on the potential of neoadjuvant immunotherapy to benefit at least some HNSCC patients.

We designed a Simon two-stage, phase-II, single-arm clinical trial (Figure 1) of neoadjuvant nivolumab (anti-PD-1 monoclonal antibody) before surgical resection in stage II-IVA OCSCC. Upon enrollment, patients received nivolumab every two weeks for a total of three doses prior to interval radiographic evaluation. In the event of disease progression, patients received definitive surgical resection. If stable disease or response was observed, patients received a 4th dose of nivolumab followed by definitive surgical resection (see STAR Methods). This trial incorporated a pathology-enhanced RECIST with a primary endpoint of objective response rate (ORR) defined as pathologic complete response + pathologic partial response (>30% reduction in tumor size).[21,22] Results from this trial reported herein corroborate emerging literature demonstrating the potential of neoadjuvant immunotherapy to instill response and survival benefit in subgroups of HNSCC patients and provided longitudinal tissues for in-depth molecular analysis to nominate mechanisms of response, resistance, and post-surgical recurrence.[23]

RESULTS

Patient characteristics

Beginning April 2017 through April 2020, 14 patients enrolled and received at least 1 dose of nivolumab. Two additional patients were enrolled but came off the study prior to receiving any nivolumab. For inclusion in the efficacy analysis, patients were specified to receive at least 2 doses of nivolumab (see Protocol section 11.1.2 in Methods S1). One (patient 8) experienced treatment delay due to hospitalization for dehydration between his 1st and 2nd dose of nivolumab that resulted in significant disease progression; therefore, he was removed from the trial and proceeded directly to surgical resection. This patient was not included in the study analysis per trial protocol. One (patient 11) received 4 doses of nivolumab and demonstrated a clinical response, but this patient was lost to follow-up prior to surgery and was not included in the analysis. One patient (patient 16) received only 2 doses of nivolumab prior to surgery, due to research restrictions during the COVID-19 outbreak, to prevent any delay to surgery. This patient was included in the analysis per trial protocol. All remaining patients received either 3 or 4

Table 1. Enrollment patient characteristics stratified by response group

		All patients (n = 12)
Age, years	mean ± SD	64 ± 8.0
	median (range)	62 (48–78)
Gender (%)	male	5 (42)
	female	7 (58)
Smoking status (%)	current	6 (50)
	former	3 (25)
	never	3 (25)
Alcohol use (drinks/week)	mean ± SD	13.2 ± 27.4
	median (range)	5.5 (0–98)
ECOG status	0	5 (42)
	1	7 (58)
T stage (%)	T2	3 (25)
	T3	3 (25)
	T4a	6 (50)
N stage (%)	N0	4 (33)
	N1	4 (33)
	N2b	2 (16)
	N2c	2 (16)
Clinical stage (%)	II	3 (25)
	III	1 (8)
	IVA	8 (67)
Tumor size (greatest dimension), cm	mean ± SD	3.6 ± 1.1
	median (range)	3.1 (2.1–5.4)
Histologic grade	well	1 (8)
	mod to well	1 (8)
	moderate	5 (42)
	mod to poor	3 (25)
	poor	1 (8)
	spindle	1 (8)

doses of nivolumab and curative-intent surgical resection. We did not observe substantial differences between eventual responders and non-responders in their baseline characteristics (Table 1).

Safety and feasibility

As per trial protocol, adverse events were monitored on patients throughout treatment and up to at least 100 days following the final dose of treatment. Nivolumab was well-tolerated with no grade 4 adverse events (AEs) (Table 2). One grade 3 AE of pain was reported in patient 16, which was resolved and deemed unrelated to treatment. 12 patients experienced a total of 67 grade 1 to 2 AEs, which included fatigue, nausea, diarrhea, oral pain and non-oral pain, rash/psoriasis, myalgia, constipation, cough, creatinine increase, dyspnea, back spasm, and hypertension (Table 2; Table S1). The most common AEs reported included constipation (n = 7; 58%), oral pain (n = 5; 42%), non-oral pain (n = 5; 42%), and weight loss (n = 4; 33%). Of AEs "possibly" or "definitely" related to treatment, fatigue, myalgia, nausea, diarrhea, and rash were each reported in 17% of patients (n = 2) (Table 2). At least one AE "possibly" or "definitely" related to treatment was experienced in 100% of responders (n = 4), 75% of non-responders (n = 3), and 25% of patients with stable disease (n = 1). There were no dose reductions, study withdrawals, or delays in definitive surgery due to AEs.[24] Treatment interruption was required in only 1 patient (patient 16) due to COVID-19 shutdowns, though this patient was still able to receive 2 doses of nivolumab. All other patients (11 of 12) received either 3 or 4 doses according to the study design (Figure S1). Median (range) time from 1st nivolumab dose to surgery was 40 days (38–41) for those receiving three doses (n = 6) and 52 days (47–55) for those receiving four doses (n = 5), which were all within the predefined ranges to avoid delay of surgical resection. Time from 1st nivolumab dose to surgery was 27 days for the 1 patient receiving 2 doses. Negative margins (1 close) were achieved in all patients.

Efficacy

Of the 12 enrolled patients eligible for efficacy analysis, 4 had >30% response (33.3%, 95% CI: 12%–53%), 4 had stable disease (33.3%), and 4 had progression of their disease (33.3%) resulting in an ORR of 33.3% (95% CI: 12%–53%) (Figure 2). There were no complete pathologic responses.

Based on the trial design, clinical response on radiographic imaging following the 3rd dose of nivolumab was used to determine which patients received a 4th dose prior to surgery. We sought to evaluate the validity of this design. Neither initial tumor size (Figures S1A and S1B) nor tumor size following dose 3 (Figures S1C and S1D) correlated with ultimate pathologic response to treatment. However, *changes* in tumor size from enrollment to interval imaging after 3 doses of nivolumab ("clinical response") were associated with ultimate response to treatment ("surgical-pathologic response") (Figure S2A). That is, all patients that eventually had a pathologic response demonstrated clinical reduction in tumor size on interval scan, and those with pathologic stable or progressive disease had no change or increased tumor burden on interval scan (Figure S2A). A positive correlation between clinical response on interval presurgical imaging and final pathologic response was identified (Figure S2B; r = 0.745; p = 0.011). Note that patient 16, who received only 2 doses, did not have an interval scan due to the impact of COVID-19 on clinical research at our institution and thus was excluded from this specific analysis.

At median follow-up of 2.23 years (0.43–3.32), 10 of 12 patients were alive (thus a median overall survival [OS] was not reached) (Figure 3A), and 7 patients remained recurrence-free. All responders or patients with stable disease remain alive, while 2 of 4 progressors have died of disease (Figure 3B). Median disease-free survival was 3.2 years overall (Figure 3C). By response category, median disease-free survival was 1.9 years, not reached, and 2.1 years for responders, stable disease, and non-responders, respectively (Figure 3D). 9 (75%) patients underwent adjuvant radiation (radiation was recommended but refused by patients 2, 14, and 16). 2 patients (17%) had the addition of concurrent adjuvant chemotherapy for extra-nodal extension (patients 4 and 12). Median (range) time to start of adjuvant treatment following surgery was 41 days (14–48). 2 patients

Table 2. Adverse events in study participants

	All events		"Definitely" or "possibly" related to nivolumab	
	No. (%), n = 12 patients			
Characteristic	Grades 1 and 2	Grades 3 and 4	Grades 1 and 2	Grades 3 and 4
Constipation	7 (58)	0 (0)	1 (8)	0 (0)
Pain (non-oral)	5 (42)	1 (8)	1 (8)	0 (0)
Oral pain	5 (42)	0 (0)	0 (0)	0 (0)
Weight loss	4 (33)	0 (0)	0 (0)	0 (0)
Fatigue	3 (25)	0 (0)	2 (17)	0 (0)
Myalgia	2 (17)	0 (0)	2 (17)	0 (0)
Oral mucositis/dry mouth/ dysphagia	2 (17)	0 (0)	0 (0)	0 (0)
Nausea	2 (17)	0 (0)	2 (17)	0 (0)
Diarrhea	2 (17)	0 (0)	2 (17)	0 (0)
Hypokalemia	2 (17)	0 (0)	0 (0)	0 (0)
Painful swelling	2 (17)	0 (0)	0 (0)	0 (0)
Loss of appetite	2 (17)	0 (0)	0 (0)	0 (0)
Rash	2 (17)	0 (0)	2 (17)	0 (0)
Muscle stiffness	1 (8)	0 (0)	0 (0)	0 (0)
Numbness of tongue	1 (8)	0 (0)	0 (0)	0 (0)
Cough	1 (8)	0 (0)	0 (0)	0 (0)
Neck stiffness	1 (8)	0 (0)	0 (0)	0 (0)
Actinic keratosis	1 (8)	0 (0)	0 (0)	0 (0)
Creatinine increase	1 (8)	0 (0)	1 (8)	0 (0)
Dyspnea	1 (8)	0 (0)	1 (8)	0 (0)
Thrush	1 (8)	0 (0)	0 (0)	0 (0)
Papilloma	1 (8)	0 (0)	0 (0)	0 (0)
ALT increase	1 (8)	0 (0)	0 (0)	0 (0)
Back spasms	1 (8)	0 (0)	1 (8)	0 (0)
Hypertension	1 (8)	0 (0)	1 (8)	0 (0)
Surgical site infection	1 (8)	0 (0)	0 (0)	0 (0)
Anxiety	1 (8)	0 (0)	0 (0)	0 (0)
Hearing impairment	1 (8)	0 (0)	0 (0)	0 (0)
Hypomagnesemia	1 (8)	0 (0)	0 (0)	0 (0)
Hypercalcemia	1 (8)	0 (0)	0 (0)	0 (0)
Facial edema	1 (8)	0 (0)	0 (0)	0 (0)
T8 compression deformity	1 (8)	0 (0)	0 (0)	0 (0)
Insomnia	1 (8)	0 (0)	0 (0)	0 (0)

(22%) had delays in commencement of adjuvant radiation treatment beyond 6 weeks,[25] due to patient preference.

Post-surgical recurrences

Up to a data cutoff of February 23, 2021, there was a total of 8 recurrences in 5 patients (Figure 2; Table S1). Patient 1 developed regional metastasis in the contralateral neck 5 months after surgical resection; this recurrence was treated with neck dissection and chemoradiation. This patient had not received contralateral neck dissection during her on-trial surgery, and the contralateral neck was not included in the post-operative radiation field. This same patient subsequently developed distant metastasis to the lung 1 year after on-trial surgery that was treated with stereotactic radiation therapy. This patient then developed local and regional recurrence and was treated with maxillectomy and lateral pharyngectomy. Patient 2 developed a local recurrence 3.29 years after enrollment and underwent a subtotal glossectomy. Patient 4 developed regional metastasis to the contralateral neck 9 months after on-trial surgery, despite having received post-operative platinum-based chemotherapy and radiation (including the contralateral neck). This patient was treated with neck dissection and adjuvant chemoradiation but subsequently developed a 2nd large surgical site recurrence and passed away 12 months after on-trial surgery. Patient 6 had a first local recurrence 2 years after enrollment and underwent a composite resection with segmental mandibulectomy followed

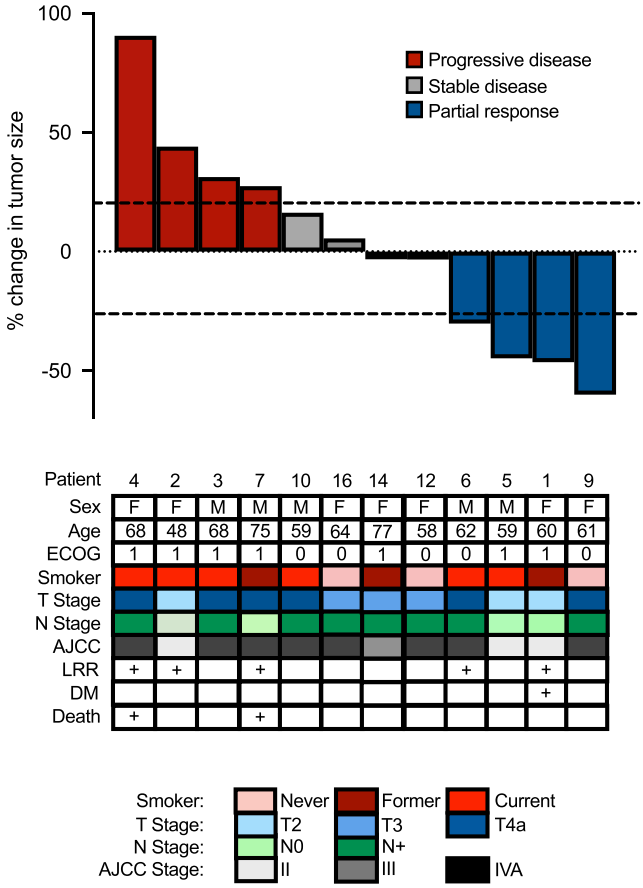

Figure 2. OCSCC response to neoadjuvant presurgical nivolumab

Waterfall plot of pathologic response (percentage change from baseline imaging to pathologic evaluation) to neoadjuvant nivolumab (33% response rate). Dashed lines indicate RECIST 1.1 cutoffs for progression (>20% change, red bars), stable disease (+20% to −30% change, gray bars), and response (>30% reduction, blue bars). There were no significant differences in baseline characteristics between responders and non-responders (see also Table 1). ECOG, Eastern Cooperative Oncology Group; AJCC, American Joint Committee on Cancer Clinical Stage; LRR, locoregional recurrence; DM, distant metastasis.

by adjuvant re-irradiation. This patient developed 2nd local recurrence 2 months following completion of adjuvant re-irradiation and received chemotherapy given the short disease-free interval; this patient has responded well to chemotherapy and is currently alive with evidence of disease. Finally, patient 7 developed local recurrence at the posterior margin of the surgical site in the oropharynx 7 months after on-trial surgery; this recurrence was treated with chemoradiation. On-trial surgery was noted to have a close margin at this site (0.3 cm) on surgical pathology, and this patient is now deceased from disease.

Immunologic evaluation

In a responder, decrease in tumor size on clinical and radiographic images was associated with marked lymphoid infiltration on hematoxylin and eosin staining of the surgical specimen (Figure 4A), compared to tumor progression and muted lymphoid infiltration (Figure 4B) in a non-responder. To further evaluate the immune infiltration in responding versus non-responding patients, we performed multiplexed immunofluorescence of post-treatment surgical specimens (Figure 5). A representative interface of tumor tissue, identified by cytokeratin expression, is presented from the most responsive tumor (from patient 9) (Figures 5A and 5B) and the most progressive tumor (from patient 4) (Figures 5C and 5D). In the responding tumor, minimal PD-L1 staining was noted to coincide with the remaining cytokeratin areas, while robust $CD4^+$ and $CD8^+$ infiltrates were observed in the surrounding tissue with scant $FoxP3^+$ expression in the infiltrative cells (Figure 5B). In contrast, in the progressive tumor, PD-L1 markedly overlapped with cytokeratin across approximately 50% of the whole-slide tissue (Figure 5C). While $CD4^+$ and $CD8^+$ T cell infiltrates were present in the surrounding tissue, relatively few of these lymphocytes overlapped with the PD-L1/cytokeratin staining, where a more exuberant $FoxP3^+CD4^+$ population was present (Figure 5D).

DISCUSSION

We report here a phase-II trial of neoadjuvant nivolumab for OCSCC, which yielded a meaningful ORR of 33%. Treatment was well-tolerated, with only one grade 3 AE, which resolved, and no grade 4 AEs. There were no delays in definitive surgery and therefore no deviations from the current standard of care. This identified response rate surpassed the total number of responders (n = 2) required to consider this a positive trial based on *a priori* statistical considerations of the Simon two-stage design. Due to the initial trial design, limited tissue availability prior to treatment precluded robust evaluation of PD-L1 expression as a predictive biomarker of response to therapy. Follow-up analysis revealed that partial response and stable disease appear to correlate with an improved overall survival. Although these findings need to be interpreted with caution, they corroborate results from previous immunotherapeutic window of opportunity trials.[17,18] Our work provides additional rationale to execute larger randomized clinical trials to this disease setting, such as the ongoing phase-III trial evaluating pembrolizumab as a neoadjuvant/adjuvant agent relative to no neoadjuvant therapy and adjuvant standard of care radiotherapy/chemotherapy (ClinicalTrials.gov: NCT03765918). This trial also generated longitudinal tissues that have been analyzed deeply to provide molecular and immune insights into the reported response, relapse, and survival patterns.[23]

Importantly, clinical response as determined by re-imaging at week 4 to 5 (after three doses of nivolumab) predicted patients that ultimately had a pathologic response. This technique of comparing radiographic response to pathologic is used frequently in other solid tumors including breast and colon cancer, where neoadjuvant therapy is the standard of care.[22,26] The ability of radiography to predict pathologic response in these OCSCC patients was unique compared to analogous immunotherapy studies in other solid tumors,[16,27] where change in tumor size on interval imaging was inconsistent with the final

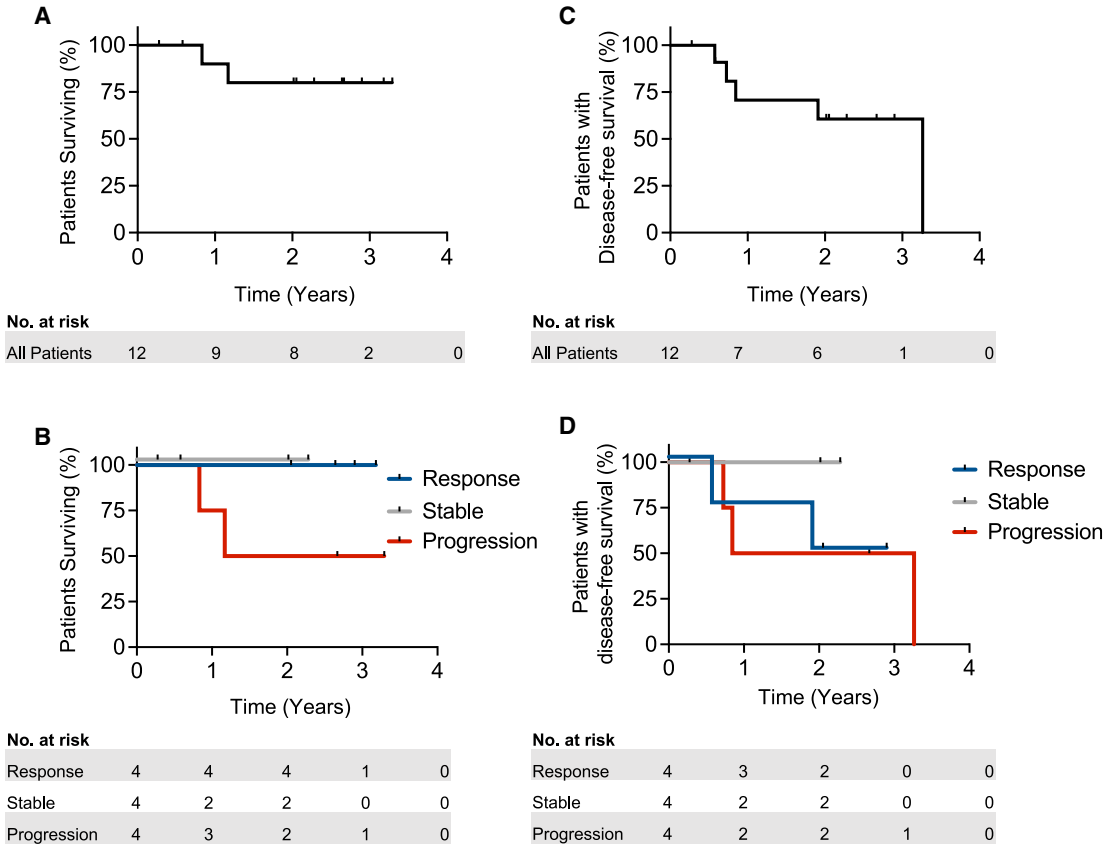

Figure 3. Overall survival and disease-free survival (DFS) in OCSCC patients treated with neoadjuvant nivolumab

(A and B) Overall survival for (A) all patients and (B) by response category displayed. Median overall survival for all patients, responders, or patients with stable disease was not reached, while median OS for progressors was 1.17 years.

(C and D) DFS for (C) all patients or (D) by response category. Median overall DFS was 3.26 years, while DFS for responders was 1.9 years, for progressors was 2.0 years, and was unreached for patients with stable disease.

n = 12 patients; n = 4 with partial response, 4 with stable disease, and 4 with progressive disease. Tick marks indicate censored data.

pathological response. Theoretically, this incongruity can be attributed to transient and unpredictable immune infiltration and inflammation of the tumor, so-called "pseudoprogression." However, reported rates of pseudoprogression are generally lower in head and neck cancer,[27] which is corroborated by our findings.

Multiplexed imaging of the post-treatment surgical specimens in our best responding patient (patient 9) relative to the worst progressing patient (patient 4) revealed striking differences at the tumor/immune interface. Both patients demonstrated significant infiltration of $CD4^+$ and $CD8^+$ T cells, suggesting that the presence of immune cells themselves was not a limiting factor for response. However, the responding patient's post-treatment surgical tumor sample showed minimal overlap of PD-L1 staining with tumor cells, in contrast to high intensity PD-L1 staining coinciding with tumor marker staining in the non-responder. While $CD4^+$ and $CD8^+$ T cells overlapped with tumors in the responder, relatively fewer immune cells but more T regulatory cells ($FoxP3^+CD4^+$) were present in cytokeratin$^+$ areas in the non-responder, who went on to relapse and die of disease. Future investigations in a comprehensive cohort will be important to validate and understand these histologic differences as mechanisms of response or relapse.

Notably, 5 patients recurred, including 2 responders and 3 non-responders, which resulted in a 41% relapse rate in our patient population over the total follow-up period. Our relapse rate at 1 year following enrollment was 3 out of 12 patients, or 25%, which is comparable to a prior report of a 16.7% incidence of relapse after 1 year in high-risk patients treated with neoadjuvant pembrolizumab.[17] Our patients who are deceased since the commencement of the study were not responsive to neoadjuvant anti-PD-1 therapy. These findings highlight the urgent need to understand mechanisms of response to anti-PD-1 therapy as well as biomarkers that will identify patients likely to benefit in order to most appropriately incorporate immunotherapy into treatment plans.

Two of the recurring patients developed regional lymph node disease in the contralateral neck. The decision to treat the contralateral neck up front (with surgery or radiation) is controversial in OCSCC, and practices vary by institution. Reported rates of recurrence in the untreated contralateral neck are ~5% and even lower when this neck is treated.[28] A past concern

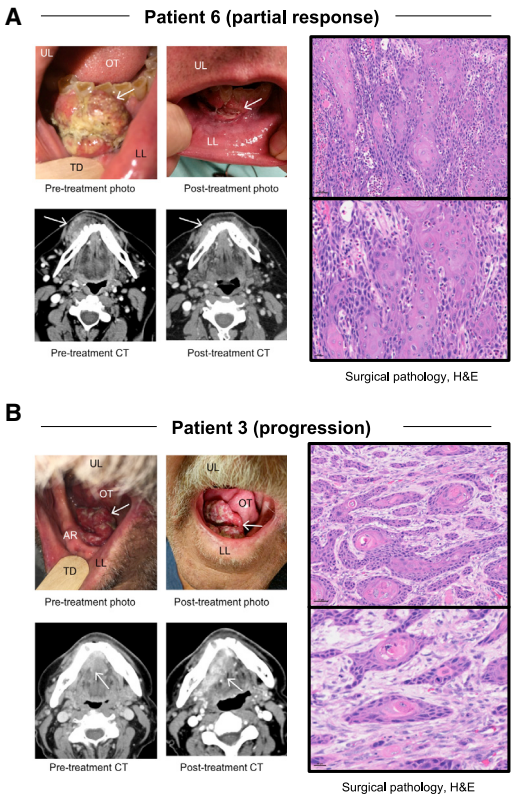

Figure 4. Clinical, radiographic, and pathologic features of response and progression after neoadjuvant nivolumab in OCSCC

(A) Patient 6 had a right gingivobuccal sulcus lesion, which measured 4.3 cm on initial imaging (left), This tumor decreased in size visually (upper right) and radiographically (to 3.6 cm; lower right) on interval evaluation following nivolumab. The lesion had decreased to 3.0 cm at surgical resection, consistent with partial response. Hematoxylin and eosin (H&E) stain on surgical pathology indicating invasive squamous carcinoma with marked acute and eosinophilic inflammatory response. 20× magnification for the top image, scale bar, 50 μM; 40× magnification for the bottom image, scale bar, 20 μM.

(B) Patient 3 had a right floor of mouth/alveolar ridge lesion measuring 3.2 cm on initial imaging (left) which increased to 3.7 cm on interval evaluation following nivolumab (right) and was 4.2 cm at surgical resection, consistent with progression. H&E stain on surgical pathology demonstrates tentacular stands of the squamous cell carcinoma with deficient inflammation. 20× magnification for the top image, scale bar, 50 μM; 40× magnification for the bottom image, scale bar, 20 μM. UL, upper lip; LL, lower lip; OT, oral tongue; TD, tongue depressor; AR, alveolar ridge. Arrow indicates tumor.

with pre-surgical treatments was how to approach the surgical margins in responders, i.e., whether the margins should be planned around the pre- or post-therapy tumor. Though all margins were negative in this study, there was 1 close margin which associated with a local recurrence in 1 patient. Cancer cells likely persist at the invasive margin even in responders. Therefore, until this is explicitly investigated, the most prudent strategy should be to plan resection based on the margins of the untreated tumor, relying on training and experience to make appropriate intra-operative decisions regarding the extent of resection. Although the current sample size is small, our recurrence rate warrants close monitoring of these metrics moving forward. Recurrences in the clinical trial setting here, where all patients were discontinued on nivolumab after in-trial surgery, cannot be considered true acquired resistance. Thus, in addition to the aforementioned issue of surgical margins, another possible contributor to clinical relapse may be inadequate $CD8^+$ T cell rejuvenation by a limited number of neoadjuvant nivolumab doses or inadequate functional persistency of tumor-specific/cytotoxic T cells due to exhaustion. The former scenario suggests the value of adjuvant anti-PD-1 therapy, whereas the latter implies combinatorial neoadjuvant immunotherapy to thwart or minimize T cell exhaustion.

Prior trials of neoadjuvant chemotherapy in patients with head and neck cancer have failed to show survival benefits.[6] The findings reported here, showing significant rates of anti-tumor activity via PD-1 blockade in the neoadjuvant setting, warrant consideration and evaluation of neoadjuvant immunotherapy for improving surgical outcomes long term. Immunotherapy is increasingly being combined in innovative ways with traditional treatment modalities including surgery and radiation, and we provide key evidence of activity for PD-1 immune-checkpoint blockade prior to surgery specifically for patients with oral cavity cancer. This therapeutic approach demonstrates an encouraging 33% ORR and is a safe and feasible modality in the overall management plan. Future investigations to determine predictive biomarkers of response are critical to stratify patients likely to benefit from neoadjuvant anti-PD-1 therapy.

Limitations of study

First, the small number of patients limits the broad applicability of our findings and their long-term implications for similar patients. Additionally, the single-arm design of our study precludes control group comparison. We enrolled a total number of 16 patients but were able to evaluate only 12 patients for efficacy endpoints.

A further limitation is that patient 8, a rapid progressor after only a single dose of nivolumab, was excluded from the primary trial analysis following the original trial protocol. At the time of study registration, there was concern that patients may come off the study early because neoadjuvant therapy is not the standard of care for oral cancer. It was hypothesized at that time that a single dose of nivolumab would not be able to demonstrate a meaningful response, as there were no published studies addressing this. Therefore, the inclusion of this patient in the response outcome would be considered a statistical *ad hoc* analysis which detracts from the prospective nature of the study.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
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  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Patients
  - Study design

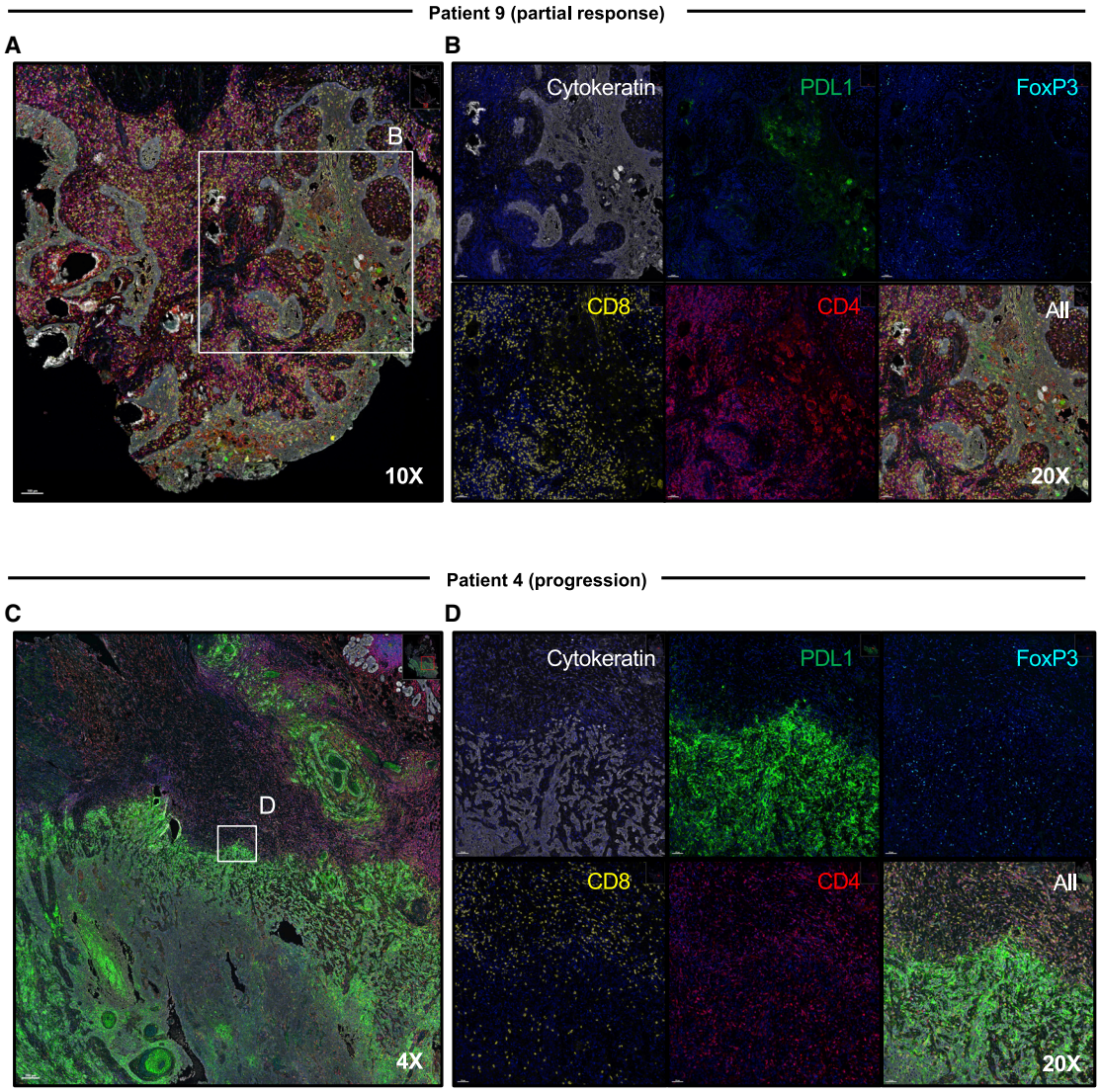

Figure 5. Immunological features of responsive and progressive tumors after neoadjuvant nivolumab

(A and B) Surgical specimen from patient 9, who experienced the greatest reduction in tumor size post nivolumab, was evaluated to characterize immune infiltrate by Vectra Polaris. Focus on cytokeratin regions revealed limited PD-L1 expression, with high levels of $CD8^+$ and $CD4^+$ but low $FoxP3^+$ infiltrates. (A) 10×, ruler, 100 μM and (B) 20×, ruler, 50 μM.

(C and D) Surgical specimen from patient 4, who represents the most rapid progressor, was analyzed for immune infiltration. Multiplexed imaging indicated robust PD-L1 staining across 50% of the pathologic section corresponding with $cytokeratin^+$ cells, with higher $FoxP3^+$ infiltrates and evident immune exclusion from tumor areas relative to non-tumor regions. (C) 4×, ruler, 200 μM and (D) 20×, ruler, 50 μM. Magnification on Phenochart analysis software.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2021.100426.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.M.K., J.D. Horton, C.M.P., and D.M.N.; methodology, E.G.-M., K.A., and D.M.N.; formal analysis, H.M.K., J.D. Horton, K.A., E.G.-M., C.M.P., and D.M.N.; investigation, H.M.K., J.D. Horton, S.L., J.M.K., M.S.R., S.H.L., Y.X., E.M.G., E.J.L., J.D. Hornig, J.S., S.S., M.V.S., E.C.O., C.D.T., M.J.R., and T.A.D.; resources, E.C.O., C.D.T., and M.J.R.; data curation, H.M.K., J.D. Horton, M.M.W., M.S.R., S.S., M.V.S., E.C.O., C.D.T., and M.J.R.; writing – original draft, H.M.K., J.D. Horton, C.M.P., and D.M.N.; writing – review & editing, all authors; visualization, H.M.K., J.D. Horton, E.C.O., C.M.P., and D.M.N.; supervision, H.M.K., J.M.W., M.R.I.Y., M.P.R., T.A.D., R.S.L., C.M.P., and D.M.N.; funding acquisition, H.M.K., C.M.P., and D.M.N.

DECLARATION OF INTERESTS

C.M.P. is the co-founder of Ares Immunotherapy. R.S.L. received research or clinical trial support from Merck, Pfizer, BMS, and OncoSec. D.M.N. received research or clinical trial support from BMS. M.R.I.Y. received research or clinical trial support Merck and BMS.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects. We worked to ensure that the study questionnaires were prepared in an inclusive way. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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STAR★METHODS

KEY RESOURCES TABLE

Reagent or Resource	Source	Identifier
Antibodies		
CD4 (SP35)	Cell Marque	Cat# 104R-1: RRID: AB_1516770
CD8 (SP16)	Cell Marque	Cat# 108R-1: RRID:AB_2892088
Foxp3 (236A/E7)	Abcam	Cat# ab20034: RRID:AB_445284
PDL1 (E1L3N)	Cell Signaling	Cat# 13684: RRID:AB_2687655
cytokeratin (AE1/AE3)	Dako	Cat# GA05361-2: RRID:AB_2892089
Reagent		
EDTA buffer (pH 9)	Agilent/Dako	Cat# S2367
Citrate buffer (pH 6)	Roche	Cat# 980-223
ProLong Gold Antifade Reagent	ThermoFisher	Cat# P36934
Opal 480	Akoya Biosciences	Cat# FP1500001KT:
Opal 520	Akoya Biosciences	Cat# FP1487001KT
Opal 570	Akoya Biosciences	Cat# FP1488001KT
Opal 690	Akoya Biosciences	Cat# FP1497001KT
Opal 780	Akoya Biosciences	Cat# FP1501001KT
Software		
Prism	https://www.graphpad.com/scientific-software/prism/	v9
inForm	https://www.akoyabio.com/phenoptics/software/inform-tissue-finder/	v2.4.10
Instruments		
Vectra® Polaris Automated Imaging System	Akoya Biosciences	N/A
Ventana Discovery Ultra Automated Research Stainer	Roche	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to the lead contact, David Neskey (neskey@musc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients

Eligible patients were 18-years old or older and had newly diagnosed histologically proven HPV-negative OCSCC. There could be no evidence of distant metastasis at enrollment. See Table 1 for specific patient demographic information. Primary tumors were required to be American Joint Committee on Cancer (AJCC) 7th Edition T stage 2-4a to ensure response to therapy could be accurately assessed clinically and radiographically. Patients were required to be Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Key exclusion criteria were T1 or unresectable tumor, prior non-surgical treatment (immunotherapy, chemotherapy including cetuximab, or radiation therapy), and active autoimmune disorder or infectious disease. Study data were collected and

managed using REDCap electronic data capture tools hosted at the Medical University of South Carolina.[29] REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Study design

Conducted at a single institution, this was a single-arm, investigator-initiated study (NCT03021993, Figure 1). A Simon two-stage design was employed,[30] with an initial recruitment of 9 patients in stage one and an additional 8 patients in stage 2 (see Study Protocol in Methods S1). Enrolled patients underwent baseline clinical and radiographic evaluation and initial pathologic diagnosis within 14 days of study registration. Clinical evaluation included standard flash or endoscopic photography of the primary tumor. Radiographic analysis included computed tomography (CT) of the head and neck. Radiographic tumor size was defined as the greatest cross-sectional dimension of the tumor on the enrollment imaging study and post-treatment size was the greatest cross-sectional dimension of the tumor on surgical pathology.

Upon enrollment, patients received a 3 mg/kg dose of nivolumab intravenously every two weeks for a total of three doses prior to interval radiographic evaluation between study days 28-35. In the event of disease progression (any increase in tumor burden or symptom progression), patients were taken for definitive surgical resection between days 36-42. In the event of stable disease (no change in tumor burden or symptoms) or response (reduction in tumor burden and symptoms), patients received a 4^{th} dose of nivolumab on day 43+/−1 followed by definitive surgical resection on day 50-56. This dosing schedule was chosen to fit within the standard of care timing between diagnosis and surgical resection to prevent delays in care.

Tumor responses on interval imaging were evaluated by averaging measurements of two radiologists blinded to the patient history and each other's measurements (SS and MVS) based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1.[21] Tumor pathologic analysis was performed with standard hematoxylin and eosin (H&E) staining. Resection of the primary tumor and draining lymph nodes along with recommendation for adjuvant radiotherapy or chemotherapy were based on National Comprehensive Cancer Network (NCCN) guidelines.[31] All patients were monitored for adverse events (AE) according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (2010).

The primary endpoint was objective response rate defined as pathologic complete response + pathologic partial response.[21] Partial response was defined as > 30% reduction in maximum tumor diameter in the surgical specimen compared to the single greatest tumor dimension on pretreatment radiographic measurement.[22] Secondary endpoints included safety and feasibility. Efficacy analysis was confined to patients who received at least two doses of nivolumab was due to concern that patients would come off study independent of tumor progression or toxicity but because neoadjuvant therapy is not the standard of care for oral cavity squamous cell carcinoma. For more details regarding study design see full clinical trial protocol (Methods S1).

Study oversight

Institutional review board and protocol review committee approvals at the Medical University of South Carolina were obtained prior to initiation of the study. The authors attest to their sole ownership of the trial design, data analysis, and drafting of the manuscript. The study was supported (with drug and funding) by Bristol-Myers Squibb; they played no role in the collection or analysis of the results.

METHOD DETAILS

Tumor histology and multiplex immunohistochemistry

Hematoxylin and eosin (H&E) stained slides were scanned using the Vectra® Polaris Automated Imaging System at 20X (Akoya Biosciences, Marlborough, MA). These slides were assessed for tumor type/differentiation and for cellularity by a surgical pathologist (MSR), and images were captured using Phenochart whole slide contextual viewer software in regions of interest (Akoya Biosciences, Marlborough, MA).

For multiplex immunohistochemistry, unstained slides with 4-5 μm sections of FFPE tissue were deparaffinized and stained using the Roche Ventana Discovery Ultra Automated Research Stainer (Roche Diagnostics, Indianapolis, IN). Heat-induced epitope retrieval was performed in EDTA buffer pH 9 (Cat# S2367 Agilent/Dako Santa Clara, CA) for 32 min at 100°C, and endogenous peroxidase was blocked with a hydrogen peroxide solution after incubation of the first primary antibody. Optimized multiplex immunofluorescence was performed using the OPAL multiplexing method based on Tyramide Signal Amplification. Antibodies used included CD4 (Cell Marque, clone SP35, 1:100), CD8 (Cell Marque, clone SP16, 1:300), FOXP3 (Abcam, clone 236A/E7, 1:600), PD-L1 (Cell Signaling Technologies, clone E1L3N, 1:200), and pan-cytokeratin (Dako, clone AE1/AE3, 1:100), as detailed in the Key Resources table. Fluorescence signals were generated using the following Akoya OPAL TSA fluorophores: OPAL 480, OPAL 520, OPAL 570, OPAL 690, and OPAL 780 (Akoya Biosciences, Marlborough, MA), and nuclei were visualized with DAPI counterstaining. Slides were incubated in citrate buffer pH 6 (Cell Conditioning Solution (CC2) Cat. #980-223, Roche Diagnostics) between each sequential antibody staining step at 90°C to remove the previous primary and secondary antibody complexes. Multiplex-stained slides were mounted with ProLong Gold Antifade Reagent (Cat. # P36934, ThermoFisher) and scanned at a 20x magnification using the Vectra® Polaris Automated Imaging System (Akoya Biosciences, Marlborough, MA). Whole slide scans were reviewed, and images were

captured using Phenochart whole slide contextual viewer software in regions of interest (Akoya Biosciences, Marlborough, MA). Spectral unmixing and elimination of autofluorescence was performed using the inForm® Software v2.4.10 (Akoya Biosciences, Marlborough, MA). Captured images were exported in TIFF format.

QUANTIFICATION AND STATISTICAL ANALYSIS

The full study used a Simon two-stage design with a null hypothesis of 2% for overall response rate and an alternative of 25%. With an alpha of 4% and power of 91%, the null hypothesis was rejected if two or more of 17 patients responded. Response rates were estimated with a 95% confidence interval with statistical inference for shortened phase II study based on the Simon two-stage design.[32] A single patient responding in the first 9 was considered sufficient evidence to progress into stage 2 in the absence of untoward AEs. The rate of grade 3-4 AEs was predicted to be $< 5\%$, and the trial would have been stopped if there was strong evidence (a likelihood ratio > 4 based on the binomial distribution) that the rate of grade 3-4 AEs was 25% or higher. Reported p values are two-sided and significant if < 0.05. Continuous variables are compared between response categories using the Kruskal-Wallis test. Continuous bivariate relationships were determined using Spearman's rank correlation.

ADDITIONAL RESOURCES

Description: https://clinicaltrials.gov/ct2/show/NCT03021993.

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Supplemental information

Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma

Hannah M. Knochelmann, Joshua D. Horton, Sixue Liu, Kent Armeson, John M. Kaczmar, Megan M. Wyatt, Mary S. Richardson, Shirley H. Lomeli, Ying Xiong, Evan M. Graboyes, Eric J. Lentsch, Joshua D. Hornig, Judith Skoner, Seth Stalcup, Maria V. Spampinato, Elizabeth Garrett-Mayer, Elizabeth C. O'Quinn, Cynthia D. Timmers, Martin J. Romeo, John M. Wrangle, M. Rita I. Young, Mark P. Rubinstein, Terry A. Day, Roger S. Lo, Chrystal M. Paulos, and David M. Neskey

Supplementary Materials for

Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma

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This file includes:

Supplementary Figures

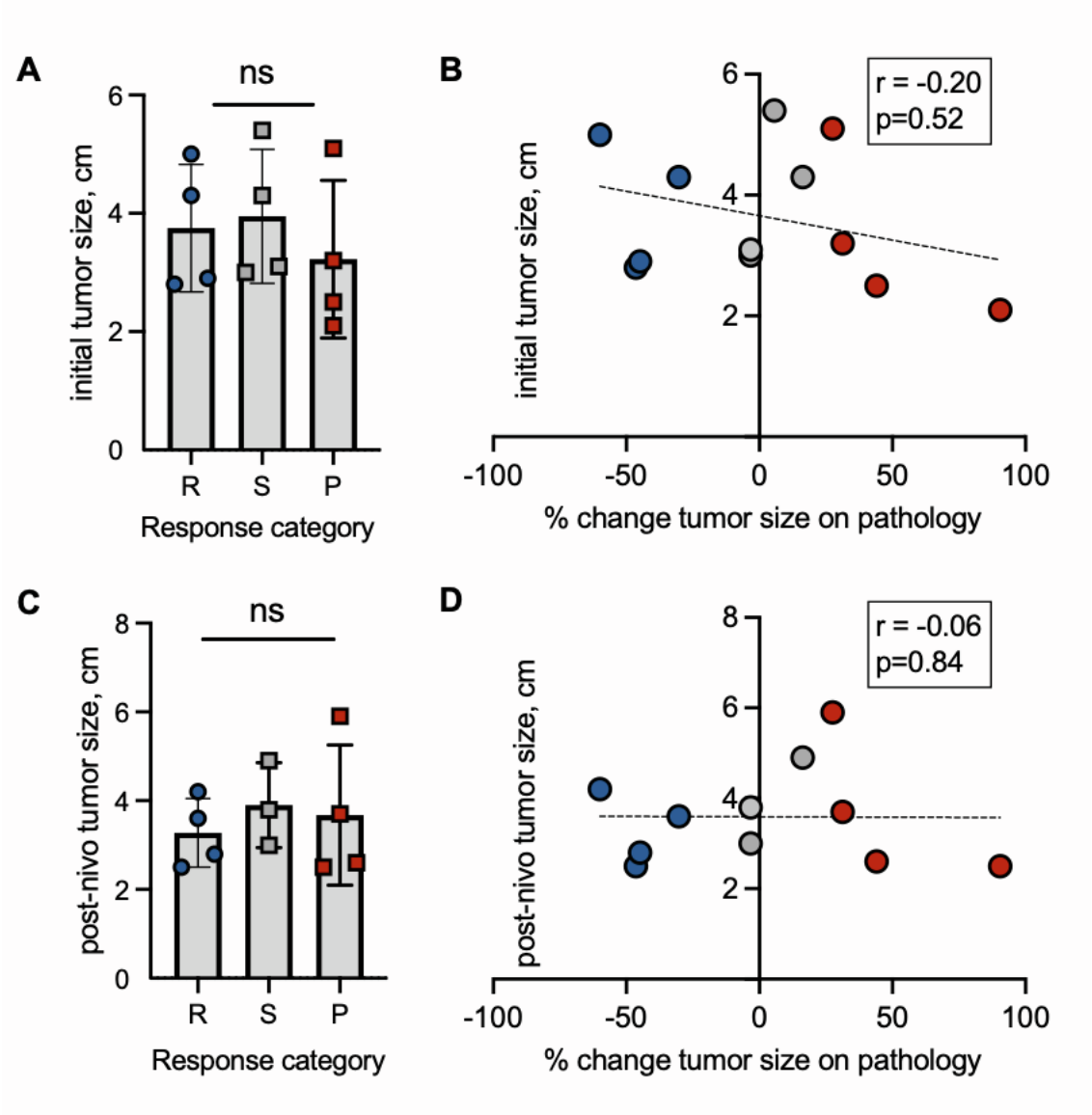

Figure S1. Absolute tumor size through treatment does not correlate with response. Related to Figures 2 and 3. A) Initial tumor size (greatest dimension, cm) is not correlated with eventual pathologic response (p=0.6255, Kruskal Wallis test). B) Correlation between initial tumor size and pathologic response (p=0.52 by Spearman correlation). C) Post-nivolumab, presurgical tumor size (greatest dimension, cm) is not associated with eventual pathologic response (p=0.6140, Kruskal Wallis test). D) Lack of correlation between post-nivolumab, presurgical tumor size and eventual pathologic response (p=0.84 by Spearman correlation). Note that patient 16 is not included in this analysis due to the absence of interval scan during the COVID-19 research shutdown. R, response; S, stable; P, progression.

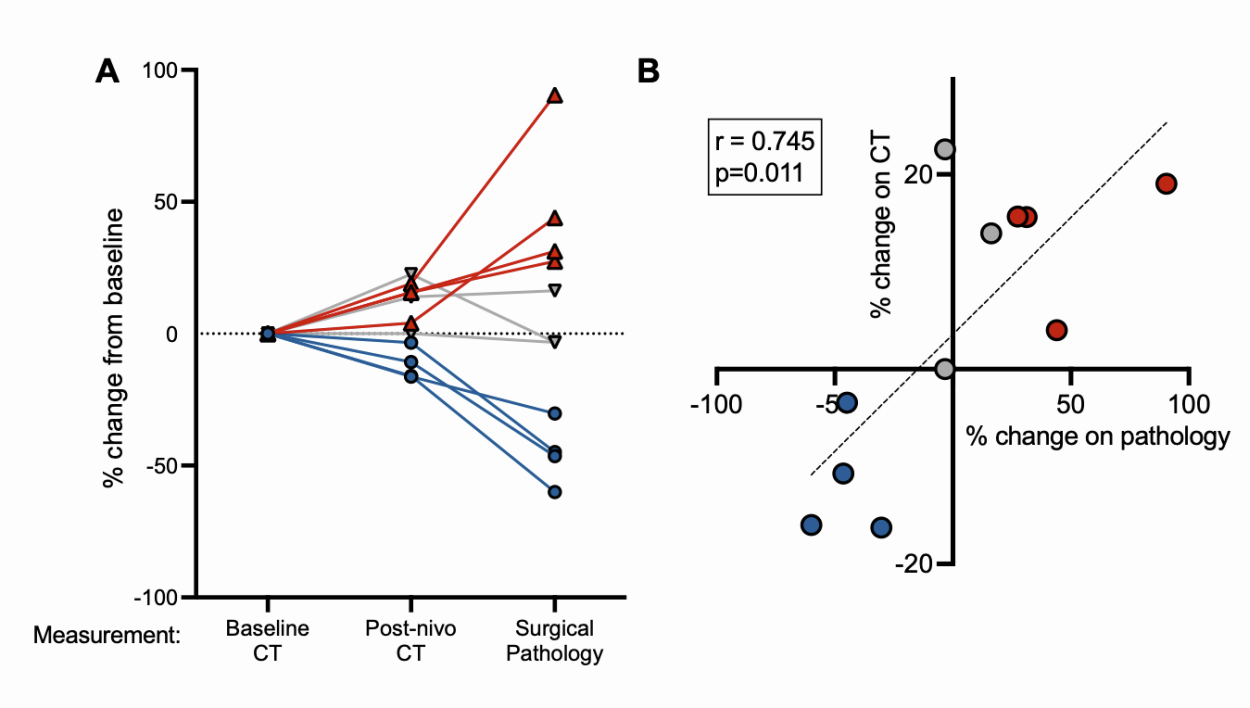

Figure S2. Post-nivolumab imaging predicts ultimate treatment response. Related to Figures 2 and 3. A) Spider plot demonstrating change in tumor dimension from baseline on interval imaging ("clinical response") and on final pathologic evaluation ("pathologic response"). B) Correlation between clinical response on post-nivolumab imaging and ultimate pathologic response (p=0.011 by Spearman correlation). R, responder; NR, non-responder; CT, computed tomography. Blue, responders; gray, stable; red, non-responders.

Supplementary Materials

Table S1. Safety, Feasibility, and Patient Follow-up after Neoadjuvant Nivolumab

Patient	Response Group	4th Nivo Dose?	Enroll to surgery, days	1st Nivo dose to surgery, days	Last Nivo dose to surgery, days	Grade 1-2 AEs (#)[a]	Grade 3-4 AEs (#)	Surgery to PORT, days	Adjuvant Chemo?	Recurrence?	Follow up, years
1	Response	+	49	49	7	8	0	14	-	Contralateral neck followed by distant metastasis	3.14
2	Progression	+	52	52	10	10[b]	0	Patient refused	-	Local recurrence	3.32
3	Progression	-	41	39	8	10[c]	0	41	-	-	2.63
4	Progression	-	41	41	9	2	0	28	+	Contralateral neck followed by local recurrence	1.15
5	Response	+	56	55	13	2[d]	0	42	-	-	2.84
6	Response	+	55	55	13	2[e]	0	41	-	Local recurrence	2.79
7	Progression	-	41	38	11	7[f]	0	45	-	Local recurrence	0.78
9	Response	+	48	47	5	6[g]	0	48	-	-	2.18
10	Stable	-	41	40	13	7[h]	0	36	-	-	2.28
12	Stable	-	44	41	13	4[i]	0	29	+	-	0.4
14	Stable	-	43	38	10	6[j]	0	Patient refused	-	-	1.86
16	Stable	-	28	27	13	3	1k	Patient refused	-	-	0.45
		median	**43.5**	**41**	**10.5**			**41**			**2.23**
		range	**28-56**	**27-55**	**5-13**			**14-48**			**0.4-3.32**

Nivo, nivolumab; PORT, post-operative radiation therapy

[a] "Possibly" or "definitely" related to nivolumab: fatigue (n=2), nausea (n=2), diarrhea (n=2), rash/psoriasis (n=2), myalgia (n=1), constipation (n=1), cough (n=1), joint pain (n=1), creatinine increase (n=1), dyspnea (n=1), back spasm (n=1), hypertension (n=1), back pain (n=1), chest wall pain (n=1)

[b] Diarrhea treated with 20mg prednisone daily x5 doses

[c] swollen right hand treated with Lovenox, 90mg subcutaneous, once per day

[d] Rash treated with triamcinolone cream

[e] psoriasis treated with clobetasol

[f] opioid induced constipation treated with laxatives, pain managed, and thrush treated with mycostatin

[g] pain, hypertension treated with steroids

[h] leg wound infection at site of graft treated with Bactrim

[I] oxycodone given for tongue pain, Miralax for constipation, and hearing aids for hearing loss

[j] magnesium given for hypomagnesia, and pain medication for oral pain

[k] Grade 3 pain was resolved

Methods S1: Clinical trial protocol, related to STAR Methods

NCT #: 03021993
MUSC CTO #: 102510
BMS Protocol #: CA209-831

PHASE II TRIAL OF NIVOLUMAB, AN ANTI-PD-1 MONOCLONAL ANTIBODY, AS A NOVEL NEOADJUVANT PRE-SURGICAL THERAPY FOR LOCALLY ADVANCED ORAL CAVITY CANCER

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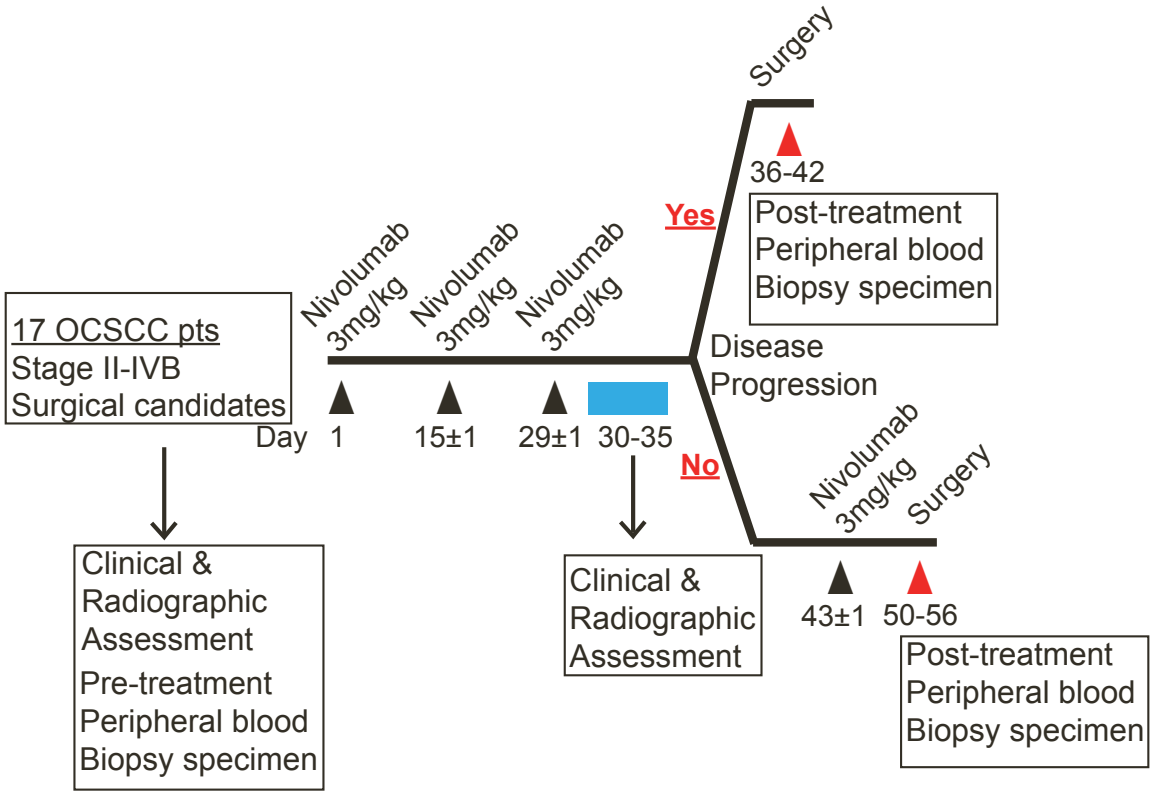
PROTOCOL VERSTION HISTORY
Version 1 dated July 11, 2016

TABLE OF CONTENTS

DEFINITIONS OF TERMS USED

AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALT	alanine transaminase
AST	aspartate transaminase
BMS	Bristol Myers Squibb
BP	blood pressure
BUN	blood urea nitrogen
CBCD	complete blood count with differential
CR	complete response
CrCl	creatinine clearance
CT	computed tomography
DILI	drug induced liver injury
DSMC	Data Safety Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
HBV	hepatitis B virus
HCC	Hollings Cancer Center
HCG	human chorionic gonadotropin
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HR	heart rate
IB	Investigator's Brochure
ICH	International Council of Harmonisation
IIT	Investigator Initiated Trial
IRB	Institutional Review Board
IV	intravenous
LFT	liver function test
MRI	magnetic resonance imaging
MUSC	Medical University of South Carolina
NCI CTCAE	National Cancer Institute Common Terminology for Adverse Events
OSCC	oral cavity squamous cell carcinoma
pCR	pathologic complete response
PET	positron emission tomography
PR	partial response
RECIST	response evaluation criteria in solid tumors
SAE	serious adverse event
SIS Unit	Sponsor-Investigator Support Unit
SOP	standard operating procedure
ULN	upper limit of normal
WBC	white blood cell
WOCBP	woman of child bearing potential

SCHEMA


17 OCSCC pts
Stage II-IVB
Surgical candidates
Nivolumab 3mg/kg
Nivolumab 3mg/kg
Nivolumab 3mg/kg
Day 1
15±1
29±1
30-35
Yes
Surgery
36-42
Post-treatment
Peripheral blood
Biopsy specimen
Disease Progression
No
Nivolumab 3mg/kg
Surgery
43±1
50-56
Post-treatment
Peripheral blood
Biopsy specimen
Clinical & Radiographic Assessment
Pre-treatment
Peripheral blood
Biopsy specimen
Clinical & Radiographic Assessment

OBJECTIVES

2.1 PRIMARY OBJECTIVE

To determine the pathological overall response rate (PR+CR) of neoadjuvant pre- surgical PD-1 inhibition in patients with surgically amenable oral cavity cancer.

2.2 SECONDARY OBJECTIVES

- To evaluate the systemic and intratumoral immune activation following PD-1 blockade.
- To determine immune reactivity to autologous OCSCC.

BACKGROUND

3.1 CLINICAL BURDEN OF HNSCC

Over 300,000 patients develop squamous cell carcinoma of the oral cavity (OCSCC) worldwide and nearly half of these patients die from the disease each year [1]. The development of regional metastases in oral cavity cancer decreases the 5-yr survival by 50%, and therefore advanced OCSCC portends a poor prognosis with estimated survival rates at 5 years ranging from 35-45% [2, 3]. Treatment of advanced oral cavity cancer often requires complex, multimodality therapy, employing surgical resection followed by post-operative radiation, with the addition of cisplatin-based chemotherapy for patients with high-risk of failure [4]. Despite these comprehensive treatment strategies, OCSCC recurs in 25-48% of patients [3, 5, 6]. Additionally, advances in reconstructive procedures have improved post-operative function of oral cavity cancer patients but these surgeries continue to be associated with significant morbidity including disfigurement, speech and swallowing deficits, and tracheostomy or gastrostomy tube dependence. Given the poor prognosis, high recurrence rates, and associated post-operative morbidities observed in OCSCC, it is evident new strategies are needed to treat and aid in the management of OCSCC [7, 8].

3.2 BENEFITS OF NEOADJUVANT TREATMENT

Neoadjuvant therapy is now being applied in multiple cancer types and has provided a new paradigm in the treatment of patients with breast and esophageal cancer in terms of overall and disease-free survival [9-12]. In the case of unresectable HNSCC, neoadjuvant chemotherapy has been applied prior to definitive radiotherapy or chemoradiotherapy with significant response rate in several phase II and III studies [13-15]. The results from these clinical trials support the concept that neoadjuvant treatment can be considered as a novel therapeutic strategy for the treatment of advanced HNSCC. Neoadjuvant chemotherapy prior to definitive surgery has been explored for the treatment of HNSCC with high rates of initial response ranging from 30-40% but a recent meta-analysis of 28 randomized trials revealed a modest survival benefit of only 6%[16-18]. Neoadjuvant treatment prior to definitive surgery has been shown to determine clinical efficacy and define molecular biomarkers predictive of response and has been tested in several studies including one at our own institution investigating the mTOR inhibitor, Rapamycin [16, 19-21]. In general, the potential benefits of effective neoadjuvant therapies include 1) the assessment of clinical response to novel, mechanism-based treatment options which can decrease tumor burden and thus reduce the extent of surgery [17]; 2) the exploration of molecular changes in cancer cells and surrogate tissues, such as blood, may provide valuable biomarkers to identify patients that may benefit the most from new targeted therapies [22]. A clinical response to neoadjuvant chemotherapy has prognostic value in multiple cancers, as pathologic complete response at the time of surgery is a recognized and validated surrogate marker for good clinical outcome [23, 24].

3.3 IMMUNOTHERAPY IN HNSCC

Because of the combination for the need of new therapies and the benefits of a neoadjuvant treatment strategy, the possibility of immunotherapeutic approaches for HNSCC patients has gained interest. Unfortunately, HNSCC patients have profound immune defects that are associated with a worse outcome and have been attributed to tumor production of inhibitory mediators and tumor-induced immune inhibitory cell populations

[25-30]. Programmed Death receptor I (PD-1) and its ligand (PD-L1) appear to contribute to this immune dysfunction as 50-60% lymphocytes from HNSCC patients have upregulated PD-1 expression and PD-1 blockade enabled lymphocyte proliferative reactivity to stimulation and indirectly overcame immune unresponsiveness by modulating immune inhibitory populations, including T regulatory cells [30-33]. Furthermore, tumor PD-L1 expression has been associated with improved objective response and clinical benefit in multiple tumor types [34]. Since 60-80% of OCSCC express PD-L1, it is anticipated PD-1 blockade will benefit 20-25% of patients with OCSCC [30, 33, 34]. Most recently the Checkmate 141 Phase III trial revealed in patients with treatment-resistant and rapidly progressive head and neck carcinoma that nivolumab treatment was associated with an improved 12-month overall survival of 36%, compared with 17% in the Investigator Choice arm [40, 41].

3.4 STUDY RATIONALE

Our past clinical trials with HNSCC patients have shown the feasibility of pre-surgical targeted or immunotherapy followed by multi-modality treatments combining surgery and adjuvant therapy leading to an improvement in recurrence free survival [35-37]. A similar multi-modality treatment is being proposed in this application to overcome immune lethargy by Nivolumab treatment to block PD 1 between the time of OCSCC diagnosis and surgical treatment. This duration of time will allow for the administration of three doses of Nivolumab given on Days 1, 15 and 29. The primary objective of the proposed study is to determine the efficacy of neoadjuvant PD-1 inhibition in patients with oral cavity cancer undergoing definitive surgical resection. In addition to assessing the clinical efficacy of Nivolumab, secondary objectives will be to analyze the effectiveness of PD-1 antibodies at restoring the immune reactivity in the peripheral blood as well as stimulating intratumoral immune activity. The rationale for this approach is based on the overexpression of lymphoid PD 1 in the HNSCC environment along with our prior demonstration of the feasibility of pre-surgical immune modulatory treatment or targeted mTOR therapy having positive post-surgical clinical effectiveness [30, 35, 36, 38]. Additionally, blocking PD 1 prior to surgical excision will allow restoration of immune competence to not only enable pre surgical immune responsiveness to HNSCC, but also post-surgical immunological surveillance for residual cancer.

We hypothesize 25-30% overall response rate (PR+CR) in patients with OSCC, and these patients will reveal a systemic and intratumoral response following anti-PD-1 therapy along with immune reactivity to autologous OCSCC.

SUBJECT SELECTION

4.1 INCLUSION CRITERIA

Patients eligible for study participation must meet all of the following criteria

1. Newly diagnosed histologically proven locoregional OCSCC (T stage 2-4) without evidence of distant metastases. OCSCC includes the subsites of oral tongue, floor of mouth, gingiva, retromolar trigone, and buccal mucosa.
2. Recurrent or persistent histologically proven locoregional OCSCC (recurrent T-stage 2-4) that was initially treated with surgery alone. To allow sufficient tumor tissue for the immunological analyses, patients with T1 OCSCC will be excluded. Eligibility criteria will also include the following:
3. Greater than or equal to 18 years of age
4. ECOG performance status of 0 or 1
5. Screening lab must meet the following criteria and must be obtained within 14 days prior to registration:
    - WBC $\geq$ 2,000/□L
    - Absolute Neutrophil Count $\geq$1,500/□L
    - Platelets $\geq$ 100 X 10^3/□L
    - Hemoglobin $\geq$ 9.0 g/dL

- Serum creatinine ≤ 1.5 X ULN or CrCl ≥ 40mL/min (if using the Cockcroft-Gault formula below):

$$\textit{Female}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{serum creatinine in mg/dL}}$$

$$\textit{Male}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$$

- AST/ALT ≤ 3 x ULN
- Total Bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)

6. Age and Reproductive Status:

   WOCBP is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 62 must have a documented serum FSH level less than 40 mIU/mL.

   WOCBP must use appropriate method(s) of contraception. WOCBP should use an adequate method to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug.

   Women of childbearing potential must have a negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab.

   Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year.

   Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product.

   Women who are not of childbearing potential (ie, who are postmenopausal or surgically sterile and azoospermic men do not require contraception.

   See appendix A for acceptable methods of contraception.

4.2 EXCLUSION CRITERIA

Patients eligible for study participation CANNOT meet any of the following criteria

1. Prior immunotherapy or treatment with another anti PD 1 agent.
2. Prior chemotherapy including Cetuximab or radiation therapy.
3. Previous severe hypersensitivity reaction to another monoclonal antibody
4. Women who are pregnant, lactating or expecting to conceive or father children within the research period
5. Known history of HIV or AIDS
6. Positive test for HBV sAg or HCV antibody indicating acute or chronic infection
7. Concomitant malignancies except cutaneous squamous cell carcinoma or basal cell carcinoma
8. Unresectable primary tumor or regional disease or distant metastases.
9. Active, known or suspected autoimmune disease. Note: Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger
10. Presence of condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

4.3 INCLUSION OF WOMEN AND MINORITIES

Both men and women of all races and ethnic groups are eligible for this trial.

4.4 SUBJECT REGISTRATION

The SIS Unit will provide subject registration services for the study. After obtaining signed informed consent and completion of required baseline assessments, eligible subjects will be registered. All study subjects will undergo an eligibility audit prior to registration. A unique subject number will be assigned to each patient. A registration confirmation will be emailed to the enrolling study team at the time of registration. This confirmation will include the subject's assigned cohort dose level and study ID number. Registrations may occur between 8AM and 5PM EST, Monday through Friday. Registrations may take place outside of this timeframe if prior arrangements are made.

STUDY INTERVENTION

5.1 NIVOLUMAB ADMINISTRATION

Nivolumab will be given every two weeks at a dose of 3mg/kg will be administered as a 60 minute IV infusion (+/- 10 minutes). Study drug will be administered on an outpatient basis. Subjects may be dosed no less than 12 days from the previous dose of drug.

REGIMEN DESCRIPTION			
Agent	**Dose**	**Route**	**Schedule**
Nivolumab	3mg/kg	IV	Cycle 1 = Day 1 Cycle 2 = Day 15 Cycle 3 = Day 29 Response assessment Cycle 4 = Day 43 if stable disease or response after day 29.

The dosing calculations should be based on the actual body weight at baseline. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the original dose, the dose must be recalculated. All doses should be rounded to the nearest milligram. **There will be no dose reductions allowed**.

Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 1 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to Protocol Section 5.3.

5.2 SURGICAL RESECTION

Between days 28-35, subjects will be evaluated for disease progression through clinical and radiographic tumor assessment, and for toxicity by history, physical and clinical labs. In the event of disease progression, subjects will proceed to definitive surgical resection between days 36-42. If disease is stable or response is observed, subjects will have the fourth administration of nivolumab on Day 43±1 followed by definitive surgical resection on day 50-56.

5.3 PRE AND POST MEDICATIONS

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest

with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms of allergic-like reactions.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE v. 4.0 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

- **For Grade 1 symptoms:** (Mild reaction; infusion interruption not indicated; intervention not indicated)

  Monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

- **For Grade 2 symptoms:** (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

  Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the eCRF. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

- **For Grade 3 or Grade 4 symptoms:** (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

  Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

  In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

DOSE MODIFICATIONS

Dose reductions or dose escalations are not permitted. Dose delays are not permitted. If an AE occurs causing a missed dose, the subject will stop the nivolumab administration schedule and proceed to surgery.

6.1 MANAGEMENT ALGORITHMS

Immuno-oncology agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity.

Management algorithms have been developed to assist investigators in assessing and managing the following groups of AEs: Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, Neurological.

For subjects expected to require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider recommendations provided in the algorithms. These algorithms can be referenced in the Nivolumab IB. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

6.2 DISCONTINUATION CRITERIA

Subjects may discontinue study treatment at any time. Subjects may be removed from study for any of the following reasons:

- Radiographic disease progression that is not amendable to resection
- Clinical disease progression that would not benefit from surgery
- Inter-current illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or require discontinuation of study drugs
- Completion of protocol treatment.
- Death
- Subject non-compliance
- Treatment should be permanently discontinued for the following:
  - Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
  - Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
  - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
  - Any drug-related LFT abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 8 x ULN
    - Total bilirubin > 5 x ULN
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do **not** require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
  - Grade 4 lymphopenia or leukopenia
  - Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Investigator.

CONCOMITANT THERAPY

7.1 PERMITTED MEDICATIONS – USE WITH CAUTION

As there is potential for hepatic toxicity with nivolumab or nivolumab/ipilimumab combinations, drugs with a predisposition to hepatoxicity should be used with caution in patients treated with nivolumab-containing regimen.

Subjects are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if > 10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (eg, delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

STUDY ASSESSMENTS

8.1 GENERAL GUIDELINES

- Baseline evaluations are to be done within 14 days of registration with the following exceptions:
    - informed consent and radiographic disease assessments to be done within 28 days of registration
    - hepatitis B and C testing and thyroid function testing to be done within 28 days of registration
    - serum pregnancy test must be done within 24hours of day 1 drug administration
- After screening, subjects should begin study drug within 72 hours.
- On study assessments to be completed +/-3 days, radiographic assessments will be completed within +/- 7 days.

8.2 PREGNANCY TEST FOR WOCBP

A serum pregnancy test is required within 24 hrs of study enrollment and prior to surgery. After discontinuation from nivolumab these should be repeated at approximately 30 days and approximately 70 days.

8.3 BASELINE ASSESSMENTS:

- Medical history will be obtained to capture relevant underlying conditions. The baseline examinations should include weight, height, ECOG Performance Status, BP, HR, temperature, and oxygen saturation by pulse oximetry at rest (also monitor amount of supplemental oxygen if applicable) should be performed within 28 days prior to first dose. Baseline signs and symptoms are those that are assessed within 14 days prior to first dose. Concomitant medications will be collected from within 14 days prior to the first dose through the study treatment period.
- Laboratory assessments will be done within 14 days prior to registration and are to include: CBCD, LFTs (ALT, AST, total bilirubin, alkaline phosphatase), BUN or serum urea level, creatinine, calcium, Magnesium, Sodium, Potassium, Chloride, phosphate, LDH, glucose; TSH, free T3, and free T4.
- The following baseline local laboratory assessments should be done within 28 days prior to first dose: Hepatitis B and C testing (HBV sAg and HCV Ab or HCV RNA).
- **After patients are entered into this study**, the clinical team will oversee collection of primary tumor biopsy if not previously performed.

8.4 RADIOGRAPHIC ASSESSMENT:

- Initial evaluation will include CT scan of the head and neck to assess local and regional disease extent. PET/CT or CT of the chest, abdomen, and pelvis will also be utilized to assess for distant disease. Restaging scans of the head and neck will be performed following the 3rd cycle between Days 28-35.
- MRIs may be substituted in place of CT scan for clinical reasons such as subject intolerance of intravenous contrast material. The same method of assessment (i.e. CT, MRI) and the same technique should be used to

characterize each identified and reported lesion at baseline and during follow-up. Use RECIST 1.1 guidelines.

- Radiographic assessments for restaging must be done within 7 days of 3rd cycle.

8.5 CLINICAL ASSESSMENT:

- Tumors will be photographed at time of clinical assessment on day 1 and following the third cycle of treatment for a qualitative assessment of possible effects on reduction in tumor size, necrosis, erythema/inflammation, and vascularity. Standard flash or endoscopic digital photography may be used depending on the site and accessibility for photography.
- Following completion of the trial, two blinded head and neck tumor surgeons (Photograph Examiner #1 and #2) will review the photographs separately. Each examiner will record estimated size, percent increase or decrease, and anatomic descriptive terms related to change in size, shape, texture, and color over time from the photographs.
- Primary tumors will be assessed for progression using caliper measurement on Day 1 and following the third cycle of treatment Day 28-35.

8.6 ON STUDY ASSESSMENTS

- Toxicity assessments will be continuous during the treatment phase. Adverse events and laboratory values will be graded according to the NCI-CTCAE version 4.0.
- On-study weight, ECOG performance status, and vital signs should be assessed at each on-study visit prior to dosing. Vital signs should also be taken as per institutional standard of care prior to, during and after dosing. Oxygen saturation by pulse oximetry at rest (also monitor amount of supplemental oxygen if applicable) should be assessed at each on-study visit prior to dosing. The start and stop time of the study therapy infusions should be documented.
- Physical examinations are to be performed as clinically indicated. If there are any new or worsening clinically significant changes since the last exam, the changes should be reported as AEs.
- Laboratory testing prior to each dose: Within 72 hours prior to re-dosing to include CBCD, LFTs, BUN or serum urea level, creatinine, Ca, Mg, Na, K, Cl, LDH, Glucose, amylase, lipase, TSH (with Free T4 and Free T3). Thyroid function testing should be done every 6 weeks (every 3 cycles) for subjects receiving nivolumab.
- Four 5-mL tubes of peripheral blood will be collected for correlative studies. See section 12 for more details.
- Additional measures, including non-study required laboratory tests, should be performed as clinically indicated or to comply with local regulations. Laboratory toxicities (eg, suspected drug induced liver enzyme evaluations) will be monitored during the follow-up phase via on site/local labs until all study drug related toxicities resolve, return to baseline, or are deemed irreversible.
- If a subject shows changes on pulse oximetry or other pulmonary-related signs (hypoxia, fever) or symptoms (eg. dyspnea, cough, fever) consistent with possible pulmonary-related signs (hypoxia, fever) or symptoms (eg, dyspnea, cough, fever) consistent with possible pulmonary adverse events, the subject should be immediately evaluated to rule out pulmonary toxicity.
- **At the time of surgery**, portions of the excised HNSCC tissues that are not required for clinical purposes (existing material) will be used for correlative research. See section 12 for more details.
- Some of the assessments referred to in this section may not be captured as data in the eCRF. They are intended to be used as safety monitoring by the treating physician. Additional testing or assessments may be performed as clinically necessary or where required by institutional or local regulations.

8.7 OFF STUDY AND FOLLOW UP

Subjects will be followed every 3 months until progression or until death, whichever comes first, for a maximum of 12 months after the last subject has been registered to the trial. AEs will be recorded for 6 weeks after surgery.

Activity	Screening			Patient Registration	Nivolumab Administration			If stable or response only:	Surgery [c]	Follow Up
	Within 28 days of Registration	Within 14 days of Registration	Within 24 hours of registration		Day 1	Day 15±1	Day 29±1	Day 43[c]	Day 36-42 or Day 50-56	
Informed consent	X									
History and Physical examination		X			X[a]	X[a]	X[a]	X[a]		
ECOG PS		X			X	X	X	X		
Vital signs, weight		X			X	X	X	X		
CBCD & CMP, mag, phos		X			X	X	X	X		
HVB and HVC screening	X									
TSH, free T3, free T4	X				X			X		
Serum Pregnancy test			X				X			X[d]
Assessment of Concomitant medications		X			X			X		
Adverse events assessment					X	X	X	X		X
Radiographic Tumor Assessment	X						X			
Survival Status and disease status[b]										X
Nivolumab Administration					X	X	X	X		
Surgery									X	
Tumor biopsy	X								X	
Research blood collection[e]					X				X	

8.8 STUDY CALENDAR

a. if clinically indicated
b. follow up every three months until progression or death, for up to 12 months after the last subject has been registered to the trial
c. If disease is stable or response is observed at days 28-35, subjects will have the forth administration of nivolumab on Day 43, followed by definitive surgical resection on day 49.
d. Serum pregnancy test should be done 30 days and 70 days after last drug administration.
e. Four 5-mL tubes of peripheral blood will be collected for correlative research. See section 12 for more details about tumor biopsy and research blood collection.

DRUG INFORMATION - NIVOLUMAB

9.1 DRUG ORDERING AND ACCOUNTABILITY

BMS is supplying study drug. Please see the pharmacy manual for instructions on how to order study drug from BMS.

9.2 PRODUCT DESCRIPTION AND DOSAGE FORM

Nivolumab (BMS-936558-01) Injection drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL.

- **Other names**: MDX-1106, ONO-4538 and antiPD-1.
- **Potency:** 100mg/Vial (10mg/mL)
- **Primary Packaging (volume)/Label type:** Carton of 5 or 10 vials
- **Secondary packaging (Qty)/Label type:** 10-cc Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals.
- **Appearance:** clear to opalescent, colorless to pale yellow liquid. May contain particles

9.3 STORAGE CONDITIONS:

Investigational product must be stored at 2-8 degrees C (36-46 degrees F) and protected from light and freezing.

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.
For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for "Recommended Storage and Use Conditions"

9.4 LABELING:

Nivolumab may be labeled as BMS-936558-01 Solution for Injection.

9.5 HANDLING AND DISPENSING

The investigator should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as per product information and the Investigator Brochure and per local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact BMS immediately.

Please refer to the current version of the Investigator Brochure and/or shipment reference sheets for additional information on storage, handling, dispensing, and infusion information for nivolumab.

9.6 DESTRUCTION

Sponsor/Investigator drug destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the Sponsor SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, ie, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for BMS to review throughout the clinical trial period as per the study agreement.

If conditions for destruction cannot be met, please contact BMS.

It is the Sponsor Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

DEFINITION OF ENDPOINTS

10.1 PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is objective response rate: pathologic complete response + pathologic partial response. Objective response rate: the sum of patients with either a pCR defined as no invasive and no in situ residuals present in the surgical specimen or partial pathologic response defined at least a 30% reduction in the size of the lesion in the surgical specimen. The reduction in size will be determined by comparing the pretreatment clinical measurements (the sum of the greatest axial measurement obtained with calipers at the time of initial evaluation) with the final pathologic measurements. Partial response has been chosen as an efficacy endpoint because of the two stage design and small sample size of the proposed trial (*i.e.* a objective response must be observed in at least 1 patient in first nine to proceed with the second stage). The proposed study is based on the current available head and neck squamous cell carcinoma data and is not powered to assess an objective response rate defined as pathologic complete response + major pathologic response.

10.2 SECONDARY EFFICACY ENDPOINTS

Immune capability will be measured as follows:

1. Levels of Treg cells in pre and post treatment peripheral blood will be evaluated using immunostaining for CD4 and flow cytometric analysis of Foxp3. Differences will be calculated (absolute change and percentage change) between pre and post treatment measures.
2. Levels of activated T-cells in peripheral blood will be assessed using flow cytometry for expression of CD69, IFN γ, T-bet and ICOS in CD4+ cells. Differences will be calculated (absolute change and percentage change) between pre and post treatment measures.
3. Intratumoral immune activity assessed by levels of immune stimulatory cytokines including IL-2, IFN γ, and IL-12 or inhibitory cytokine, IL10 and TGF-beta, in OCSCC tumor lysates will be measured flow cytometrically by cytokine bead array. Differences will be calculated (absolute change and percentage change) between pre and post treatment measures.

Immune reactivity against autologous OCSCC will be measured by T cell responsiveness of their pre and post treatment peripheral blood following a challenge with autologous OCSCC.

1. Expression of IFN γ, IL-2 (Th1 responses) and IL 10 (Th2 responses) in CD4+ cells from peripheral blood of patients following PD-1 inhibition therapy will be compared between pre-treatment peripheral blood samples and post-treatment samples from the same patient.
2. Expression of CD8+ cells expressing granzyme B (cytolytic response) from peripheral blood of patients following PD-1 inhibition therapy will be compared between pre-treatment peripheral blood samples and post-treatment samples from the same patient.

Definition of secondary outcomes/endpoints:

1. Increased immune capability will be defined as decrease in Treg population with subsequent increase in activated T cells population.
2. Immune reactivity will be defined as increased Th1 response and CD8+ cytolytic response following challenge to autologous OCSCC in post treatment peripheral blood relative to pretreatment peripheral blood samples.

10.3 SAFETY ENDPOINTS

Adverse events, assessed using the CTCAE v4, occurring between enrollment and 6 weeks following surgery will be collected.

STATISTICAL CONSIDERATIONS AND DETERMINATION OF SAMPLE SIZE

11.1 DEFINITION OF EVALUABLE PATIENTS

11.1.1 EVALUABLE FOR SAFETY

Any patient who receives one or more doses of nivolumab will be included in the safety population.

11.1.2 EVALUABLE FOR EFFICACY

Patients who receive at least two doses of nivolumab and undergo surgery will be included in the efficacy population.

11.1.3 EVALUABLE FOR IMMUNE ACTIVATION AND REACTIVITY

Patients who have received at least two doses of nivolumab and have both a pre-treatment and a post-treatment sample available for measuring immune markers will be included in the analyses for the secondary objectives of the study.

11.2 DATA ANALYSIS PLANS

11.2.1 PRIMARY OBJECTIVE

The clinical, radiographic and pathologic response rates will each be estimated with a 95% confidence interval, accounting for the two-stage design [41]. The null hypothesis is a 2% pathological response rate and the alternative is a 25% pathological response rate. If no patients respond in the first nine evaluable patients enrolled, the study will be terminated and we will conclude that there is not sufficient activity to reject the null hypothesis. The trial may pause enrollment after the first nine patients (if needed) to follow the ninth patient for a pathologic response. Note, however, that it is possible that an earlier patient may respond which would negate the need to stop enrollment (e.g. the 2nd patient may respond which would satisfy the criteria of at least one patient response in the first nine patients). If at least one patient has a pathologic response among the first nine evaluable patients enrolled, an additional eight patients will be enrolled. If two or more patients among 17 evaluable patients respond, we will reject the null hypothesis. The reported p-value will be based on two-stage design and any deviations from the intended sample size will be accounted for using the approach by Koyama and Chen [41]. Deviations from the planned sample size would only include the need for replacement patients in the event of a patient withdrawal following a single dose of Nivolumab secondary to toxicity or patient preference. If this were to occur these withdrawn patients would be excluded from the efficacy analysis. Only patients that receive at least 2 doses of Nivolumab will included in the efficacy analysis. Briefly, Koyama and Chen's approach adjusts the final results of the Simon two-stage design (point estimate, 95% confidence interval, and p-value) by considering the evaluation of the data at the end of the 1st stage of the design. Ignoring the two-stage nature of the design, the estimated response rate (i.e. number of responders divided by the total number of patients at the end of stage 2) tends to underestimate the true response rate. In addition, the p-value and 95% confidence interval based simply on the maximum likelihood estimate using the total number of responses and total sample size will not be valid if the futility stopping evaluation is ignored. The approach for implementing the adjustment can be found here:
http://biostat.mc.vanderbilt.edu/wiki/Main/TwoStageInference

11.2.2 SECONDARY OBJECTIVES

Immune activation markers will be graphically displayed at baseline (pre-treatment) and at time of surgical resection, and summary statistics will be reported for baseline, follow-up and changes over time. Based on our prior studies a 50% increase in immune stimulatory cytokines and 50% decrease in Treg levels is anticipated.

Transformations will be taken as needed (e.g. log transform) to adhere to assumption of statistical methods. Comparisons of baseline vs. follow-up measures will be made using paired t-tests. Logistic regression will be used to evaluate the association between baseline immune markers and clinical response and to evaluate associations between changes in immune markers and response. Both absolute change and percentage change in markers will be considered

11.3DETERMINATION OF SAMPLE SIZE

This study will enroll up to 19 patients. The Simon two-stage design is based on a total of 17 patients who are evaluable for efficacy. We may enroll up to 19 to allow for up to two patients to be inevaluable.

Without treatment, the rate of response would be expected to be very low (<2%). With treatment, we anticipate a meaningful pathological response rate of 25%. Using a Simon optimal two-stage design with alpha of 4% and power of 91%, we can detect this difference with a sample size of no more than 17 patients. In stage 1, 9 patients will be enrolled. If none respond, the study will be stopped. If one or more responds, we will continue to the 2nd stage and an additional 8 patients will be enrolled. If a total of 2 or more patients respond out of 17 we will reject the null hypothesis and conclude that treatment with Nivolumab results in a meaningful pathological response for patients with HNSCC.

11.4SAFETY ANALYSIS

Adverse events occurring between enrollment and 6 weeks after surgery will be tabulated by type and grade. Adverse events that are at least possibly related to nivolumab will be tabulated separately.

Safety monitoring will occur continuously during the study. We anticipate nivolumab to be well-tolerated in this patient population and would anticipate a low (≤5%) rate of grade 3 and 4 adverse events that are at least possibly related to nivolumab. If there were strong evidence during the course of the trial that the rate of grade 3 and 4 adverse events was 25% or higher, we would stop the study. Using a likelihood-based approach, the study will stop if the likelihood ratio for a grade 3-4 AE rate of 25% vs. a grade 3-4 AE rate of 5% is 4 or higher. This is based on the binomial distribution. The stopping boundaries based on this approach are shown in the table below. Note that the upper limit on the sample size 19 due to the potential need to enroll up to 19 patients in the case of up to two patients inevaluable for response.

Stop the trial if:	Observed rate	Likelihood Ratio
2 of 3-9 pts have grade 3-4 AEs	22% - 67%	≥ 4.78
3 of 10-17 pts have grade 3-4 AEs	18% - 30%	≥ 4.57
4 of 18-19 pts have grade 3-4 AEs	21% - 22%	≥ 4.36

11.5INTERIM ANALYSIS

There are no interim analyses planned for this study. However, given that the study design is a Simon two-stage design, the number of responses in the first nine patients will be evaluated and the study will be terminated after the ninth patient if none of these nine patients have achieved a clinical response.

CORRELATIVE STUDIES

After patients are entered into this study, the clinical team will oversee collection of primary tumor biopsy if not previously performed and peripheral blood at the onset of the study prior to initiating treatment. Prior to surgical resection, peripheral blood will again be collected in an effort to measure pre- and post-treatment immune reactivities. At each blood draw, each patient will have peripheral blood drawn into four 5-ml tubes. Portions of the excised HNSCC tissues that are not required for clinical purposes (existing material) will be used in addition to the peripheral blood for immunological assessments. The peripheral blood and HNSCC tissues will be used to assess immunological responsiveness to the immunotherapy (see “Correlative Research” below)

12.1 EVALUATION OF THE SYSTEMIC AND INTRATUMORAL IMMUNE ACTIVATION FOLLOWING PD-1 BLOCKADE:

Increased immune capability will be defined as follows:

a. Reduced levels of Treg cells in pre and post treatment peripheral blood will be evaluated using immunostaining for CD4 and flow cytometric analysis of CD3 and Foxp3.

b. Increased levels of activated T-cells in peripheral blood will be assessed using flow cytometry for expression of CD69, IFN-γ, T-bet and ICOS in $CD4^+$ cells.
c. Intratumoral immune activity assessed by increased levels of immune stimulatory cytokines including IL-2, IFN-γ, and IL-12 or inhibitory cytokine, IL10 and TGF-beta, in OCSCC tumor lysates will be measured flow cytometrically by cytokine bead array.

12.2DETERMINING IMMUNE REACTIVITY TO AUTOLOGOUS OCSCC:

Immune reactivity against autologous OCSCC will be measured by T-cell responsiveness of their pre and post treatment peripheral blood following a challenge with autologous OCSCC.

a. Following a challenge with autologous OCSCC, increased expression of IFN-γ, IL-2 (Th1 responses) and decreased IL-10 (Th2 responses) in $CD4^+$ cells from peripheral blood of patients following PD-1 inhibition therapy will be compared to pre-treatment peripheral blood samples from the same patient.
b. Following a challenge with autologous OCSCC, Increased expression of $CD8^+$ cells expressing granzyme B and perforin (cytolytic response) from peripheral blood of patients following PD-1 inhibition therapy will be compared pre-treatment peripheral blood samples from the same patient.

ADVERSE EVENT REPORTING REQUIREMENTS

13.1 PURPOSE

AE data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AE are reported in a routine manner at scheduled times during a trial. Additionally, certain AEs must be reported in an expedited manner to allow for more timely monitoring of patient safety and care. The following guidelines prescribe routine and expedited AEs reporting for this protocol.

Throughout the study, the Investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safety of the drug under investigation.

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

13.2 DEFINITION OF SEROUS ADVERSE EVENT

An SAE is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Potential DILI is also considered an important medical event.
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

The following hospitalizations are not considered SAEs for this protocol:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEsPotential drug induced liver injury is defined as:

1) ALT or AST elevation > 3 times ULN

AND

2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

3) No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety).

The investigator must immediately notify Worldwide Safety @BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

13.3SAE REPORTING

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (eg, a follow-up skin biopsy). The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies on a Pregnancy Surveillance Form.

SAE Email Address: **WORLDWIDE.SAFETY@BMS.COM**
SAE Facsimile Number: 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS (or designee) using the same procedure used for transmitting the initial SAE report.
All SAEs should be followed to resolution or stabilization.

The Sponsor/Investigator will ensure that all SAEs in the clinical database are reported to BMS and any applicable health authority during the conduct of the study including periodic reconciliation.

Any event that is both serious and unexpected must be reported to the FDA as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at:
http://www.accessdata.fda.gov/scripts/medwatch/.
MedWatch SAE forms should be sent to the FDA at:
MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to BMS at:
Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

The SIS Unit will be responsible for submitting the SAEs to the FDA and other regulatory agencies/oversight committees as required.

13.4DEFINITION OF ADVERSE EVENT

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all AEs. The casual relationship can be one of the following:

Definitely related:	An adverse event occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary and feasible.
Possibly related:	An adverse event with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Not related:	An adverse event with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying diseases provide plausible explanations.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

13.5NONSERIOUS ADVERSE EVENT COLLECTION AND REPORTING

The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

13.6DEFINITION OF SEVERITY

Adverse events will be graded according to the revised NCI CTCAE v. 4.0. If toxicities are not defined by the NCI CTCAE v. 4.0, the intensity of each adverse event should be graded as outlined below:

GRADE 1	MILD: Sign or symptom noticeable, but does not interfere with normal daily activities.
GRADE 2	MODERATE: Sign or symptom sufficient to interfere with normal daily activities.
GRADE 3	SEVERE: Sign or symptom is incapacitating, with inability to perform daily activities.
GRADE 4	LIFE-THREATENING: sign or symptom poses immediate risk of death to this patient.

13.7DOCUMENTATION OF ADVERSE EVENTS

The Investigator will monitor and/or ask about or evaluate AEs using non leading questions at each visit or evaluation. The occurrence of all AEs will be documented in the CRF with the following information, where appropriate:

- AE name or term
- When the AE first occurred (start date)
- When the AE stopped (stop date), (or an indication of “ongoing”)
- How long the AE persisted (optional)
- Severity of the AE
- Seriousness
- Actions taken
- Outcome
- Investigator opinion regarding the relationship of AE to the study drug(s)

ETHICAL AND REGULATORY CONSIDERATIONS

This study will be conducted in accordance with ICH CGP, the FDA, local IRB and in compliance with the protocol. The protocol, any amendments and the subject informed consent will received IRB approval before initiation of the study or implementation of any protocol change.

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

14.1INFORMED CONSENT

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Informed consent will be obtained by personnel who are qualified by education, training and experience to perform the task. The study will not sure the services of study personnel for whom sanctions have been invoked where there has been scientific misconduct or fraud.

Investigators must enrusre that subjects are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical studies in which they volunteer to participate. The approved consent form will adhere to the ehical principles that have their origin in the Declaration of Helsinki.

14.2INSTITUTIONAL REVIEW

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46)

MONITORING

The SIS Unit will be responsible for the monitoring of study patient data and records; monitoring will be performed centrally. The SIS Unit will be responsible for forwarding any applicable reports to the HCC DSMC for review.

The SIS Unit will conduct patient eligibility audit reviews for all patients prior to patient registration. During the course of the study, subjects will be selected for an audit at least once a year. Progress reports will be submitted to the HCC DSMC at least once a year.

15.1 PROTOCOL DEVIATIONS

For the purposes of this study, a **protocol deviation** is any variance from the protocol involving a subject or subjects that is not approved by the IRB prior to its initiation or implementation, and occurs when a member of the study team departs from the IRB-approved protocol in any way without the investigator first obtaining IRB approval. Protocol Deviations have special reporting requirements. Any protocol deviation and any supporting documentation will be submitted to the SIS Unit within 10 days of notification as outlined in the Operations Manual for this study.

15.2 DATA SAFETY MONITORING BOARD

The HCC DSMC will have oversight of the protocol. The HCC DSMC will meet at a minimum on a semi-annual basis to review the audits and progress reports for this IIT.

In addition, all protocol deviations and SAEs as defined above will be reviewed by the HCC DSMC at monthly meetings. As new protocol deviations or serious adverse events are reported to the SIS Unit, the SIS Unit will review these reports for form completion and follow up if more information is warranted. The SIS Unit will forward the event report to the HCC DSMC so that the information can be reviewed at the next available DSMC meeting. During the DSMC review, the DSMC can make recommendations for any further study action. The SIS Unit will maintain a copy of the DSMC approval letters for each event reviewed for this study within the site's central study file and will distribute to the participating site, if applicable.

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APPENDIX A: ACCEPTABLE FORMS OF CONTRACEPTION

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

- Male condoms with spermicide
- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena® by WOCBP subject or male subject's WOCBP partner Female partners of male subjects participating in the study may use hormone based contraceptives as one of the acceptable methods of contraception since they will not be receiving study drug
- Nonhormonal IUDs, such as ParaGard®
- Tubal ligation
- Vasectomy
- Complete Abstinence*

*Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

LESS EFFECTIVE METHODS OF CONTRACEPTION

- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal sponge
- Male Condom without spermicide
- Progestin only pills by WOCBP subject or male subject's WOCBP partner
- Female Condom*.
- A male and female condom must not be used together

APPENDIX B: MANAGEMENT ALGORITHMS

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Medical Monitor representing the Sponsor. The guidance applies to all immuno-oncology agents and regimens. A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated. Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended. The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

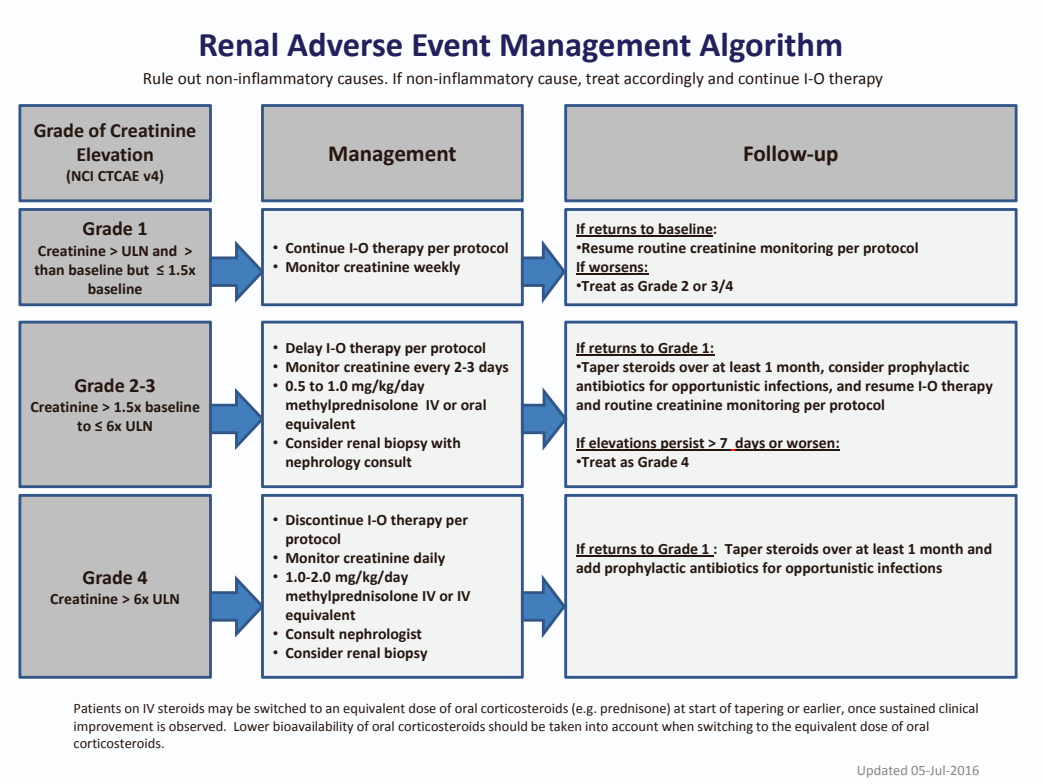
GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hrs; not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with ADL Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

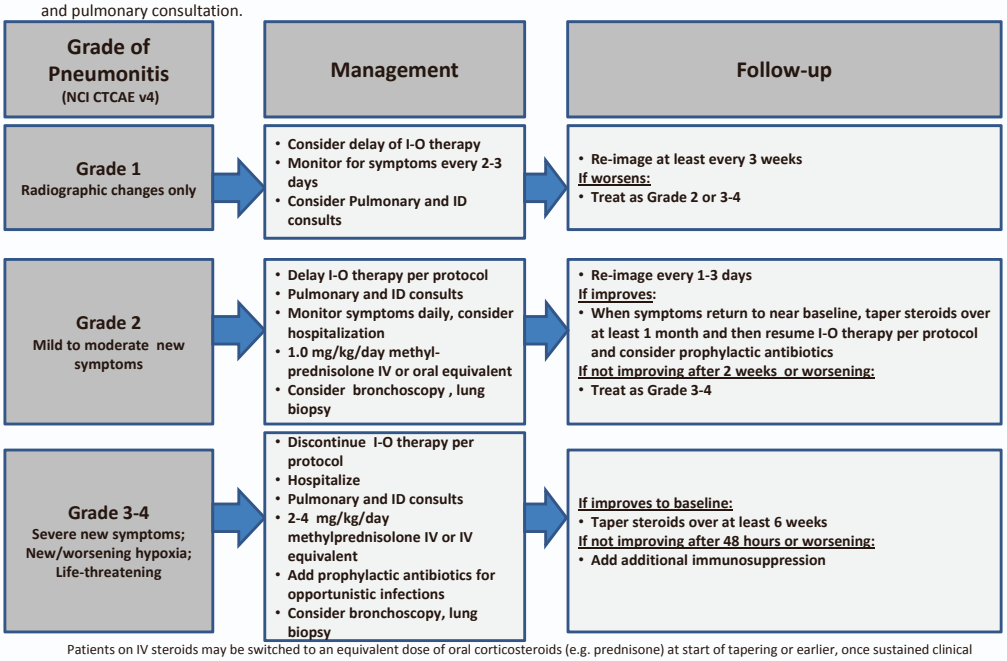
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Updated 05-Jul-2016


Renal Adverse Event Management Algorithm
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy
Grade of Creatinine Elevation (NCI CTCAE v4)
Management
Follow-up
Grade 1
Creatinine > ULN and > than baseline but ≤ 1.5x baseline
• Continue I-O therapy per protocol
• Monitor creatinine weekly
If returns to baseline:
•Resume routine creatinine monitoring per protocol
If worsens:
•Treat as Grade 2 or 3/4
Grade 2-3
Creatinine > 1.5x baseline to ≤ 6x ULN
• Delay I-O therapy per protocol
• Monitor creatinine every 2-3 days
• 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent
• Consider renal biopsy with nephrology consult
If returns to Grade 1:
•Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol
If elevations persist > 7 days or worsen:
•Treat as Grade 4
Grade 4
Creatinine > 6x ULN
• Discontinue I-O therapy per protocol
• Monitor creatinine daily
• 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent
• Consult nephrologist
• Consider renal biopsy
If returns to Grade 1 : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Updated 05-Jul-2016

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

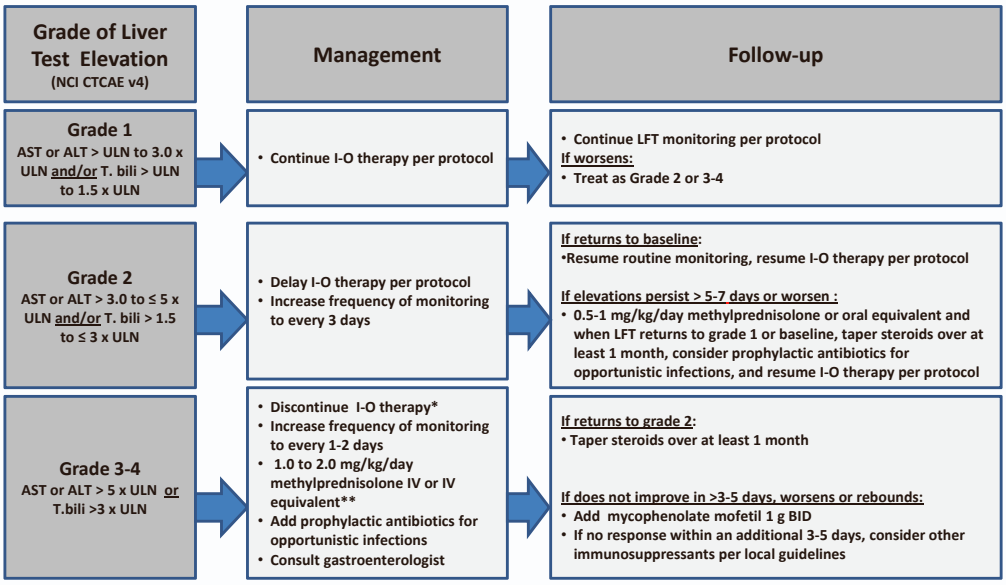


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Updated 05-Jul-2016

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

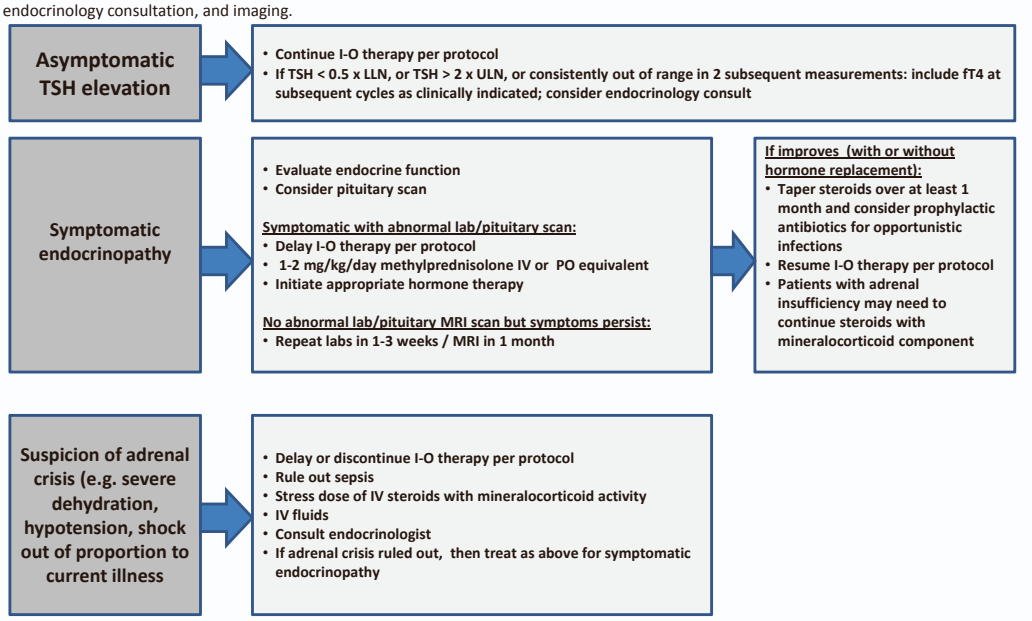
***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T.bili ≤ 5 x ULN.**

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Updated 05-Jul-2016

Endocrinopathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.

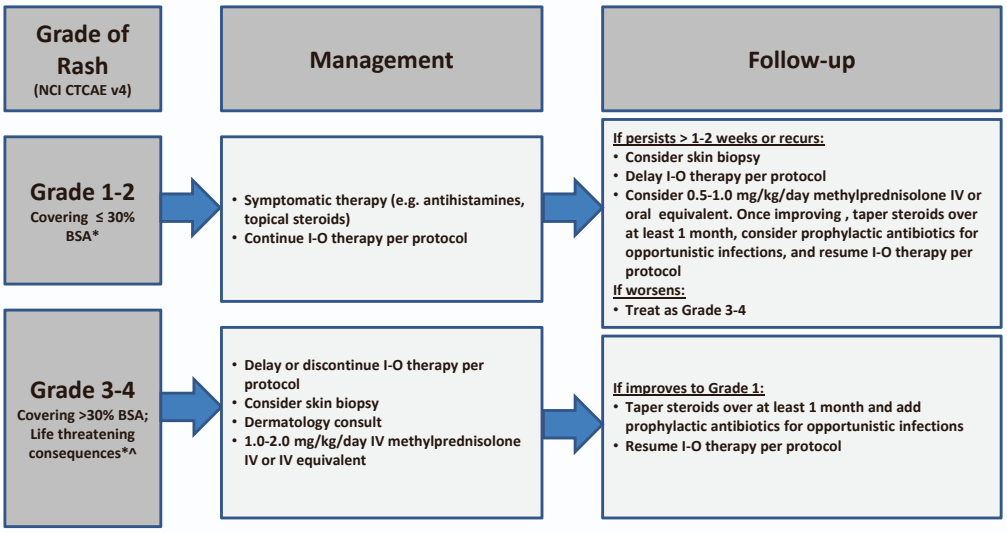


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Updated 05-Jul-2016

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

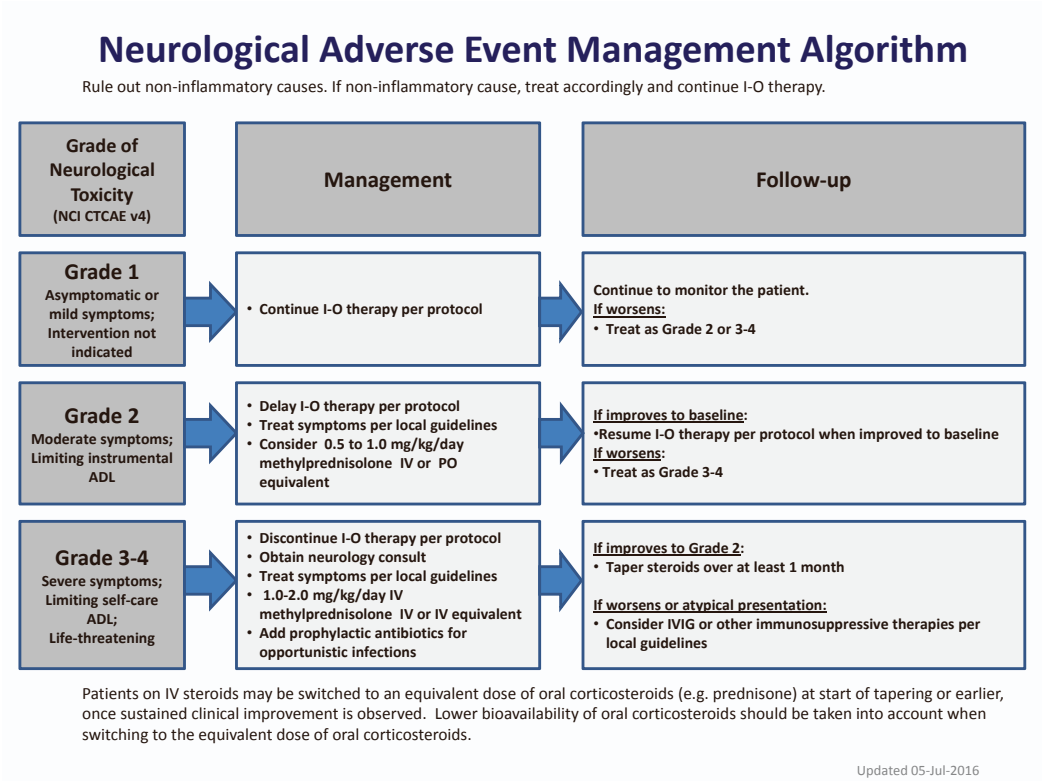


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v4 for term-specific grading criteria.

^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Updated 05-Jul-2016


Neurological Adverse Event Management Algorithm
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Grade of Neurological Toxicity (NCI CTCAE v4)
Management
Follow-up
Grade 1
Asymptomatic or mild symptoms; Intervention not indicated
• Continue I-O therapy per protocol
Continue to monitor the patient.
If worsens:
• Treat as Grade 2 or 3-4
Grade 2
Moderate symptoms; Limiting instrumental ADL
• Delay I-O therapy per protocol
• Treat symptoms per local guidelines
• Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent
If improves to baseline:
•Resume I-O therapy per protocol when improved to baseline
If worsens:
• Treat as Grade 3-4
Grade 3-4
Severe symptoms; Limiting self-care ADL; Life-threatening
• Discontinue I-O therapy per protocol
• Obtain neurology consult
• Treat symptoms per local guidelines
• 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent
• Add prophylactic antibiotics for opportunistic infections
If improves to Grade 2:
• Taper steroids over at least 1 month
If worsens or atypical presentation:
• Consider IVIG or other immunosuppressive therapies per local guidelines
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Updated 05-Jul-2016