Preoperative Immune checkpoint inhibitor therapy for patients with primary untreated or recurrent/metastatic squamous cell carcinoma of the head and neck (RM-SCCHN)

Acronym: **The SPARK study**

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Center: Johns Hopkins University School of Medicine,
Sidney Kimmel Comprehensive Cancer Center

Principal Investigator: Tanguy Seiwert, MD (Tanguy Seiwert)
201 North Broadway, Room 8156
Baltimore, MD 21287
Telephone: 443-287-8312
Fax: 410-614-9334
e-mail: tseiwert@jhmi.edu

Surgical Investigator: Christine Gourin, MD
JHOC Otolaryngology HNS
601 North Caroline Street
Baltimore, MD 21287
Telephone: 443-287-2016
Fax: 410-955-0035
e-mail: cgourin@jhmi.edu

Biostatisticians: Hao Wang, MD

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SUMMARY

The primary objective of this study is to assess safety and feasibility of pre-operative nivolumab in patients with squamous cell carcinoma of head and neck (SCCHN) who will undergo surgery. The study will enroll a total of 26 patients and include patients with primary untreated, newly diagnosed SCCHN (Cohort 1), or patients with recurrent/metastatic SCCHN Cohort 2). Patients with primary untreated locoregional disease will undergo surgical resection as originally planned, right after study treatment. Patients with recurrent/metastatic disease will also be enrolled ideally if disease can be resected, but distant metastatic disease is acceptable if an indication for surgery is clinically appropriate (e.g. for symptomatic control, or if a distant lesions is addressed separately (e.g. SBRT or resection, or with further palliative treatment). In such patients distant metastatic disease can be addressed at an interval with systemic therapy or other local therapy/ies (RT, metastectomy) as indicated. All patients will be treated with 1 dose of nivolumab 480mg IV over 30 minutes 4-6 weeks prior to surgery. Subjects who sign the consent form but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), and patients who do not undergo surgical resection (for reasons unrelated to the study drug or toxicity) will be replaced and will not count towards the accrual goal.

Overall study design

The ***primary objective*** is to establish safety and feasibility of preoperative treatment with immune checkpoint inhibitor in patients with newly diagnosed or recurrent or metastatic SCCHN undergoing surgery.
Secondary objectives are to 1) determine the pathologic response using immune related response criteria (irPRC)[51], 2) determine the changes in tumor-specific T cell responses and tumor microenvironment and compare these with baseline analyses in similar populations of patients undergoing resections without neoadjuvant therapy, 3) compare immunologic parameters between locoregional untreated and recurrent/metastatic sites, 4) compare irPRC[51] with radiographic evidence of response to immunotherapy, and 5) determine progression free survival.

Once patients are consented, they will be screened with pretreatment biopsy and images. They will receive 1 dose of nivolumab on Day -28 prior to planned surgery on or after Day 0 and up to/including Day +14. A 14 day window is allowable (as needed for scheduling purposes, or if clinically appropriate for patients with clinical benefit/improvement, if deemed safe by the treating physicians (surgeon & medical oncologist). Furthermore if clinically indicated patients may undergo surgery earlier (see Section 4.1.2 regarding early surgery, salvage treatment).

Surgical specimens and blood will be collected for correlative analyses at various time points pre- and post-treatment.

1. Endpoints

1.1 Primary Endpoint

Safety and feasibility of neoadjuvant nivolumab administration in patients with newly diagnosed or locoregional recurrence or metastatic disease undergoing surgical resection, measured by 1) frequency of drug-related adverse events occurring up to 100 days after the last dose of nivolumab or 30 days after surgery (whichever is longer), and 2) successful completion of preoperative treatment and proceeding to surgery without any extended treatment related delays (defined as >28 days from preplanned Day 0) from preplanned surgery date (Day 0) (or e.g. >14 days if surgery is on Day +14).

1.2 Secondary Endpoints

1) Immune related pathologic response (irPRC)
   → irPRC criteria[51] will be used – to assess resection specimen

2) Major pathologic response (MPR)

3) Progression free survival
   Progression-free survival will be measured from the time of start of neoadjuvant nivolumab until radiologic or clinical progression or death.

4) Radiographic response rate
   Response will be determined by RECIST version 1.1.

Exploratory

1. To determine changes in expression of selected immune markers and responses compared to baseline, in the blood, primary tumor tissue and draining lymph nodes from patients receiving neoadjuvant therapy; to determine changes in the quality and quantity of tumor infiltrating lymphocytes; and to compare findings in tumor and draining lymph nodes from treated patients, to findings in a parallel stage-matched cohort of untreated patients on a companion tissue collection protocol

2. To evaluate the potential effects of neoadjuvant therapy on normal head and neck mucosal tissue, by comparing tissues obtained on this study to those obtained from untreated patients undergoing tumor resection on a parallel tissue collection protocol.

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3. To compare immunologic markers and responses in HPV+ vs HPV(-) tumors. This includes

responses to HPV16 E6 and E7 antigens in patients with HPV+ cancers.

4. To explore features of the gut and oral microbiota of SCCHN patients before and after neoadjuvant nivolumab that may correlate with pathologic response.

5. To response to immunotherapy from pre- and on-treatment tissues in a histoculture model.

6. To assess recurrence-free survival in patients receiving preoperative therapy in this study.

7. To assess overall survival in high-risk patients with SCCHN receiving neoadjuvant therapy.

8. To study response to metabolic and or immunotherapy from pre- and on-treatment tissues in functional models (histocultures (slices/explants) and short term (< 4 weeks) in-vitro organoids) with in-vitro antibody testing.

9. To assess baseline immune and genomic tumor characteristics by performing multiplex immunofluorescence/immunochemistry (e.g., CD45, CD3, CD4, CD8, PDL1, etc.) and genetic sequencing (targeted and whole exome) on tissues slides/cryopreserved tissue blocks.

2. BACKGROUND

2.1 Squamous cell carcinoma of head and neck (SCCHN)

SCCHN is the sixth most common cancer in the world, and approximately 50,000 new cases are diagnosed in a year in the United States[1,2]. SCCHN can arises from different sites in upper aerodigestive tract, which include oral cavity, oropharynx, hypopharynx and larynx. A subset of SCCHN is caused by human papillomavirus (HPV) and represents a biologically distinct process[3,5]. Expression of viral oncoproteins E6 and E7 result in rapid degradation of two important tumor suppressors, p53 and pRb[6,8]. Disruption in pRb function induces a compensatory increase in expression of $p16^{INK4A}$ (p16)[9], which has been used as a surrogate marker for HPV in HNSCC occurring in the oropharynx (oropharyngeal SCC)[10,11]. HPV-related oropharyngeal SCC is associated with unique demographic characteristics such as male gender, better performance status, and lower consumption of tobacco and/or alcohol[12]. Additionally, survival for HPV-positive patients is higher than for HPV-negative SCCHN patients[13], when treated with current standard of care, which is either surgery followed by adjuvant radiotherapy with or without concurrent chemotherapy or definitive concurrent chemoradiotherapy[14,13,16,17]. HPV-negative SCCHN is typically associated with heavy use of tobacco and alcohol.[15]

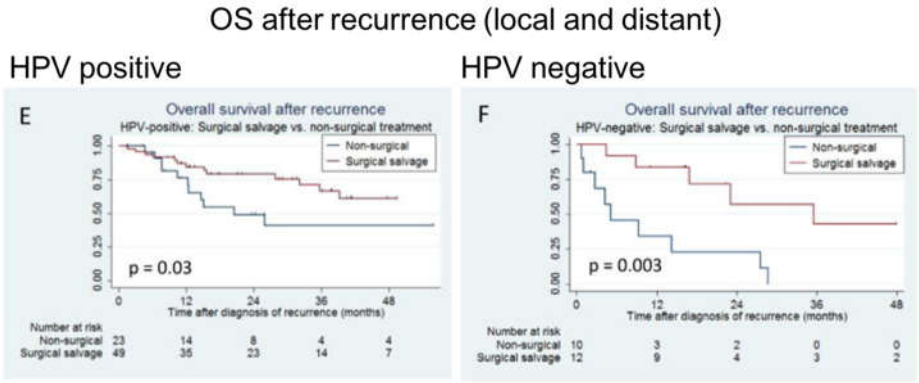

Figure 1. Overall Survival after locoregional and distant recurrence by receipt of surgical salvage.

Although the majority of patients with SCCHN present with locally advanced disease, approximately 30-40% of patients develop recurrence even with multimodality treatment[16]. For selected patients with locoregional recurrence, surgical salvage may be a viable therapeutic option[17]. Prospective cooperative group trial data suggests that surgical salvage for locoregional and distant failure in oropharyngeal SCC is independently associated with improved overall survival[18] (Figure 1). Furthermore in retrospective case series, this survival advantage of surgical salvage applies to both HPV-positive and negative patients, although the benefit in oligometastatic disease was largely limited to HPV-positive disease[19]. However, median disease free survival after salvage surgery remains poor for both HPV-positive and HPV-negative patients (1.28 year and 0.78 year, respectively)[20]. Combination chemotherapy such as platinum, fluorouracil and cetuximab (EXTREME regimen) does improve survival for RM-SCCHN[21], but the absence of measurable disease burden precludes from the use of chemotherapy after salvage surgery as there is no measurable disease to serve as an endpoint to assess the response. Adjuvant radiotherapy or chemoradiotherapy can be performed for patients with locoregional recurrence after surgical salvage, but it is challenging and no survival benefit has been documented as most patients already had received radiotherapy or chemoradiotherapy as part of their initial treatment[22].

2.2 Programmed death-1 – Preclinical and Clinical Studies

Programmed death-1 (PD-1 or CD279), primarily expressed on activated T cells, NK cells and B cells[23] is a 55 kD type I transmembrane protein that is a member of the CD28 family of T-cell co-stimulatory/co-inhibitory receptors that also includes CD28, CTLA-4, ICOS BTLA and many others[24]. Two ligands specific for PD-1 have been identified: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273)[25-27]. While PD-L1 can be expressed by many cell types, PD-L2 primarily expressed on antigen presenting cells, in particular dendritic cells. PD-L1 and PD-L2 have been shown to downregulate T-cell activation upon binding to PD-1 in both murine and human systems. PD-1 has been shown to inhibit CD28-mediated upregulation of IL-2, IL-10, IL-13, IFN-γ and Bcl-xL. PD-1 expression has also been noted to inhibit T cell activation and expansion of previously activated cells. PD-1 contains an intracellular membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal immunoreceptor tyrosine based switch motif (ITSM). Both Src homology region 2 domain-containing phosphatase (SHP) -1 and -2 have been found to bind to the cytoplasmic tail of PD-1 and mediate its signaling. Once PD-1 is engaged by one of its ligands, SHP-1 and -2 bind to the phosphorylated tyrosine residue in the ITSM in its cytoplasmic region and mediate the suppressive effects of PD-1 via dephosphorylation of immunoreceptor tyrosine activating mitofs (ITAM) on TCR-triggered signaling adaptors such as the zeta chain. As such, PD-1 engagement down-modulates antigen-specific T cell activation.

Programmed death-1 – Preclinical Studies

Further evidence for a negative regulatory role of PD-1 comes from studies of PD-1-deficient mice. PD-1-deficient mice develop various autoimmune phenotypes later in life, including dilated cardiomyopathy, a lupus-like syndrome with arthritis and nephritis, and accelerated diabetes mellitus. The emergence of these autoimmune phenotypes is dependent upon the genetic background of the mouse strain and many of these phenotypes emerge at different times (almost always after 6 months of age) and show variable penetrance[28,29]. Thus PD-1 plays a more subtle regulatory role than CTLA-4, whose gene knockout results in lethal autoimmunity within 3-4 weeks of birth. In addition to the phenotypes of null mutations, PD-1 inhibition by antibody-mediated blockade in several murine models has been found to accelerate autoimmune diseases such as encephalomyelitis, graft-versus-host disease, and type I diabetes in genetically prone mouse strains[30,31]. Taken together, these results suggest that PD-1 modulates immune responses in tissues undergoing inflammatory responses and PD-1 blockade has the potential to enhance inflammatory (including “anti-self”) responses in tissue, but these responses are variable and dependent upon various host genetic factors. Thus, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens. Preclinical animal models of tumors have shown that blockade of PD-1 by monoclonal antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection[32]. The effects of anti-PD-1 blockade in combination with vaccines and many other immunomodulatory agents have been tested in multiple murine tumor models.

2.2.1 **Clinical development of anti-PD-1 antibodies**

Nivolumab (BMS-936558, ONO-5438, MDX-1106) is a fully human, IgG4 (kappa) monoclonal antibody

that binds PD-1 with high affinity blocking its interactions with its ligands PD-L1 (B7- H1) and PD-L2 (B7-DC) and increasing tumor antigen specific T cell proliferation and cytokine secretion[33]. Nivolumab has been approved by FDA for melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), SCCHN, bladder cancers, gastric cancer, hepatocellular cancer, MSIhigh colon cancer and classical Hodgkin's lymphoma as single agent and for melanoma in combination with ipilimumab.

A phase 1 single dose, dose-escalation study of nivolumab in 39 heavily pretreated patients with solid tumors (CA209-001, NCT00441337) demonstrated good tolerance and signals of efficacy[33]. Patients with advanced NSCLC, melanoma, RCC, metastatic castration-resistant prostate cancer (mCRPC) and metastatic colorectal cancer (CRC) in this study received a single IV infusion of nivolumab over 1 hour in escalating cohorts of 0.3, 1, 3 or 10 mg/kg. Restaging was performed radiologically at 8 and 12 weeks, and patients with no adverse event (AE) ≥3 and stable disease or response by RECIST received additional doses of nivolumab at weeks 12 and 16 followed by further restaging at 3 months. Those with continued clinical benefit could receive two more doses, spaced by 4 weeks. Treatment could continue for up to 2 years.

Nivolumab was in general well-tolerated with most frequent adverse events being hematologic (notably a grade 3 reduction in CD4 count in 17.9% of patients), fatigue and mild musculoskeletal symptoms. A maximum tolerated-dose (MTD) was not reached.

Immune-related colitis, well described with CTLA-4 inhibition, occurred in 1 patient and resolved with treatment with infliximab and steroids. Grade 2 hypothyroidism and grade 2 polyarthritis were noted in 1 and 2 patients respectively. Efficacy was promising with a durable complete response (CR) in a CRC patient, 2 partial responses (PRs) in RCC and melanoma patients, and a transient response not meeting PR criteria in a NSCLC patient. PD-L1 (B7-H1) expression was assessed by immunohistochemistry in pretreatment tumor specimens from 9 patients. Of these, 3 of 4 patients with membranous (cell surface) tumor cell expression of PD-L1 experienced tumor regression; none of 5 patients without expression of PD-L1 experienced a tumor response, suggesting a marker for further investigation. Pharmacodynamic analyses suggested that high level occupancy of the PD-1 receptor on circulating T cells persisted for up to 85 days after a single dose of nivolumab.

Subsequent to this original study, antibodies targeted to PD-1 or PD-L1 that block this ligand-receptor interaction have been tested extensively in most human cancers[34, 36]. Clinical responses have been seen in a variety of cancers, many of which are extremely durable. Immunologic studies support the notion that clinical responses are due to "unleashing" of endogenous anti-tumor T cells from PD-1 pathway restraint. Virtually all side-effects of these antibodies are immune-related (autoimmune or hyperimmune), with 9-15% grade 3/4 drug-related adverse events. As of 2017, two anti-PD-1 antibodies (nivolumab, pembrolizumab) and three anti-PD-L1 antibodies (durvalumab, atezolizumab, avelumab) have been approved for various cancers including melanoma, renal cancer, NSCLC, hepatocellular cancer, Merkel Cell cancer, HNSCC, bladder cancer, gastric cancer, Hodgkin lymphoma and mismatch repair deficient cancers of all types. Clinical responses are higher in patients with increased PD-L1 expression in their cancer and in cancers with high mutational load. Expression of viral antigens in virus-associated cancers (MCPyV in Merkel Cell, HPV in some HNSCC and EBV in some

Hodgkin lymphomas) are thought to generate tumor-specific antigens that can be targeted by T cells. There have been over 2000 clinical trials, completed or ongoing, testing PD-1 pathway blocking antibodies either alone or in combination with other agents. While initial trials tested these antibodies in refractory metastatic cancer, success in the adjuvant and neoadjuvant setting has prompted the therapeutic blockade of the PD-1 pathway much earlier in the course of disease.

Further information on nivolumab is available in the current version or the investigator's brochure

2.2.2 Immune checkpoint inhibitors in SCCHN

SCCHN has been associated with dysregulation and evasion of the immune system. It has been described as an immunosuppressive disease, with lower absolute lymphocyte counts than those found in healthy subjects, impaired natural killer (NK) –cell activity, and poor antigen-presenting function. Impairment of tumor-infiltrating T lymphocytes has also been reported in SCCHN and other cancers, with a strong impact on clinical outcome. In addition, suppressive regulatory T cells (Tregs) have been linked to SCCHN tumor progression[37].

Immune checkpoint inhibitors offer promise as a novel therapeutic approach for RM-SCCHN. Especially, anti-PD-1 antibodies have shown notable response rates in heavily pre-treated RM-SCCHN patients[38] and also showed a significant survival benefit compared to conventional chemotherapy in platinum refractory patients. Keynote-012 was a phase 1b study of pembrolizumab in SCCHN patients with recurrent/metastatic SCCHN. 192 patients were treated at two different dose levels; in the combined analysis the response rate was 18% and OS at 12 months was 38%[39]. A single arm phase II study of pembrolizumab, an anti-PD1 antibody, in SCCHN patients who had failed platinum and cetuximab, pembrolizumab showed robust overall response rate of 18%[40]. These data led to approval of this agent in platinum refractory RM-SCCHN patients.

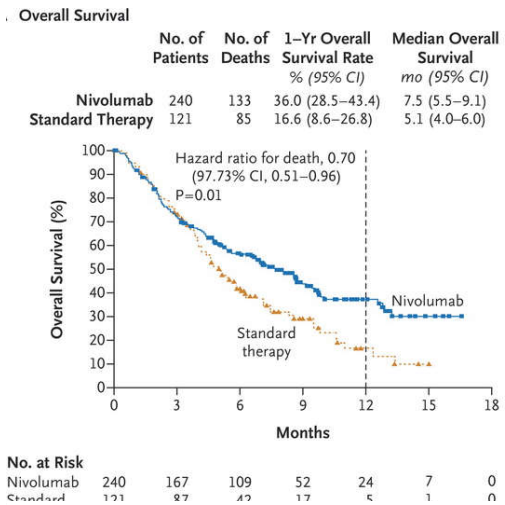

Figure 2. Nivolumab improves overall survival in RM-SCCHN patients.

In a large randomized double blinded phase III clinical trial, nivolumab, another anti-PD1 antibody, almost doubled 1-year overall survival[37] (Figure 2) and was approved for RM-SCCHN as well. Both of these agenets are approved without a companion biomarker for patient selection. Given these successes of immune checkpoint inhibitors in RM-SCCHN, there is enthusiasm to bring these agents in earlier stage of SCCHN. Pembrolizumab alone or in combination with chemotherapy is being compared with combination of chemotherapy and cetuximab in treatment naïve RM-SCCHN patients (NCT02252042), and nivolumab is being compared with combination of nivolumab and ipilimumab in a randomized phase 2 trial in platinum eligible RM-SCCHN patients (NCT02823574). An anti-PD-L1 antibody, durvalumab, is being tested against durvalumab in

combination with tremelimumab (anti-CTLA4 antibody) and standard of care chemotherapy (NCT02551159). Even in locally advanced disease, anti-PD1 therapy is being widely adopted in various clinical setting, in combination with radiotherapy or surgery. For example, nivolumab is being incorporated with definitive radiotherapy or chemoradiotherapy in locally advanced SCCHN patients (NCT02764593), and pembrolizumab was successfully administered without significant delay for patients undergoing definitive surgical management for locally advanced SCCHN[41]. Based on these studies, it is timely and reasonable to ask whether immune checkpoint inhibitors could be incorporated in treatment of patients with primary untreated locally advanced disease, or recurrent and/or metastatic RM-SCCHN who are undergoing surgical salvage. Currently there are no prospectively validated biomarkers to assist with identifying patients who are at higher chance of obtaining a response to PD-1 inhibitors. Thus, there is a need to further understand characteristics of tumors that will respond in the newly diagnosed and locally advanced setting.

2.3 Rationale for immune checkpoint inhibitors in pre-operative treatment

Immune checkpoint inhibitor therapy has shown to have activity in RM-SCCHN[42]. In addition, immune checkpoint inhibitors have been shown to be a very effective treatment in non-small cell lung cancers (NSCLC)[43], including squamous cell carcinoma of lung, which shares biologic characteristics with HPV negative SCCHN[44]. A neoadjuvant nivolumab study demonstrated the safety of pre-operative immune checkpoint inhibitors in resectable NSCLC and has shown that 2 doses of nivolumab could be safely administered without significant delay in surgery.

Adults with untreated surgically resectable stage I-IIIA NSCLC received two doses of nivolumab (anti-PD-1) preoperatively. The primary endpoints of the study were safety and feasibility. Tumor pathologic response, PD-L1 expression, mutation burden and mutation-associated neoantigen-specific T-cell responses were evaluated. Neoadjuvant nivolumab had an acceptable side effect profile without surgical delays, and 20 of 21 tumors were completely resected. Major pathologic response occurred in 45% (9/20) of resected tumors. Responses occurred in both PD-L1 positive and negative tumors. Multispectral imaging demonstrated robust influx of PD-1+ CD8+ T cells in resected tumors. Pathologic response significantly correlated with pre-treatment tumor somatic mutation burden. T cell clones shared between the tumor and peripheral blood increased systemically upon anti-PD-1 treatment in 8 of 9 patients analyzed. Mutation-associated neoantigen-specific T-cell clones, from a primary tumor that underwent pathologic complete response, rapidly expanded in peripheral blood at 2-4 weeks post-treatment, some of these clones were not detected before anti-PD-1. These findings underpin multiple neoadjuvant trials of anti-PD-1 and anti-PD-L1-based immunotherapies.

2.3.1 Neoadjuvant PD-1 blockade in SCCHN

To date, two studies have reported preliminary experiences with neoadjuvant treatment for newly diagnosed squamous cell carcinoma of the head and neck. Dr. Uppaluri and colleagues treated 21 patients with 1 dose of pembrolizumab after surgical resection. A pathologic response was noted in 43% of patients and after 1 year none of the first 10 patients had a locoregional or distant metastatic

disease recurrence[45]. Another study, 29 patients (12 HPV+, 17 HPV-) received two doses of nivolumab followed by surgical resection. Treatment was able to be administred without protocol specified delays in surgery. In addition, 48% of evaluable patients experiences pre-surgical tumor reduction[46]. Neither of these studies reported any analysis of genomic landscape of the tumor, T cell responses to either HPV antigens or neoantigens, nor was there any detailed analysis of the tumor immune microenvironment in response to neoadjuvant PD-1 blockade.

Both of these reports demonstrate early data regarding the feasibility and treatment related impact of neoadjuvant PD-1 inhibitor therapy in previously untreated patients with SCCHN. To date, there is no data on the feasibility of neoadjuvant therapy among patients with recurrent disease. These patients are often at higher risk of surgical complications related to prior treatment related morbidity. In addition, it is unclear what the expected pathologic response to PD-1 inhibitors would be in a prior treated population.

In addition, there is a great need in SCCHN to further understand which patients are likely to respond to PD-1 inhibitors. In our study, we hope to provide further data on the feasibility of neoadjuvant therapy in the untreated and recurrent population of SCCHN. We also seek to obtain greated clarity on the immune response in both populations with focus on PD-L1 expression, mutation burden and mutation-associated neoantigen-specific T-cell responses. The rationale of studying both previously untreated and recurrent populations is to obtain a greater understanding or PD-inhibitor activity and to compare the T cell responses in both groups.

2.4 **Rationale for dosing and schedule**

The planned schedule of a preoperative dose of nivolumab, flat dose 480 mg, given once 28 days prior to surgery (with an additional 14 day window). The rationale for flat dosing is noted below. The duration of preoperative treatment of approximately 28 days was initially chosen as a short time period is unlikely to allow significant progression of disease which might preclude complete surgical resection. However, a 14 day window is allowable (as needed for scheduling purposes, or if clinically appropriate for patients with clinical benefit/improvement, if deemed safe by the treating physicians (surgeon & medical oncologist). Furthermore if clinically indicated patients may undergo surgery earlier (see Section 4.1.2 regarding early surgery, salvage treatment).
Waiting 28-42 days after dosing before surgery would still allow for time to appreciate immune mediated cellular changes.

Flat (standardized) dosing of nivolumab as a single agent:

Nivolumab monotherapy has been extensively studied in a number of tumor types including NSCLC, MEL, RCC, and CRC with body weight normalized dosing (mg/kg). Nivolumab pharmacokinetics (PK) and exposures of subjects in these studies have been characterized by population pharmacokinetic (PPK) analysis of data collected in these studies, together with PK data from several phase 1, 2, and 3 clinical studies of nivolumab monotherapy in solid tumors. Population PK (PPK) analyses have shown

that the PK of nivolumab are linear, with dose proportional exposures over a dose range of 0.1 mg/kg to 10 mg/kg, and are similar across tumor types. Nivolumab clearance and volume of distribution were found to increase with increasing body weight, but the increase was less than proportional, indicating that a mg/kg dose represents an over-adjustment for the effect of body weight on nivolumab PK. Given the relationship between nivolumab PK and body weight, a flat dose is expected to lead to lower exposures in heavier patients, relative to the exposures in lighter patients.

Using the PPK model, nivolumab steady-state trough, peak and time-averaged concentration (Cminss, Cmaxss, and Cavgss, respectively) were predicted for a flat nivolumab dose of 240 mg Q2W and compared to those following administration of 3 mg/kg Q2W in NSCLC subjects. A dose of 240 mg nivolumab is identical to a dose of 3 mg/kg for subjects weighing 80 kg, which is the approximate median body weight of NSCLC subjects in the 3 Phase 2 and 3 BMS clinical studies of nivolumab monotherapy. The geometric mean values of Cminss, Cmaxss, and Cavgss with flat dosing are slightly (< 15%) higher than that produced by a 3 mg/kg dose, and the coefficient of variation (cv%) in these measures of exposure are only slightly (< 10%) greater than that of 3 mg/kg dosing.

Across the various tumor types in the BMS clinical program, nivolumab has been shown to be be safe and well tolerated up to a dose level of 10 mg/kg, and the relationship between nivolumab exposure produced by 3 mg/kg and efficacy has been found to be relatively flat. Taken together, the PK, safety, and efficacy data indicate that the safety and efficacy profile of 240 mg nivolumab will be similar to that of 3 mg/kg nivolumab. Receptor occupancy studies now support a dose of 480mg IV every 4 weeks, which is thought to be equivalent to the 240mg IV every 2 week schedule. This flat dose maintains saturation receptor occupancy for greater than 4 weeks. Thus a single flat dose of 480 mg given 4 weeks prior to resection is recommended.

3. PATIENT SELECTION

3.1 Eligibility Criteria

Cohort 1: Subjects must have histologically confirmed previously untreated squamous cell carcinoma of the head and neck that is amenable to surgical resection as part of standard of care.

Cohort 2: Subjects must have histologically confirmed recurrent squamous cell carcinoma of head and neck, that is amenable for salvage surgery. Sites of recurrence may either be locoregional and/or distant. Distant metastatic disease is permissible in this cohort and can be addressed at an interval with systemic therapy (e.g. chemotherapy, targeted therapies), or local therapies (e.g. radiation, metastatectomy) as clinically indicated.

Distribution between both patient cohorts is aimed to be 1:1 but may vary.

For both cohorts:

3.1.1 The primary site should be a head and neck squamous cell carcinoma (including, but not limited to oral cavity, oropharynx, hypopharynx, or larynx, paranasal sinuses, nasal cavity). Squamous cell carcinoma of unknown primary, diagnosed in lymph nodes in neck, can be included but should be tested for p16 and confirmed with an HPV specifc assay (testing NOT required for enrollment; can be done at an interval).

3.1.2 Subjects with oropharyngeal primary tumors must have confirmation of HPV tumor status per clinical standards, although not necessary at enrollment.

3.1.3 Subjects must have been determined to be candidates for surgical resection by a multidisciplinary team including a surgeon, a medical oncologist and a radiation oncologist.

3.1.4 Subjects must have at least one lesion that can be (or has been) biopsied at baseline.

3.1.5 Eastern Cooperative Oncology Group (ECOG) performance status 0 to 1 (see Appendix A for definitions).

3.1.6 Age ≥18 years.

3.1.7 Life expectancy of greater than 6 months.

3.1.8 Patients must have normal organ and marrow function as defined below:

leukocytes	≥ 1,500/mcL
absolute neutrophil count	≥ 1,000/mcL
platelets	≥ 100,000/mcL
total bilirubin	≤ 1.5 X institutional upper limit of normal (except subjects with Gilbert syndrome, who can have total bilirubin < 3.0 mg/dL)
AST(SGOT)/ALT(SGPT)	≤ 3 X institutional upper limit of normal
Creatinine OR creatinine clearance	within normal institutional limits OR ≥ 40 mL/min (using modified Cockcroft-Gault formula) for patients with creatinine levels above institutional normal.

3.1.9 The effects of nivolumab on the developing human fetus are unknown.

1) Women of childbearing potential (WOCBP) must have a negative urine or

serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of study treatment.
2) Women must not be breastfeeding
3) Women of childbearing potential (WOCBP) must agree to follow instructions for method(s) of contraception for the duration of treatment with study treatment and for 5 months post-treatment completion. Women should use an adequate method(s) of contraception (Refer to nivolumab IB for WOCBP and methods of contraception to be provided).
4) Males who are sexually active with WOCBP must agree to follow instructionsfor method(s) of contraception for the duration of treatment with study treatment(s) and 7 months post-treatment completion. In addition, male participants must be willing to refrain from sperm donation during this time. Men who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception.

3.1.10 Patient understands the study regimen,
its requirements, risks and discomforts and is able and willing to sign the informed consent form. Voluntary signed and dated IRB/IEC approved written informed consent form in accordance with regulatory and institutional guidelines must be obtained before the performance of any protocol related procedures that are not part of normal patient care. Subjects must be competent to report AEs, understand the drug dosing schedule and use of medications to control AEs.

3.1.11 Measurable disease – either radiologically (per RECIST V 1.1) or clinically measurable on exam in order to assess treatment response.

3.2 Exclusion Criteria

3.2.1 Any active history of a known autoimmune disease. Subjects with vitiligo, type 1 diabetes mellitus, residual hypothyroidism requiring hormone replacement, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.

3.2.2 Patients who have had prior chemotherapy for newly diagnosed (cohort 1) or recurrent (cohort 2) head and neck cancer. In cohort 2 only, previous perioperative chemotherapy or chemoradiation for the management of localized or locally advanced disease is permitted.

3.2.3 Patients who received prior therapy with anti-PD-1, anti-PD-L1, anti-PD-L2, anti CD137, anti-CTLA-4 antibody therapies, any other antibody or drug specifically

targeting T-cell co-stimulation or checkpoint pathways.

3.2.4 Any live / attenuated vaccine (e.g. varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMR) etc.) within 30 days of first treatment.

3.2.5 Patients with uncontrolled brain metastases

Patients with brain metastases must have stable neurologic status following local therapy (surgery and/or radiation) for at least 2 weeks without the use of steroids or on stable or decreasing dose of ≤ 10mg daily prednisone (or equivalent), and must be without neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with a history of carcinomatous meningitis are not eligible.

3.2.6 Patients who have an active concurrent malignancy that is not controlled/cured and could impact life expectancy within the next 3 years. E.g. patients with localized cutaneous squamous cell carcinoma or basal cell carcinoma or treated prostate cancer with no evidence of disease progression may be allowed to enroll after review by the study team and principal investigator.

3.2.7 Uncontrolled inter-current illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, myocardial infarction or new onset angina within six months of enrollment, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Women who are pregnant or nursing.

3.2.9 Men with female partners who are not willing to use contraception.

3.2.10 Active infection with hepatitis B or hepatitis C (active infection is defined by abnormal liver function tests (=elevated AST/ALT) or ongoing use of an antiviral hepatitis treatment). Patients with **normal liver function tests** (defined by normal AST/ALT) per definition do not have an active infection and are eligible to enroll without additional testing).

3.2.11 Patients with a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of randomization. However, inhaled or topical steroids and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune

disease.

3.2.12 EBV(+) head and neck cancer (e.g. EBV(+) nasopharyngeal carcinoma)

3.2.13 Patient with HIV are excluded given the unknown risk of interaction with HAART and the unknown benefit of immunotherapy in this population.

3.3 Inclusion of Women and Minorities

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

4. TREATMENT PLAN

4.1 Overall Study Design and Toxicity Assessment

The study will acrrue to 2 cohorts, each of which consists of 12 patients who undergo surgical resection. Total of 26 patients will be accrued to the study. Patients who do not undergo surgery for reasons unrelated to toxicity of the study treatment will be replaced and will not count toward the accrual goal of 12 surgically resected patients in each cohort. If enrollment on 1 cohort is significantly faster, the lesser enrolling cohort number of patients may be decreased in favor of the better enrolling cohort to keep the total number of evaluable patients at 26 pts (also see statistical section).

4.1.1 **Treatment overview (Cohorts 1 & 2)**

After being consented, patients will undergo baseline evaluations including clinical exam, complete blood count, blood chemistry, and thyroid function test. Baseline core or surgical biopsy and images with either CT scan or MRI scan will be obtained at screening. Blood collection for correlative analyses will be performed anytime after consent and prior to Nivolumab administration. Nivolumab 480mg will be administered intravenously on approximately Day -28 prior to planned surgery on Day 0 to Day +14 (14 day window as needed for scheduling surgery/logistics). An earlier surgery date is permissible if clinically indicated – see section 4.1.2 (salvage treatment).

Patients will undergo post-treatment images and specimen collection ideally within 7 days from planned surgery date, or as clinically indicated.

Post-operative adjuvant treatment with radiotherapy or chemoradiotherapy can be delivered, if clinically indicated at the discretion of treating physician following the standard of care. All subjects will

get follow up imaging about 3 months after completion of curative therapy (e.g. after adjuvant therapy) as per clinical routine.

After the initial post-treatment images, further imaging will be obtained as clinically indicated. In case of recurrence, a biopsy will be obtained and the specimen will be secured for future analyses if clinically feasible.

To explore oral and gut microbial correlates of response to neoadjuvant nivolumab, stool samples and oral rinses will be collected at baseline prior to infusion and then again prior to surgery. Subjects will also be asked to fill out a medical, antibiotic use and dietary questionnaire that will be used to assess whether antibiotic use or dietary patterns correlate with features of the oral and gut microbiome.

4.1.2 **Early salvage treatment options: salvage surgery, salvage chemotherapy**

In patients with clinically worsening disease, or concern that resectability may be at risk, or due to extenuating scheduling circumstances, an earlier surgery date can be approved in consultation with the treating surgeon, medical oncologist, and study team.

Alternatively, neoadjuvant chemotherapy can be given in patients with clinically worsening disease, or concern that resectability may be at risk after approval and review by the treating surgeon, and medical oncologist, and study team.

Such 'salvage' patients will continue on study, unless deemed non-evaluable for other reasons.

4.2 Screening and pre-treatment criteria

4.2.1 **Recruitment**

Patients will be recruited through the head and neck oncology clinic at Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (SKCCC) including medical oncology, radiation oncology, multidisciplinary, dental/ oral surgery and head and neck surgical oncology. Each enrolled subject will be assigned a participant sequential subject number at the time of consent with site number assigned in advance, e.g., 1-001, 1-002, etc.

4.2.2 **Determination of Eligibility**

After eligibility is established, the study staff will register participants. The following are required to be submitted for successful registration:

- Copy of subject consent
- Copies of the following documents:
  - Diagnostic pathology reports (with HPV status for oropharyngeal SCC)

  - Pathology report(s) from initial diagnosis
  - Pathology report(s) from the recurrence
- Baseline cross-sectional imaging (as clinically indicated, report sufficient for enrollment)
- Laboratory reports including:
  - Complete blood count (CBC) with differential (including absolute lymphocyte count) and direct platelet count.
  - Chemistry: Albumin, SGOT (AST), SGPT (ALT), Bilirubin, Calcium, Creatinine, Glucose, Total protein, Urea nitrogen, Electrolytes (including sodium, potassium, chloride and bicarbonate).
  - Baseline thyroid function assay: Thyroid Stimulating Hormone (TSH). Abnormal endocrine results should be followed up per standard of care, and may require an endocrine consult and additional testing.
- Medical records documenting treatment history of the initial therapy for SCCHN before recurrence
- Other documents, if requested.

Study treatment cannot begin until the patient is registered and assigned to a treatment cohort.

Subjects who sign a consent form but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), and patients who do not undergo surgical resection will be replaced and will not count towards our accrual goal of 26 patients (Patient allocation ideally should be 1:1 between both cohorts but may vary). All patients who receive a dose of nivolumab will be evaluable for safety and feasibility.

4.2.3 **Pre-treatment criteria**

Enrolled patients will need to meet the following criteria before receiving nivolumab.

leukocytes	≥ 1,500/mcL
absolute neutrophil count	≥ 1,000/mcL
platelets	≥ 100,000/mcL
total bilirubin	≤ 1.5 X institutional upper limit of normal (except subjects with Gilbert syndrome, who can have total bilirubin < 3.0 mg/dL)
AST(SGOT)/ALT(SGPT)	≤ 3 X institutional upper limit of normal
Creatinine OR creatinine clearance	within normal institutional limits OR ≥ 40 mL/min (using modified Cockcroft-Gault formula) for patients with creatinine levels above institutional normal.

4.2.4 **Diagnostic and surgical evaluations**

All patients enrolled on this protocol must be surgical candidates with either locally recurrent SCCHN or oligometastatic SCCHN. Patients will have undergone radiographic evaluation indicating no evidence of disease outside the planned surgical field and main/target lesion/s should be determined to be amenable for resection by the treating surgeon.
Distant metastatic disease is permissible if a clinical indication for surgery exists (e.g. in order to provide symptom control) and such distant disease can be addressed at an interval with systemic therapy (e.g. chemotherapy, targeted therapies, etc), or local therapies (e.g. radiation, metastatectomy) as clinically indicated.
Any further preoperative testing that is recommended by the surgeon or anesthesiologist will be performed as part of standard of care. Surgery for patients enrolled on this protocol will be according to generally accepted standards of care. Patients should have baseline imaging as clinically appropriate of the affected area (neck/chest/abdomen and/or pelvis) – e.g. a CT or MRI with IV contrast and appropriate imaging should be repeated after completing neoadjuvant therapy, within the 7 days prior to planned surgery to assess response.

4.3 Study drug administration

Patients will receive 1 dose of nivolumab on approximately Day -28 prior to planned surgery on or after Day 0 and up to/including Day +14. A 14 day window is allowable (as needed for scheduling purposes, or if clinically appropriate for patients with clinical benefit/improvement, if deemed safe by the treating physicians (surgeon & medical oncologist). Furthermore if clinically indicated patients may undergo surgery earlier (see Section 4.1.2 regarding early surgery, salvage treatment).

Nivolumab is to be administered as a 30 minute IV infusion.

Nivolumab may be diluted in 0.9% Sodium Chloride Solution or 5% Dextrose solution.
All subjects will receive nivolumab at a flat dose of 480 mg IV.

Please refer to the current nivolumab investigators brochure for storage conditions. 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) current Investigator Brochure section for “Recommended Storage and Use Conditions”. There will be no dose escalations or reductions of study drugs allowed. There are no premedications recommended for nivolumab on the first dose.

4.4 **Management of Nivolumab -related infusion reactions**

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.

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 (Insert version e.g.: 4.0) guidelines.

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

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

Remain at bedside and 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.

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 electronic case report form (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 administration. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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]).

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).

4.5 Surgical resection

4.5.1 Surgery for SCCHN patients with newly diagnosed and untreated locoregional disease: Cohort 1

Extent of primary surgery and reconstruction will be decided by treating surgeons following the best clinical practice.

4.5.2 Surgery for SCCHN patients with locoregionally recurrent or oligometastatic disease: Cohort 2

Salvage local surgery is a standard practice for patients with resectable locoregional recurrence. Metastatic disease is permissible and can be addressed with systemic or local therapies (radiation, surgery etc), at an interval as clinically indicated in addition to the local treatment. Surgical technique and extent will be decided by treating surgeons following the best clinical practice.

4.6 Adjuvant treatment post surgery

Postoperative radiotherapy with or without concurrent chemotherapy for subjects in both cohorts (cohort 1 and 2) will be determined based upon pathologic assessment and administered at the discretion of the treating multidisciplinary team based on established standard of care. Common scenarios indicating radiotherapy with concurrent chemotherapy may include high-risk pathologic findings such as positive margin or extranodal extension. Decision for or against postoperative radiotherapy with or without concurrent chemotherapy will be recorded.

4.7 Evaluation of peri-operative safety

Subject safety will be assessed at the regular trial safety meeting and adhoc as part of clinical care (including at the weekly tumor board with the surgeons) from the start of therapy until 100 days after the last dose of study treatment, or 30 days following surgery, whichever is longer, for information regarding operative complications including treatment-related delay in planned surgery and in particular potential immune related toxicities (Note: Only those subjects who initiate protocol treatment will be followed). Toxicities will be reviewed at regular meetings of study investigators and research staff and minutes of these meetings will be documented by the clinical research staff. In the event that a subject does not continue his or her peri-operative care at the institution, every attempt will be made to collect this information either by direct contact or through communication with the

subjects outside physician(s).

4.8 Discontinuation, withdrawal and replacement of subjects

All patients who receive at least one dose of study treatment (including those who do not undergo surgical resection of their tumor) will be included in the overall evaluation of safety (intention-to-treat analysis). All reasons for discontinuation of therapy should be documented clearly in the medical record. If a subject discontinues or withdraws from the study, every attempt will be made to obtain an off-study blood collection if the subject is able and willing to do so.

Subjects who do not undergo surgical resection of their tumor, while evaluable for safety and feasibility, will not be evaluable for the key secondary endpoint of pathologic response. Subjects who sign a consent form but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), and patients who do not undergo surgical resection (for reasons unrelated to the study drug or toxicity) will be replaced and will not count towards our accrual goal.

4.9 Discontinuation of treatment

The reasons for discontinuation of protocol treatment include:

- Evidence of significant disease progression during the preoperative phase at the discretion of the treating investigator.

- Non-compliance with the study protocol; including, but not limited to not attending the majority of scheduled visits. The principal investigator will determine when non-compliance should lead to removal from study. **Note:** The patients will still be included in the overall evaluation of safety (intent-to-treat analysis).

- Unacceptable toxicity. **Note:** The patients will still be included in the overall evaluation of safety (intent-to-treat analysis).

- Intercurrent illness or condition that would, in the judgment of the treating investigator, affect assessment of clinical status to a significant degree or require discontinuation of study treatment.

- At subject's own request. **Note:** The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of safety (intent-to-treat analysis) if any protocol therapy was administered prior to withdrawal.

- Study is closed for any reason (e.g. new information shows that the patient's welfare would be at risk if he or she continued study treatment).

4.10 **Study calendar**

Procedure	Screening (Days -42 to -29)[a]	Day -28	Pre-surgical evaluation (Days -7 to -1)**	Day 0 Surgery (up to day +14)	Post-surgical evaluation (Days 21 to 42)	Follow-up visits (Every 3-4 months for 1 yr; then every 6 months for 2nd yr.)[$$]	At the time of recurrence	Survival Follow-up (every 12 months)
Clinical Assessments								
Physical Exam	X	X	X		X	X	X	
Vital Signs	X	X	X		X	X	X	
Performance status (ECOG)	X	X	X		X	X	X	
Concomitant medicines	X	X	X		X	X	X	
Toxicity assessments	X	X	X		X	X	X	
Electrocardiogram (EKG)	X							
Laboratory Tests								
CBC with diff	X	X****	X		X	X	X	
Chemistry including LFT	X	X****	X		X	X	X	
[#] *IF abnormal LFTs draw:* Hepatitis B/C serology	(X)[#]							
TSH	X				X	X		
Free T4 and T3	X						X	
PT/INR and PTT	X		X				X	
Pregnancy Tests (WOCBP only)	X		X		X		X	
Treatment								
Nivolumab		X						
CT neck/chest with IV contrast*	X		X			(X)	(X)	
MRI neck with IV contrast, optional†	(X)		(X)			(X)	(X)	

Procedure	Screening (Days -42 to -29)	Day -28	Pre-surgical evaluation (Days -7 to -1)	Day 0 (and up to day +14) Surgery	Post-surgical evaluation (Days 21 to 42)	Follow-up visits (Every 3-4 months for 1 yr; then every 6 months for 2nd yr.)$$	At the time of recurrence	Survival Follow-up (every 12 months)
Correlative Blood/Tissue/Stool Studies								
PBMCs, Serum, plasma (Blood collection 100 cc's)		X[b]		X	X	X	X	
Tumor biopsy/sample	X			X (resection specimen)			X	
Stool Sample		X[C]	X		X	X	X	
Oral Rinse[d]		X			X	X[d]	X	
Medical & Dietary Questionnaire		X			X	X	X	
Survival								X

a. screening labs are permited to be used for treatment if completed within three (+3) days of day -28
b. Research blood permited to be collected anytime after consent and prior to Nivolumab infusion (Days -42 to day -28)
c. Patients are to be provided with a stool collection kit during screening (days -42 to -29) and instructed to collect a stool sample within 48 hours of their scheduled infusion day (day -28).
d. Oral rinse is considered an optional collection during the first and second year follow up visits.

*CT abdomen/pelvis can be added if there's a clinical indication. MRI may replace or supplement CT scans if clinically appropriate. Ideally same imaging modality should be used for sequential imaging assessments.
** can be done on day of sugery also if necessary.
† MRI can be used at the treating physician's discretion; ideally same modality should be used throughout.
(X) as clinically indicated
*** Blood for correlates may be collected anytime after consent and prior to Nivolumab infusion.

***** Days -42 until/including Day -28

$$ F/u visits are allowable +/- 28 days or as clinically indicated

See Appendix D for Sociodemographic Characteristics Questionnaire

5. DOSE DELAY AND TOXICITY MONITORING

Dosing delays will not be permitted for this study. Prior to surgery, any treatment related toxicity should have resolved to ≤ Grade 2. Decision for surgery should be made by the treating medical oncologist and the surgeon.

General management algoirthms for potential nivolumab-related toxicities are contained in Appendix of this protocol and in the Investigators Brochure.

5.1 **Dose-Limiting Toxicity**

Dose-Limiting toxicity (DLT) is defined as any of the items listed below that occur from the first dose of nivolumab through day 100 following the last dose of nivolumab (or day 30 post surgery, whichever is longer). Any patient who experiences a DLT will proceed to surgery after standard preoperative evaluation by a surgeon and anesthesiologist.

- Any ≥ Grade 2 drug-related pneumonitis or interstitial lung disease that does not resolve to Grade 0 or 1 within 2 weeks with systemic steroids. The management algorithm for pneumonitis or pulmonary toxicity can be found in the appendix of current Investigator Brochure for nivolumab
- Any ≥ Grade 2 drug-related uveitis or eye pain that does not respond to topical therapy and does not improve to Grade 1 severity within the retreatment period OR requires systemic treatment. If uveitis occurs during nivolumab treatment, workup and treatment should follow the nivolumab uveitis toxicity treatment algorithm located in the appendix of the nivolumab Investigators Brochure.
- Any Grade 3 non-skin drug-related adverse event lasting ≥ 7 days with the exception of asymptomatic laboratory abnormalities.
- Grade 3 drug-related bronchospasm, allergic reaction, or infusion-related reaction will be recorded and treated; however, it will not count as a dose-limiting toxicity for study purposes.
- Any Grade 3 drug-related diarrhea that does not respond to dose delay and the use of systemic steroids within 2 weeks. If diarrhea occurs during nivolumab treatment, workup and treatment should follow the nivolumab diarrhea toxicity treatment algorithm located in the appendix of the nivolumab Investigators Brochure.
- Any Grade 4 drug-related adverse event, including laboratory abnormalities apart from isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelaeand are corrected with appropriate management within 72 hours of their onset.
- Any of the following drug-related hepatic function laboratory abnormalities or potential Drug Induced Liver Injury (DILI):
    - AST or ALT > 5-10x ULN for > 2 weeks

- AST or ALT >10x ULN
- Total bilirubin > 5 x ULN
- Concurrent AST or ALT >3 x ULN and total bilirubin >2 x ULN

- If suspected DILI occurs, workup and treatment should follow the nivolumab DILI treatment algorithm located in the appendix of the nivolumab Investigators Brochure.
- Any other toxicity which is assessed by the principal investigator as having directly led to a delay in surgical resection more than 28 days past the planned Day 0 (or 14 days past D +14).
- Failure to complete all protocol specified treatment doses due to toxicity (at the discretion of the PI).

Note - Adverse events of special interest are nivolumab-related events with potential immune-mediated causalities. For example, this may include cutaneous toxicities, colitis, liver function abnormalities (AST, ALT, total bilirubin, alkaline phosphatase), endocrine abnormalities (hyperthyroidism, hypothyroidism, hypophysitis and secondary adrenal insufficiency), interstitial pneumonitis and nephritis. These events will be noted. However, it may not constitute DLT's unless they fulfill the previously outlined DLT criteria described above.

6. ADVERSE EVENTS

This study will use the descriptions and grading scales found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 for adverse event reporting that can be found at https://ctep.cancer.gov/.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

All adverse events experienced by subjects will be collected from the time of first dose of study medication, throughout the study and until 100 days post last dose of study drug. Subjects continuing to experience toxicity after discontinuation of the study drug may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

Any adverse event experienced during additional preoperative treatment or after the surgical procedure that the investigator feels is related to study treatment will be captured.

6.1 **Serious Adverse Events**

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant 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.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

NOTE: (PI determines if this information regarding hospitalizations are considered SAEs and should be included in the protocol. This is supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- 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).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

6.2 Adverse Events

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug 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 adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

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

Adverse events can be spontaneously reported or elicited during open-endedquestioning, 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.)

6.2.1 Unexpected adverse event

An adverse event, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the Investigator's Brochure, package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

6.2.2 Expected (known) adverse event

An adverse event, which has been reported in the Investigator's Brochure. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

6.3 Nonserious Adverse Events

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from the start of a placebo lead-in

period or other observational period intended to establish a baseline status for the subjects.

- A ***non-serious adverse event*** is an AE not classified as serious.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at initiation of study drug. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of (provide # of days depending on the asset and study type) days following the last dose of study treatment.

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

6.4 Laboratory Test Abnormalities

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 to BMS 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 participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

6.5 Potential Drug Induced Liver Injury (DILI)

_____ Specific criteria for identifying potential DILI have not been identified for this protocol. Standard medical practice in identifying and monitoring hepatic issues should be followed.

_____ 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 SAEs.

Potential drug induced liver injury is defined as:

1. ALT or AST elevation > 3 times upper limit of normal (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 AT 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.

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 SAEs.

6.6 **Pregnancy**

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com of this event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

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. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

6.7 **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 SAEs (see Section 6.3 for reporting details).

6.8 Other Safety Considerations

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 non-serious or serious AE, as appropriate, and reported accordingly.

6.9 Adverse Event Attributions

The attributions are assessed by an investigator and assigned as followings:

- Attribution of the AE:
  - Definite – The AE is clearly related to the study treatment.
  - Probable – The AE is likely related to the study treatment.
  - Possible – The AE may be related to the study treatment.
  - Unlikely – The AE is doubtfully related to the study treatment.
  - Unrelated – The AE is clearly NOT related to the study treatment.

6.10 Serious Adverse Event 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 Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation for those subjects that receive study therapy (within 30 days of last visit for enrollment failure) The Investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its status of seriousness. If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE report form.
- All SAEs, whether related or unrelated to nivolumab and all pregnancies must be reported to BMS (by the investigator or designee) within 24 hours.
- The principal investigator or their designee will notify the appropriate regulatory agencies of any serious adverse event due to any cause during the course of this investigation. These include the Johns Hopkins Cancer Center Data and Safety Monitoring Committee, and the Johns Hopkins Medical Institutional Review Board (JHM-IRB) of the Johns Hopkins Medical Institutions. The required reporting time period is 3 days for fatal events, and 10 days for all other events.

- For studies conducted under and Investigator IND, any event that is both serious and unexpected must be reported to the Food and Drug Administration (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 institutuion'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 or similar form It MUST include the institutional AND BMS study ID.

MedWatch SAE forms should be sent to the FDA with reference to the IND under which this study is being conducted.

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

The study period during which adverse events will be reported is form the initiation of study procedures to the end of the study treatment follow-up, defined as 100 days following the last administration of nivolumab treatment.

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 to BMS using the same procedure used for transmitting the initial SAE report.

In accordance with local regulations, BMS will notify investigators of all SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the Investigator Brochre). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR).

Investigator notification of these events will be in the form of an expedited safety report (ESR) provided through the FastTrack portal system.

SAEs must be recorded on the SAE Report Form. 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.

All SAEs should be followed to resolution or stabilization.

The Sponsor will reconcile the clinical database SAE cases (case level only) transmitted to BMS Global Pharamacovigilance (WoldwideWorldwide.Safety@bms). Frequency of reconciliation should be every 3 months and prior to the database lock or final data summary. BMS GPV&E will email, upon request from the Investigator, the GPV&E reconciliation report. Requests for reconciliation should be sent to aepbusinessprocess@bms.com. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS.

6.11 **Non-serious Adverse Event Collection and Reporting**

The collection of non-serious AE information should begin at initiation of the study drug. Non-serious AE information should also be collected from the start of the observational periodintended to establish a baseline status for the subjects.

All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 90 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cuase interruption or discontinuation of study drug, or those that are present at the end of study treatment as appropriate. All identified non-serious AEs must be repocrded and described on the non-serious AE page of the CRF (paper or electronic).

7. DATA AND SAFETY MONITORING

7.1 Data Management

7.1.1 **Confidentiality**

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

7.1.2 **Source Documents**

Source data include all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

7.1.3 **Case Report Forms**

The study case report forms (CRFs) are the primary data collection instrument for the study. All data requested on the CRF will be recorded for each subject. If a procedure was not done or a

question was not asked, this will be recorded as "N/D". If the item is not applicable to the individual case, this will be recorded as "N/A". CRFs will be built electronically in Johns Hopkins RedCap (or CRMS). All data will be entered electronically onto the electronic CRF through Johns Hopkins RedCap (or CRMS) by the Study Coordinator and/ or Data Manager from each site.

The completed forms will be forwarded for central review and inclusion in the study dataset with relevant source documentation as outlined in the case report forms. The data submission schedule is as follows:

At the time of registration:

- Informed Consent Form (signed by the subject)
- Eligibility Checklist
- Source documents related to eligibility

Within 2 weeks after registration:

- Baseline study case report forms
- Pertinent source documents

Within 2 weeks after final dose of study medication:

- On study case report forms
- Pertinent source documents

7.2 Safety Monitoring

7.2.1 Auditing and Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, University compliance and quality assurance groups. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

The PI shall internally monitor the progress of the trial, including review and confirmation of all safety/treatment-related outcomes, response assessments, safety reports and/or any related source documentation.

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.

7.3 **Ethical Considerations**

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made to the investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the agents administered in this study can be found in Section 6 and Investigator's Brochures of nivolumab.

8.1 Nivolumab

8.1.1 Fully human anti-PD-1 IgG4:κ monoclonal antibody
Other names: BMS-936558, MDX-1106
Classification: Monoclonal antibody; impedes PD-1/PD-L1 interaction

8.1.2 How Supplied: Nivolumab is provided as a sterile liquid in vials each containing 100mg/10ml of the monoclonal antibody and 5 vials per carton.

8.1.3 Mechanism of Action: Nivolumab is a monoclonal antibody that binds to the cell surface protein PD-1, a key regulator of cytotoxic T lymphocyte activation. Nivolumab inhibits PD-1/PD-L1 and PD-1/PD-L2 interaction, facilitating CTL reactivity and promoting an anticancer cytolytic response.

8.1.4 Route of Administration: Nivolumab is to be administered as an intravenous infusion, using a volumetric pump with a 0.2 micron in-line filter at the protocol-specified doses. It is not to be administered as an intravenous push or bolus injection. At the end of the infusion, the line should be flushed with a sufficient quantity of normal saline. A 30 minute infusion, can be diluted with 0.9% NS for delivery but the total drug concentration of the solution cannot be below 0.35mg/ml.

8.1.5 Storage and Stability: Nivolumab vials must be stored at a temperature of 2°C to 8°C and should be protected from light. 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. After Nivolumab has been prepared for administration, the total storage time (combination of refrigeration and room temperature) is not to exceed 24 hours. Stability data for Nivolumab following dilution and transfer to the IV bag supports either: 24 hours at 2°C to 8°C in the refrigerator, or 8 hours at room temperature/under room light and 18 hours at 2°C to 8°C in the

refrigerator. Care must be taken to assure sterility of the prepared solution as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between Nivolumab and polyolefin bags have been observed.

8.1.6 Potential Drug Interactions: Studies examining interactions between Nivolumab and other agents are ongoing.

8.1.7 Nivolumab will be supplied directly from BMS Inc.. The IDS Pharmacy will be responsible for ordering the study drug directly from BMS Inc.

8.1.8 Pharmacy: Prepare in Non-PVC bag. Final concentration will be > 0.35mg/ml. Mix to total volume of 60ml NSS. If dose volume > 60ml, use straight drug. Prime tubing with NSS and attach to bag. Infuse via 0.2 micron filter. Send inline filter to clinic.
Nurse: Attach inline filter to patient side of pump
Investigational supply of Nivolumab is obtained from Bristol Myers Squibb. Drug supplies, will be kept in a secure, limited access storage area under the storage conditions. Where necessary, a temperature log must be maintained to make certain that the drug supplies are stored at the correct temperature.

The responsible person must maintain records of the product's delivery to the study site, the inventory at the site and the use by each patient. The Principal Investigator (and participating site investigators) are responsible for all destructions/disposals of partially used and unused supplies. Supplies are not to be shipped back to BMS. A copy of the drug destruction certificate must be maintained for provision to BMS at the end of the study.

These records will include dates, quantities, batch/serial numbers, expiry ("use by") dates, and the unique code numbers assigned to the investigational product(s) and study patients. The responsible person will maintain records that document adequately that the patients were provided the doses specified by the CSP and reconcile all investigational product(s) received from BMS.

9. MEASUREMENT OF EFFECT

9.1 Pathologic evaluation

Assessment of immune related pathologic response (irPRC) is the first secondary endpoint of the study as defined by Stein et al.[51]
Diagnostic pathology specimen will be collected and processed following the treating institution's standard operating procedures and will be evaluated by a head and neck pathologist.

Major pathologic response (mPR) will also be assessed and is defined as $\leq$ 10% residual tumor

tissue on all reviewed specimens of each subject.

9.2 Radiographic evaluation

Radigraphic response is the third secondary endpoint of the study. On this study, planned radiologic evaluations will be performed at baseline and after 4 weeks of therapy prior to planned surgical resection.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009], Changes in the longest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Evidence of PD will be based on a comparison with baseline (or nadir) scans, in which there is either an increase of 20% or more in the sum of the longest diameters (SLD) of target lesions taking as reference the smallest sum of the longest diameters (nadir) recorded since starting nivolumab treatment, and/or unequivocal progression of non-target lesions, with or without the development of 1 or more new lesions. Because all subjects are intended to get surgical resection, confirmatory scans are not required.

9.2.1 **Definitions**

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment on protocol.

Evaluable for objective response. All patients will be evaluable for objective response after completing the first treatment on protocol, as long as they get radiographic evaluation with either CT scans or MRI scans on the specified timeframe.

9.2.2 **Disease Parameters**

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion.

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray or as >10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitic involvement of the skin or lung, inflammatory breast disease, and abdominal masses/abdominal organomegaly (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

9.2.3 **Methods for Evaluation of Measurable Disease**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

9.2.4 **Response Criteria**

9.2.4.1 Evaluation of Target Lesions

Immune related pathologic response criteria (irPRC)
irPRC response will be assessed as described by Stein et al by Dr. Lisa Rooper/a board certified pathologist trained in irPRC assessment.[51]

Radiologic Response:
Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Special notes on the assessment of target lesions:
Lymph nodes: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded and should be measured in the same anatomical plane as the baseline examination, even if the nodes regress to below 10mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm.

Target lesions that become 'too small to measure': All lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2mm). If the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm. When such a lesion becomes difficult to assign an exact measurement, it is recommended to: If it is in the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (note: in case of a lymph node believed to be present and faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness).

Lesions that split or coalesce on treatment: When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

9.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator). When the patient also has measurable disease: To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. When the patients has only non-measurable disease: To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If

'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point.

9.3 **Progression-Free Survival**

PFS is defined as the duration of time from start of nivolumab treatment to time of progression or death, whichever occurs first.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

10.1.1 **Study Design**

Patients will receive 1 dose of nivolumab, 480mg IV on Day -28 prior to planned surgery on or after Day 0 and up to/including Day +14. A 14 day window is allowable (as needed for scheduling purposes, or if clinically appropriate for patients with clinical benefit/improvement, if deemed safe by the treating physicians (surgeon & medical oncologist). Furthermore if clinically indicated patients may undergo surgery earlier (see Section 4.1.2 regarding early surgery, salvage treatment).

Patients will be observed for **perioperative grade 3-4 adverse events through day 100 (or day 30 post surgery – whichever is longer)** following the last dose of study drug(s). Safety and feasibility will be monitored continually throughout the study through biweekly meetings with investigators. A set of markers of immune reactivity measured in the tumor resection specimens, draining lymph nodes, and peripheral blood will be evaluated. Feasibility in this study means the successful completion of preoperative treatment and proceeding to surgery without any extended treatment related delays. Extended treatment-related delay is defined as > 28 days from preplanned Day 0 in this context (or accordingly >14 days from day +14).

10.1.2 **Endpoints**

Primary Endpoint

Safety and feasibility of neoadjuvant nivolumab administration in patients with newly diagnosed head and neck cancer and those with locoregional recurrence or oligometastatic disease undergoing surgical resection, measured by 1) frequency of drug-related adverse events occurring up to 100 days after the last dose of nivolumab or 30 days after surgery (whichever is longer), and 2) successful completion of preoperative treatment and proceeding to surgery without any extended treatment-related delays greater than 28 days from the preplanned day 0 (or 14 days from day +14).

Secondary Endpoints

Immune related pathologic response (irPRC)
As defined by Stein et al (CCR 2019)[51]

Major pathologic response rate
Defined as ≤ 10% residual tumor in the resection specimen after neoadjuvant nivolumab

Progression-free survival
Progression-free survival will be measured from the time of initiation of nivolumab until radiologic or clinical progression or death.

Radiographic response rate
Response will be determined by RECIST version 1.1.

Laboratory correlates of immune activity and response

10.2 Sample Size, Analysis, and Accrual Rate

This study is investigating the safety and feasibility of neoadjuvant nivolumab administration in patients with locoregional newly diagnosed, recurrent or oligometastatic disease undergoing surgical resection.The study will enroll patients in two cohorts with an expected 12 patients in each cohort (although exact allocation may vary), who will undergo surgery in each cohort; the total sample size will be up to 26 patients. Subjects who sign a consent form but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), and patients who do not undergo surgical resection (for reasons unrelated to the study drug or toxicity) will be replaced and will not count towards our accrual goal.

The goal for the primary analysis of safety is to estimate the rate of DLTs (as defined in section 5.1) (AEs). We will provide the proportion of patients experiencing AEs, along with 95% confidence interval. Based on our study of neoadjuvant nivolumab in resectable lung cancer, we assume that at most 5% of patients will develop drug-related AEs of grade 3 or higher. The estimate and corresponding two-sided 95% confidence interval (CI) for each cohort is listed in the table below and may be updated if allocation between cohorts is not balanced:

Observed number of patients with grade 3 or higher toxicity	Observed rate	95% CI (Clopper-Pearson)
0	0	0% - 26.5%
1	8.3%	0.2% - 38.5%
2	16.7%	2.1% - 48.4%

For feasibility, the estimate of percent patient proceeding to surgery without any extended treatment related delays and the corresponding 95% CI based on 11-12 patients is, but may be updated if allocation between cohorts varies further:

Observed number of patients proceeding to surgery without extended delay	Observed rate	95% CI (Clopper-Pearson)
12	100%	73.5% - 100%
11	91.7%	61.5% - 99.8%

However, cohort size (e.g. in the case of imbalanced enrollment) may be split 13/11, or 14/10. after consultation with the study statistician.

Surgical resection for head and neck cancers is standard of care. With regards to the recurrent population, this study targets a niche population, and historically there has been no dedicated trial for these patients. Typically surgeons perform at least 2-4 operations in patients appropriate for this study per month at a high volume academic institution, and thus expected accrual is 1-4 per month. For 26-patient study, it will take approximately 12-18 months with a high volume academic site such as Johns Hopkins University.

Endpoints:
Feasibility will be evaluated as the successful completion of preoperative treatment and proceeding to surgery without any extended treatment-related delays defined as >28 days from preplanned Day 0 in this context (or 14 days if surgery is performed Day +14).

Safety will be measured by:

- Frequency of drug-related adverse events occurring from the (first) dose of nivolumab up to 100 days after the last dose of study treatment or 30 days after surgery (whichever is longer).
- Frequency of serious adverse events occurring from the (first) dose of nivolumab up to 100 days after the last dose of study treatment or 30 days after surgery (whichever is longer).
- Frequency of clinical laboratory test by worst toxicity grade using NCI CTCAE v5 (as assessed at the time intervals outlined in the study calendar 4.10).

Safety and feasibility will be monitored continually throughout the study.
Safety stopping guideline: For the neoadjuvant nivolumab cohorts we will temporarily halt the accrual if the risk of grade 3-4 toxicities appears to be higher than 25% (i.e., posterior probability that the risk > 25% is 0.7 or higher). We use a stopping rule based on a Beta prior distribution with parameters 1 and 3. With this prior, there is 90% probability that this risk of high-grade toxicity is between 1.7% and 63.2%. The safety stopping rule for the two single-agent cohorts applies this prior distribution to the observed number of patients experiencing high-grade toxicity and to compute the resulting risk of such toxicities. If, given the data from patients already treated, the probability is at least 70% that the risk is greater than 25%, we will consider stopping treatment. Starting from the 2nd patients, the safety stopping guidelines are

shown in Tabel 1.

Table 1. Stopping rule for safety.

Stop if there are this many grade 3 or higher AEs	2	3	4	5
In this many patients	2-4	5-8	9-11	12

Operating characteristics of the safety stopping rule are shown below and are based on 5,000 simulatins:

Risk of AE	0.15	0.20	0.25	0.30	0.35	0.40
% of Time study stops	16.7%	30%	42.2%	55.4%	69%	78.7%
Expected sample size	10.8	10	9.1	8.2	7.1	6.3

Feasibility stopping guideline: We will temporarily halt the accrual if the risk of delayed surgery due to neoadjuvant nivolumab appears to be higher than 25%. We use a stopping rule based on a Beta prior distribution with parameters 0.1 and 0.9. With this prior, there is 90% probability that this risk of high-grade toxicity is between 0 and 65%. If, given the data from patients already treated, the probability is at least 70% that the risk is greater than 25%, we will consider stopping accrual pending feasibility evaluation. Starting from the 2nd patients, the stopping rules are shown in Table 2.

Table 2. Stopping rule for feasibility.

Number of patients whose surgery is delayed for >28 days	2	3	4	5
Out of this many patients	2-4	5-8	9-11	12

Operating characteristics of the feasibility stopping rule are shown below and are based on 5,000 simulatins:

Risk of delayed surgery	0.15	0.20	0.25	0.30	0.35	0.40
% of Time study stops	17.3%	29.3%	43.1%	56.2%	68.3%	79.2%
Expected sample size	10.8	10	9	8.1	7.1	6.2

We will report the proportion of patients experiencing high-grade toxicity with exact binomial 95% confidence intervals. The safety analysis population includes patients who receive one dose of nivolumab. We will also describe patient experiences with respect to feasibility as defined above. The analysis population for feasibility includes patients who receive one dose of nivolumab. All other adverse events will be similarly summarized by type and grade. Analyses of immune markers will be descriptive and/or graphical in nature.

10.3 **Analysis of secondary endpoints**

We will obtain pathology reports from all surgical specimens and tabulate the data to obtain rate of pathologic CR. Descriptive reports of pathology findings for each cohort will also be reported. We will estimate progression-free survival probabilities using the Kaplan-Meier method. Progression-free survival is defined as the time from the first dose of nivolumab until progression or death from any cause. Toxicities will be categorized by frequency and type, and reported in tabular form for each cohort and all subjects on the trial. All patients will be evaluable for toxicity from the time of their first treatment with any study therapy.

11. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

(Please see lab manual for full details. The lab manual will be continually updated, and in case of discrepencies for specific items (e.g. a specific tube color) the lab manual will be followed as long as it is consistent with the intent of the protocol.

Five categories of exploratory correlative immune responses will be analyzed:
1) Expression of molecules in the tumor microenvironment (TME) via IHC and multispectral imaging of immunofluorescence staining (IF),
2) Analysis of T cell responses in peripheral blood to mutation associated neoantigens (MANA) and HPV-16 E6 and E7,
3) Flow cytometry and single cell analysis of TIL
5) Genomic markers assessed via Exome sequencing and RNAseq used to assess MANA and HPV specific immune responses.

Examples of membrane immunomodulatory molecules to be analyzed by IHC/IF as well as flow cytometry include, but are not limited to, PD-L1, PD-1, LAG3, TIM3, TIGIT, PVRIg. The neoadjuvant trial design also allows for the isolation of enough TIL to analyze coordinate expression of multiple membrane and intracellular molecules by multiparameter flow cytometry as a complement to IHC/IF. Multiparameter flow cytometry analysis of TIL will utilize a 25-color T cell staining panel that can be assessed on the BDnSymphony50, which currently simultaneously analyzes 28 colors and will have the capability to analyze 48 colors in 2019. Competing multiplex flow cytometers have achieved similar results (e.g. Cytek Aurora). In addition to cell membrane receptors and ligands, a number of transcriptional activators and repressors have been associated with the anergic or “exhausted” T cell functional state vs an active effector or effector-memory state. For example, Tbethi Eomeslo cells represent renewable effector memory cells where as TbetloEomeshi cells are terminal cells on their way to full exhaustion or apoptosis. Other more recently defined transcriptional regulators, such as TCF-1, are important in maintenance of effector function.

Comprehensive genomic, transcriptomic and neoantigen analyses in tumor biopsies will be performed and MANA and HPV E6/7 antigen recognition will be analyzed via the MANAFEST assay for MANA and the VIRAFEST assay for HPV. Whole-exome sequencing utilizing the Personal Genome Diagnostics (PGDx) next-generation platform will be utilized and performed on pre-treatment and resected tumor samples. This will allow detection of mutations in DNA-

repair and other relevant genes. Exome data will be applied in a neoantigen prediction pipeline that evaluates antigen processing, MHC binding and gene expression to generate neoantigens specific to the patient's HLA haplotype. Truncal neoantigens will be identified by correcting for tumor purity and ploidy. Putative neoantigens will then be used to generate peptides and stimulate autologous T cells, followed by T Cell Receptor (TCR) next-generation sequencing (MANAFEST).

The MANAFEST assay will determine if treatment increases the frequency and/or magnitude of anti-tumor T cell responses, based on the parameters described above. For each patient, the comparison of MANA-specific T cell clonality pre-and post-treatment among the peptides that are positive at both time points will be analyzed using a paired-sample t-test, and the difference of clonality of peptides that were MANAFEST-positive at either time points will be assessed using a mixed effect model to account for the correlation of common peptides. The analysis of MANAFEST results across patients will apply mixed effect models with each patient as a cluster, to account for the correlations due to the common microenvironment within a patient. Data transformation will be considered if the distribution of MANAFEST data is not normal. The VIRAFEST assay is an adaptation of the MANAFEST that analyzes T cell responses to predicted viral antigens in the identical fashion. For patients on this protocol with HPV+ HNSCC, we will analyze responses to a set of HPV-16 E6 and E7 peptides in addition to MANA peptides identified via WES.

11.1 Collection of tumor tissue specimens

11.1.1 Collection of pretreatment tumor and/or lymph node biopsies

Archived FFPE specimens from the original diagnostic tumor biopsy may be utilized. If archival tissue does not provide sufficient material for study, then new biopsies is recommended as clinically feasible. Ideally 6 (and at least 4) core needle biopsies of the tumor will be required at the time of diagnosis (prior to first dose of study drug) and in the event of a recurrence if clinically feasible; fine needle aspiration biopsy is sufficient for hilar or mediastinal lymph node sampling, but not for primary tumor biopsy. Where possible, and after a consent form has been signed, attempts will be made to coordinate diagnostic and study biopsies.

Previously collected tissue/blood/material can be accessed for this study to fulfill study requirements including fresh/frozen/FFPE/etc biologic material to fulfill eligibility/study requirements.

11.1.2 Pretreatment biopsy handling, transportation, storage and processing

Please see flow diagram in Appendix B for overview of tissue collection and the laboratory manual for detailed procedures. The study staff will be notified when a biopsy is taking place. The following procedures will be followed:

If a core needle biopsy is being performed specifically for entry to the study, then at least four

(and ideally 6) core-biopsy specimens will be obtained, the first 3-4 of these cores obtained will be suspended in 10% buffered formalin while subsequent cores will be flash frozen in liquid nitrogen (see lab manual). After approximately 24 hours of suspension in formalin, the cores will be embedded in paraffin. As required for correlative analyses slides will be cut for the appropriate studies listed below. For fine needle aspiration biopsies of lymph nodes from bronchoscopy, cells will be collected by centrifugation, fixed in formalin and embedded in a paraffin block using standard pathology procedures.

The study coordinator will keep a log with the study number, the patient's study number, the date and time, and a consecutive sample number; thus, the samples will be numbered serially and will not contain identifying information.

11.1.3 Surgical specimens (tumor and lymph nodes)

Tissue specimens obtained at the time of surgery will be dissociated enzymatically into single cell suspensions and will be viably cryopreserved according to a protocol provided in a companion laboratory manual. Additional specimens will fixed in formalin and embedded in paraffin blocks, for routine pathologic studies and immunohistochemistry. Tissue will also be flash-frozen at -80oC for subsequent RNA/DNA analysis. If there is additional tissue available, it will be embedded in OCT (Optimal Cutting Tissue) compound for analysis of frozen sections.

11.2 Collection of blood samples

11.2.1 Collection schedule

Blood samples will be drawn and collected at the time points shown in the study calendar (section 4.10). These time points include:

Day -28 (Prior to treatment): 100 mL whole blood or local protocol for collection accepted for peripheral blood mononuclear cell (PMBC) isolation, and 20 mL whole blood for serum isolation
Day 0 (prior to surgery): 100 mL and 10 mL whole blood, for PBMCs and serum, respectively

11.2.2 Specimen handling, transportation, storage and processing

- Serum samples: Whole blood will be collected in serum tubes, processed per manufacturer's instructions and stored at -70°C or below until transfer for analysis.

- PBMCs: Whole blood will be collected into EDTA tubes (Becton-Dickinson ETDA tube or equivalent) and processed per manufacturer's instructions. Viable PBMCs will be stored in cryopreservation medium, at 5-10 ul per vial, in liquid nitrogen.

Analysis of exploratory features of oral and gut microbiota that correlate with clinical response.

Integration of microbiome science into cancer therapeutics is a new field of study. Three recent papers published in Science have suggested that responses in melanoma and lung cancer are impacted by the composition of the microbiome and that certain bacterial species can promote improved therapeutic responses to checkpoint inhibitor therapy[47-49] (). Thus, the goal of this sample collection is to facilitate exploratory, correlative analyses between the oral and gut microbiome communities and responses to neoadjuvant checkpoint blockade therapy.

We will consider using 16S rRNA, shotgun metagenomics and/or RNA-seq for analyses. 16S rRNA sequencing data will be filtered for poor quality and contaminant/chimeric sequences, followed by taxonomic assignment using standard bioinformatic pipelines such as QIIME and Resphera Insight. To detect differentially abundant taxa between responders and non-responders, we will utilize the nonparametric Mann-Whitney test with correction for multiple hypothesis testing using the False Discovery Rate. To evaluate beta-diversity between responders and non-responders (i.e. shared total community composition), we will compute the UniFrac distance metric for all sample pairs, followed by principal coordinate analysis and significance testing with PERMANOVA[50]. Functional inference of gene content from 16S rRNA data may also be performed using tools such as PICRUSt. While 16S rRNA identifies only bacterial sequences, metagenomics permits detection of viruses and fungi among others. Metagenomic analyses will be designed to remove human contaminant sequences followed by taxonomic assignment using Kraken and Pathoscope. Functional characterization of metagenomic data will be performed using the HUMAnN tool. Additionally, RNA-seq analysis (meta-transcriptomics) enables characterization of actively transcribed microbial genes and RNA viruses, with the potential to explore the combined host:microbial interaction(s).

11.2.3 Leftover samples

Any leftover study blood and tissue samples will be stored in the Laboratories of coinvestigators at Johns Hopkins for future research studies. These samples may be released for use in future studies after approval by the principal investigator and other regulatory bodies, as appropriate. Subjects will be asked to consent to the future use of samples in the consent document.

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Appendix A. ECOG Performance Status Scale

Score	Definition
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed less than 50% of day
3	Symptomatic, in bed more than 50% of day, but not bedridden
4	Bedridden

Appendix B. Guidelines for Tissue Banking Process (may be modified based on guidelines in correlative SOP, which will be continually updated)

Note: Only tissue that is absolutely not needed for clinical diagnosis or staging should be collected for tissue banking. If in doubt about this, do NOT submit specimens for banking.

Banking of Frozen Tissue

1. Place a single tissue specimen flat in the plastic bag. A single tissue specimen's overall volume should be at least 1 cm3, and at most 3–4 cm3, with at least one dimension measuring 0.5 cm thick or less to facilitate quick freezing.
2. For a given case (patient), please collect sufficient non-malignant and malignant tissue. Tissue selected should be grossly viable, and grossly consistent with tumor or adjacent normal tissue (see #5 below for contraindications). Non-malignant (i.e. "adjacent normal") tissue should be collected at least 2 cm from the primary tumor, subject to any limitations from the specimen's physical dimensions. Do not place tumor and non-malignant tissue in the same bag. For large tumors, do not place large pieces of tissue in a single bag. Rather, divide the tissue according to size guidelines in #1 above, and place each in an individual bag. Collect and separately identify both: 1) primary tumor and 2) metastatic lesions to lymph nodes or other tissues. Tissue will typically be taken by scalpel or dissection blade, though the use of 5 -7 mm skin punch biopsy tools could be considered in certain situations.
3. Immediately place the specimens for freezing in an isopentane or 2-methylbutane cryobath, or other effective liquid freezing agent. If no cryobath is available, then liquid N2 can be used as the freezing agent, in a properly insulated container and with sufficient safety precautions. The goal is to have bankable tissue immersed in the bath within 30 minutes of the OR's procurement from the patient. If more cryobath space is needed, move already frozen tissue to a -80C freezer in order to make sufficient room. Make sure to check periodically for cryobath problems (e.g. not maintaining temperature, refrigerant level low), and call for appropriate maintenance as needed. Do not freeze tissue by placing it fresh directly in the -80C freezer.
4. On receipt by the tissue bank laboratory, the frozen tissue is embedded in OCT (Optimal Cutting Temperature medium), and a frozen section is stained with H&E and the section evaluated by the tissue bank pathologist for quality assurance (QA) purposes. A report on the histopathologic findings is filed or communicated as needed. The frozen section evaluation can also count for adjacent pieces of tissue if they were taken as a "mirror image" section to the surface cut for the frozen section.
5. General contraindications to tissue banking

DON'T bank tissue from these specimen types or situations:

- small tumors and other cases where all or most of the lesional tissue is needed for diagnosis

- surgical margins of resection specimens where tumor and benign areas cannot be clearly delineated grossly grossly visible areas of primarily necrosis, hemorrhage, or fat

- specimens which are known to have been delayed significantly more than 30 minutes past their procurement time in the OR

- tissue previously freeze-thawed, or frozen slowly (e.g. in the cryostat or -80 freezer)

- areas of deepest invasion, tumor/normal interface, tumor/capsule interface, extranodal extension of tumor, and other key landmarks needed for surgical pathology evaluation and/or tumor staging

- diagnostic biopsies where most or all tissue must be submitted for pathology evaluation: most lymph node, GI, bone marrow, and liver biopsies fall in this category

- tissue clearly marked as intended for a special study such as immunofluorescence

Appendix C. Immune-Related Adverse Events Management Algorithms

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Principal Investigator. 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 and SITC or ASCO irAE management guidelines should be consulted . 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 llow-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.

Appendix D: Sociodemographic questionaires (Initial and follow-up)

Initial Sociodemographic Characteristics Questionnaire

Date: Month_____ / Day _____ / Year_____
Study ID Number: _____
Medical Records Number: _____

SOCIODEMOGRAPHIC CHARACTERISTICS QUESTIONNAIRE

1. **Name: _____**
2. **Birthdate: month _____/day _____/year _____**
3. **Sex: ☐ Male ☐Female**
4. **What is your race or ethnic group? ❒ Caucasian ❒ Hispanic ❒ African American ❒ Asian ❒ Other _____ ❒ I choose not to disclose**

FAMILY QUESTIONS

Both genetics and environment could be risk factors for the development of cancer. For this reason, it is important to determine your biological relationship with your family.

5. Are you adopted? □ Yes □ No
6. How many of each of the following family members do you have?
Brothers: _____Sisters: _____Sons: _____ Daughters: _____

SMOKING QUESTIONS

7. **Do you smoke cigarettes? ☐ Yes ☐ No, never ☐ Not currently but I have in the past**
8. **At what age did you start smoking? _____**
9. **When did you quit smoking cigarettes? _____**
10. **How many total years have you or did you regulary smoke cigarettes? _____**
11. **During the time you usually smoked regularly, how many cigarettes do or did you usually smoke per day? _____**
12. **Do you smoke cigars? □ Yes □ No, never □ Not currently but I have in the Past**
13. **At what age did you start smoking cigars? _____**

14. When did you quit smoking cigars? _____

15. How many years in total did you regularly smoke cigars? _____

16. Do you use smokeless tobacco or other nicotine products? (i.e. chewing tobacco, snuff, e-cigarette, nicotine patch or gum) ☐ **Yes** ☐ **No**

If yes, please indicate type(s):_____

17. Were you exposed to asbestos, that you know of? ☐ **Yes** ☐ **No**

18. Were you exposed to any other potential harmful exposures to your lung?
☐ **Yes** ☐ **No/never**

ALCOHOL QUESTIONS

19. Have you ever drunk alcoholic beverages, such as beer, wine, or liquor regularly, that is at least once a month? ☐ **Yes** ☐ **No**

20. At what age did you start drinking alcoholic beverages regularly, i.e. at least once a month?
_____years of age

21. Before the age of 40, how many drinks of beer (12 oz.), wine (5oz.), or liquor (1 oz.) did you usually drink per week?

More than one per week. Please indicate number_____
Less than one per week_____
Never drank before age 40 _____

22. After the age of 40, how many drinks of beer (12 oz.), wine (5 oz.), or liquor (1 oz.) did you usually drink per week?

More than one per week. Please indicate number _____
Less than one per week _____
Never drank after age 40 _____
Currently aged less than 40 years _____

MEDICAL QUESTIONS

23. Have you taken antibiotics in the last 3 months? ☐ **Yes** ☐ **No**
A list of antibiotics is attached for you to refer to. If no, skip to question 25.

24. If you know the name of the antibiotic(s), please write it here.

_____
_____

25. Have you had a bronchoscopy in the last 3 months? ☐ **Yes** ☐ **No**

26. Have you taken oral corticosteroids in the last 2 weeks? ☐ **Yes** ☐ **No**
(Oral corticosteroids examples: prednisone, dexamethasone, methlyprednisone, hydrocortisone)

27. If you answered Yes to (26), please write the name and dose here and when you took these.
_____
_____

28. Have you taken inhaled corticosteroids in the last 2 weeks? ☐ **Yes** ☐ **No**
(Inhaled corticosteroids examples: budesonide, fluticasone, beclomethasone, ciclesonide)

29. If you answered Yes to (28), please write the name and dose here and when you took these.
_____
_____

30.Do you have sleep apnea? ☐ **Yes** ☐ **No**

31.Do you have reflux disease? ☐ **Yes** ☐ **No**

32.Do you have any other chronic lung conditions? ☐ **Yes** ☐ **No**

33. If you answered Yes to (32), please write the name of the condition here
_____
_____

DENTAL HEALTH

34. About how often do you visit a dentist?
- ☐ **Less than every 6 months.**
- ☐ **Between every 6-12 months.**
- ☐ **Greater than every 12 months.**
- ☐ **I have never been to a dentist.**

35. Overall, how would you rate the health of your teeth and gums?
☐ **Excellent** ☐ **Good** ☐ **Fair** ☐ **Poor**

36. Have you ever had treatment for gum disease such as scaling and root planing, sometimes called deep cleaning? ☐ **Yes** ☐ **No**

37. Have you ever had any teeth become loose on their own, without an injury? (Not including baby teeth). ☐ **Yes** ☐ **No**

DIET QUESTIONS

38. Do you eat meat? Meat is defined as beef, chicken, pork, lamb or venison:

- **A. I do not eat meat.** ☐
- **B. I have eaten meat in the last year.** ☐
- **C. I have eaten meat one or more times a month during the last year.** ☐
- **D. I have eaten meat one or more times a week during the last year.** ☐

39. Do you eat Fish? Fish is defined as all fish and shellfish:

- **A. I do not eat fish.** ☐
- **B. I have eaten fish in the last year.** ☐
- **C. I have eaten fish one or more times a month during the last year.** ☐
- **D. I have eaten fish one or more times a week during the last year** ☐

40. Do you eat Eggs?

- **A. I do not eat eggs** ☐
- **B. I have eaten eggs in the last year** ☐
- **C. I have eaten eggs one or more times a month during the last year** ☐
- **D. I have eaten eggs one or more times a week during the last year** ☐

41. Do you eat Cheese? (This includes fresh, soft, aged or cottage cheese as well as sour cream):

- **A. I do not eat cheese** ☐
- **B. I have eaten cheese in the last year** ☐
- **C. I have eaten cheese one or more times a month during the last year** ☐
- **D. I have eaten cheese one or more times a week during the last year** ☐

42. Do you drink Milk? Milk is defined as milk from a cow, goat or sheep (not soy, coconut or almond milk, for example). If you put milk on your cereal, you should not answer (A).

- **A. I do not drink milk** ☐
- **B. I drank milk in the last year** ☐
- **C. I drank milk one or more times a month during the last year** ☐
- **D. I drank milk one or more times a week during the last year** ☐

43. Do you eat yogurt?

- **A. I do not eat yogurt** ☐
- **B. I have eaten yogurt in the last year** ☐
- **C. I have eaten yogurt one or more times a month during the last year** ☐
- **D. I have eaten yogurt one or more times a week during the last year** ☐

44. Do you take probiotics (live bacteria supplement)? ☐ **Yes** ☐ **No**

If yes (question 44) and if you know the name of the probiotic product(s), please write here:

_____

_____

45. Do you take vitamin supplements? ☐ **Yes** ☐ **No**

If yes (question 45) and if you know the name of the vitamin supplement(s), please write it/them here:

_____

_____

WORK AND PHYSICAL ACTIVITY

46. What is your current employment status?

☐ **Employed/self-employed** ☐ **Unemployed** ☐ **Retired** ☐ **Disabled**

47. How would you categorize your physical activity on the job?

☐ **Mostly sedentary or light activity (e.g. mostly sitting, standing, lifting light objects of less than 3 kilos).**

☐ **Mostly medium activity (e.g. much walking, climbing stairs).**

☐ **Mostly intense activity (e.g. heavy construction work).**

☐ **Unemployed/retired/disabled**

48. What type of exercise (physical activity) do you do regularly (at least 3 times per week)?

☐ **Mostly moderate activity (slow walking, gardening, golfing etc.)**

☐ **Mostly vigorous activity (running, swimming, bicycling, football etc.)**

☐ **I do not exercise regularly**

49. At age 20, what type of exercise did you do regularly (at least 3 times per week)?

☐ **Mostly moderate activity (slow walking, gardening, golfing etc.)**

☐ **Mostly vigorous activity (running, swimming, bicycling, football etc.)**

☐ **I did not exercise regularly**

Follow-Up Sociodemographic Characteristics Questionnaire

Date: Month_____ / Day _____ / Year_____
Study ID Number: _____
Medical Records Number: _____

SMOKING QUESTIONS

1. Are you currently smoking? ☐Yes ☐No

If yes, how many cigarettes/day?_____

ALCOHOL QUESTIONS

2. Regularly? ☐Yes ☐No

If yes, please indicate pattern:

More than one per week_____ (If checked), please indicate number_____

Less than one per week_____

MEDICAL QUESTIONS

3. Have you taken antibiotics since completing the last questionnaire? ☐Yes ☐No

4. If you know the name of the antibiotic(s), please write it here.
_____
_____

A list of antibiotics is attached for you to refer to. If no, skip to question 5.

5. Have you had a bronchoscopy since completing the last questionnaire? ☐Yes ☐No

6. Have you taken oral corticosteroids since completing the last questionnaire? ☐Yes ☐No
(Oral corticosteroids examples: prednisone, dexamethasone, methlyprednisone, hydrocortisone)

7. If you answered Yes to (6), please write the name and dose here and when you took these.
_____
_____

8. Have you taken inhaled corticosteroids since completing the last questionnaire?

☐Yes ☐No (Inhaled corticosteroids examples: budesonide, fluticasone, beclomethasone, ciclesonide)

9. If you answered Yes to (8), please write the name and dose here and when you took these.

_____

_____

DIET QUESTIONS

10. Do you eat meat? Meat is defined as beef, chicken, pork, lamb or venison:

A. I do not eat meat. ☐
B. I have eaten meat in the last year. ☐
C. I have eaten meat one or more times a month during the last year. ☐
D. I have eaten meat one or more times a week during the last year. ☐

11. Do you eat Fish? Fish is defined as all fish and shellfish:
A. I do not eat fish. ☐
B. I have eaten fish in the last year. ☐
C. I have eaten fish one or more times a month during the last year. ☐
D. I have eaten fish one or more times a week during the last year ☐

12. Do you eat Eggs?
A. I do not eat eggs ☐
B. I have eaten eggs in the last year ☐
C. I have eaten eggs one or more times a month during the last year ☐
D. I have eaten eggs one or more times a week during the last year ☐

13. Do you eat Cheese? (This includes fresh, soft, aged or cottage cheese as well as sour cream):
A. I do not eat cheese ☐
B. I have eaten cheese in the last year ☐
C. I have eaten cheese one or more times a month during the last year ☐
D. I have eaten cheese one or more times a week during the last year ☐

14. Do you drink Milk? Milk is defined as milk from a cow, goat or sheep (not soy, coconut or almond milk, for example). If you put milk on your cereal, you should not answer (A).
A. I do not drink milk ☐
B. I drank milk in the last year ☐
C. I drank milk one or more times a month during the last year ☐
D. I drank milk one or more times a week during the last year ☐

15. Do you eat yogurt?

A. I do not eat yogurt ☐
B. I have eaten yogurt in the last year ☐
C. I have eaten yogurt one or more times a month during the last year ☐
D. I have eaten yogurt one or more times a week during the last year ☐

16. Do you take probiotics (live bacteria supplement)? ☐Yes ☐No

If yes (question 16) and if you know the name of the probiotic product(s), please write here:

_____
_____

17. Do you take vitamin supplements? ☐Yes. ☐No

If yes (question 17**) and if you know the name of the vitamin supplement(s), please write it/them here:**

_____
_____

WORK AND PHYSICAL ACTIVITY

18. What type of exercise (physical activity) do you do regularly (at least 3 times per week)?

☐ **Mostly moderate activity (slow walking, gardening, golfing etc.)**

☐ **Mostly vigorous activity (running, swimming, bicycling, football etc.)**

☐ **I do not exercise regularly**