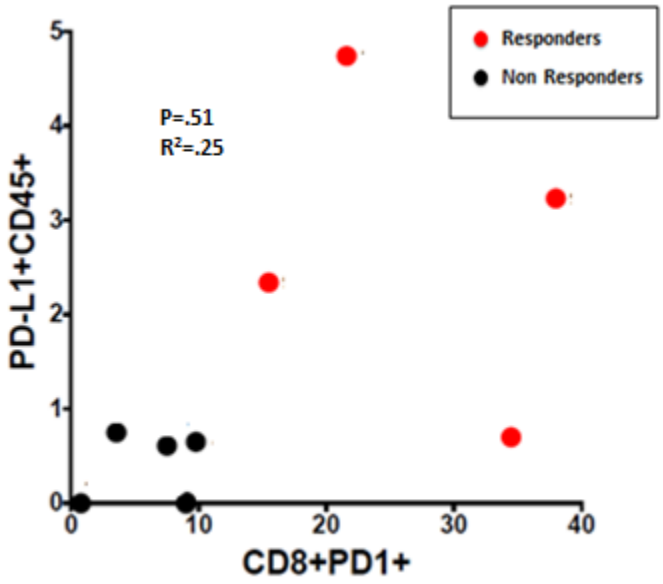
THE LANCET
Oncology

Supplementary appendix

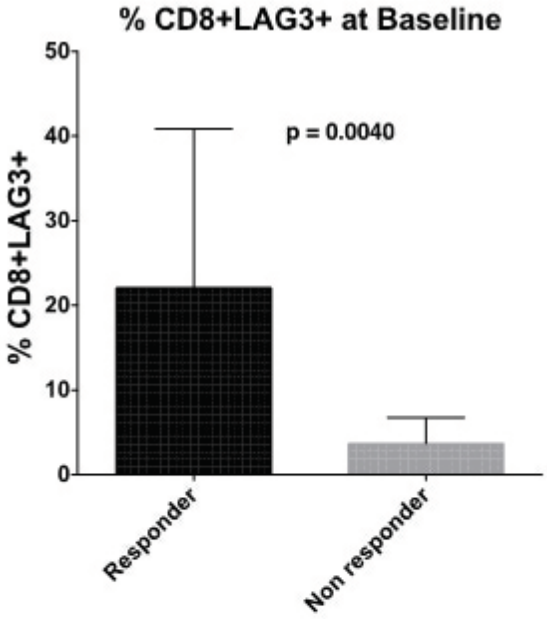
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

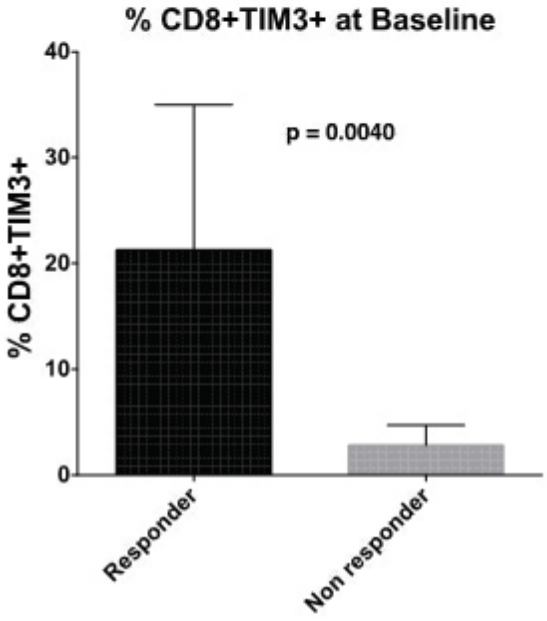
Supplement to: Morris VK, E Salem ME, Nimeiri H, et al. Nivolumab for previously treated unresectable metastatic anal cancer (NCI9673): a multicentre, single-arm, phase 2 study. *Lancet Oncol* 2017; published online Feb 17. http://dx.doi.org/10.1016/S1470-2045(17)30104-3.

Supplemental Figure 1: PD-L1 expression on CD45+ cells versus PD-1 expression on CD8+ T cells for responders and non-responders.

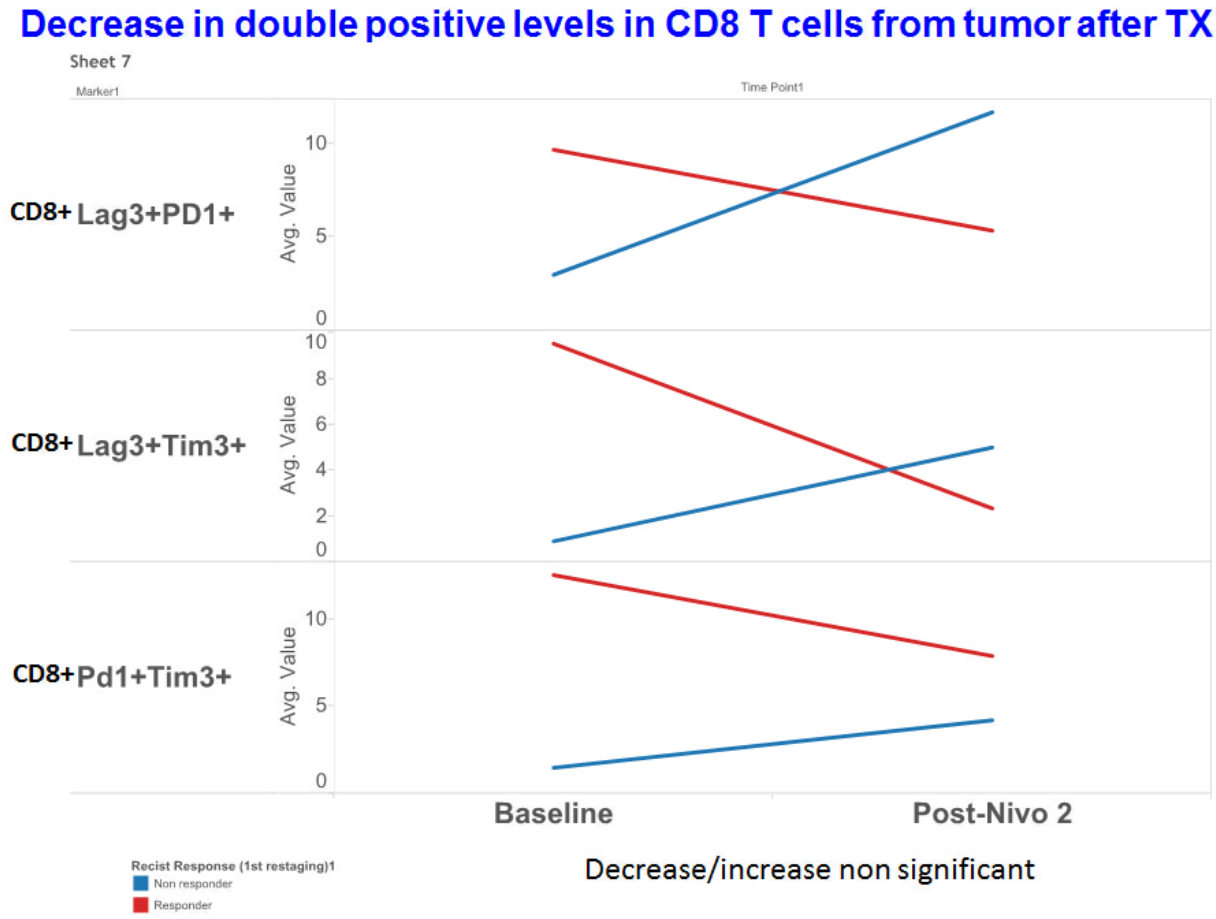


Supplemental Figure 2: Baseline LAG-3 and TIM-3 expression on CD8+ T-cells – responders vs. non-responders





Supplemental Figure 3: Prevalence of CD8+ T-cell subsets in biopsy samples – responders vs. non-responders



Supplemental Table 1: Sites of Enrollment

Site	Site Investigator	Patients Enrolled
MD Anderson Cancer Center	Dr. Cathy Eng	18
Medstar/Georgetown University	Dr. Mohamed Salem	3
Northwestern University	Dr. Halla Nimeiri	3
University of Southern California	Dr. Syma Iqbal	3
Washington University	Dr. Preet Singh	3
The Ohio State University	Dr. Kristen Ciombor	2
University of Chicago	Dr. Blasé Polite	2
University of Wisconsin	Dr. Dustin Deming	1
Vanderbilt University	Dr. Emily Chan	1
Decatur Memorial Hospital	Dr. James Wade	1

Supplemental Table 2: Mutation and Amplification Frequency

Gene	Mutation	Amplification	Total
PIK3CA	7	3	10
TP53	8	0	8
EGFR	1	2	3
FBXW7	3	0	3
BRAF	1	1	2
CTTNB1	2	0	2
KIT	2	0	2
MET	0	2	2
ARID1A	1	0	1
BRCA2	1	0	1
FGFR1	0	1	1
FGFR2	1	0	1
GNAS	1	0	1
JAK2	1	0	1
PTEN	1	0	1
RB1	1	0	1
SMAD4	1	0	1
STK11	1	0	1
CDK6	0	1	1
MYC	0	1	1
TOTAL	47	11	58

SUMMARY OF CHANGES – PROTOCOL 9673

For Protocol Amendment #5: This protocol is being submitted because the Guardant Health Assay language has been added to the protocol to describe the addition of cfDNA on the residual blood sample to optimize correlate information.

A Multi-Institutional Phase 2 Study of Nivolumab in Refractory Metastatic Squamous Cell Carcinoma of the Anal Canal

NCI Protocol #: 9673
Local Protocol #: NCI9673

NCI Version Date: April 12, 2016
Protocol Date: April 12, 2016

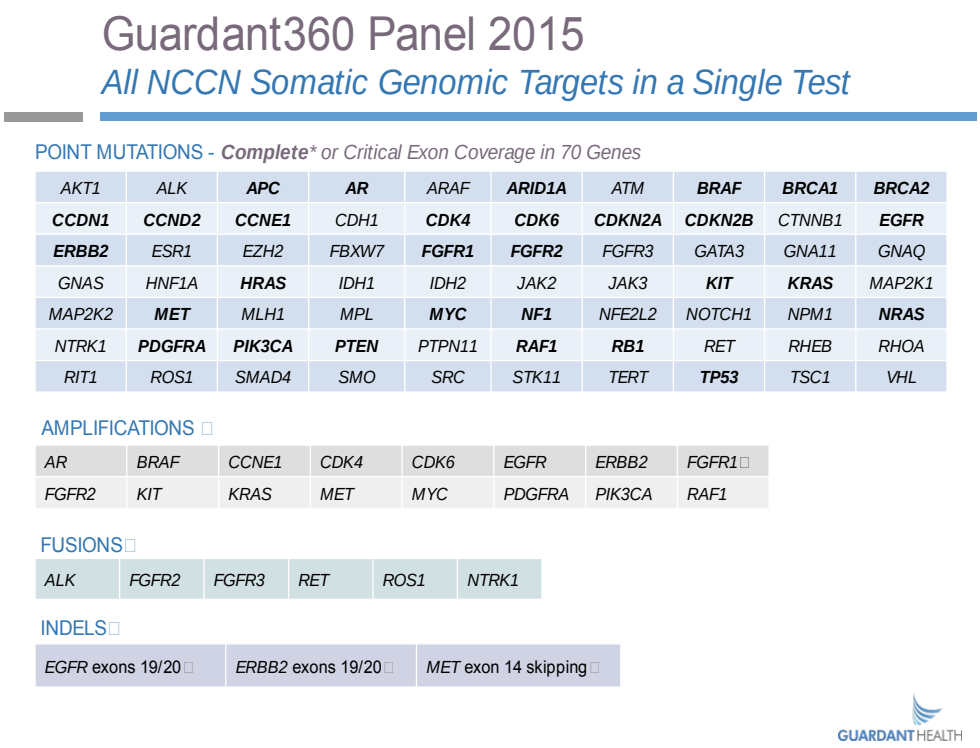
#	Section	Page	Change
1.	Table of Contents	4	**OLD TEXT:** Old table of contents **NEW TEXT:** New table of contents **RATIONALE:** Updated table of contents based on revisions and to ensure page numbers match
2.	Section 9.3.3	48	**OLD TEXT:** N/A **NEW TEXT:** **Due to the limitations of fresh tissue sampling in this high impact study, residual blood samples from MDACC and from our ETCTN collaborators will be considered for a noninvasive approach in mutation analysis to optimize all available information we have from all collaborators. For the purpose of this analysis, all correlative blood work is exploratory and will not be used for the purpose of treatment decision-making.** **RATIONALE:** Added rationale for analysis of correlative blood work.
3.	Section 9.3.4	49	**OLD:** N/A **NEW TEXT:** **Added Description of the Guardant Health Assay** **Guardant360 is a next-generation sequencing (NGS) panel of 70 clinically actionable onco- and tumor suppressor genes utilizing digital sequencing of cell-free circulating tumor DNA (cfDNA) isolated from a simple, non-invasive blood draw. It is medically indicated for the prevention of a repeat invasive biopsy in advanced cancer patients when the initial biopsy is insufficient (QNS) or unavailable/unobtainable as well as when cancer has progressed or recurred despite treatment. The test detects single nucleotide variants via complete exon sequencing in 70 genes, copy number**

			amplifications in 16 genes, small indels in *EGFR, ERBB2* and *MET* exon 14 skipping, and fusions in *ALK, FGFR2, FGFR3, RET, ROS1* and *NTRK1*. The genes are selected because mutations in these genes have FDA-approved matched therapies or are eligible for late phase clinical trials, as well as non-druggable genes with high prevalence alterations that may be helpful in monitoring for molecular response/non-response such as *TP53*. The panel also includes genomic markers of acquired resistance that may require a change in pharmacotherapy, e.g. *EGFR* T790M, *ALK* or *ESR1* mutations. **Guardant360 is an advanced diagnostic laboratory test (ADLT) offered by a sole source laboratory certified by the Clinical Laboratory Improvement Amendments (CLIA) for high complexity (molecular pathology) testing and accredited by the College of American Pathology (CAP). Due to high rates of false positives with traditional NGS assays when tumor DNA is in low concentrations, the majority of "liquid biopsy" methods interrogating cell-free DNA have been limited to hotspot analyses. In contrast, the ultra-high specificity (> 99.9999%) of the digital sequencing method enables the sequencing of long, targeted regions (146,000 base pairs) without false positives. Complete exons are sequenced for all exons in 30 genes and the critical exons (those reported as having a somatic mutation in COSMIC) in 40 additional genes. Thus, its key differentiating characteristic from other "liquid biopsy" methods is the ability to sequence complete exons in many genes, in contrast to gene hotspot testing.** **Advantages of the Guardant360 cell-free DNA (cfDNA) NGS methodology versus solid tumor tissue-based NGS are:** **1. An invasive needle or surgical biopsy is avoided with cfDNA, reducing costs and complications.** **2. CfDNA provides a *quantitative* measure (concentration or mutant allele frequency) of mutations present whereas solid tumor biopsy typically provides a qualitative result (mutation either present or not detected). The quantitative cfDNA result may be followed over time to monitor response to treatment and evolution of acquired resistance.** **3. CfDNA sequencing identifies *both* germline and somatic mutations in the same sample.** **4. The assay failure rate is for cfDNA is less than 0.5% (in the first 9,000+ samples) compared to 15%-25% failure rates of tissue-based NGS related to insufficient quantity of tissue (QNS).** **Guardant360 utilizes algorithmic methods to encode and ultimately decode inputs and outputs from massively parallel deep sequencing analysis. By leveraging signal transduction processing technology where voice or image**

			data is digitally encoded before transmission and then decoded post-transmission, this NGS method, known as Digital SequencingTM, enables signal interference to be reduced by two orders of magnitude or more [88]. Four validation studies have been published with concordance to tissue biopsy-based genomic testing [88-91]. With high enough sensitivity and specificity to robustly quantitate ctDNA from blood, this approach has the potential to evaluate the multiple genomic targets required in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with intra-tumor and inter-tumor heterogeneity, and to prevent the time delays, costs and complications inherent in invasive biopsiesThe analytical and clinical validation of Guardant360 is conducted in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), REporting of tumor MARKer Studies (REMARK), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines [92-95]. **Methodology** **The gene panel was selected to focus on those genomic alterations that are currently actionable defined as being targets of sensitivity or resistance to an FDA-approved matched therapy and/or a targeted therapy in clinical trials. The test simultaneously sequences the 70 cancer-related genes to an average depth of coverage of greater than 8,000X. To summarize, cell-free DNA is extracted from plasma and genomic alterations are analyzed by massively parallel paired end synthesis-by-sequencing of amplified target genes utilizing an Illumina Next-Seq platform complemented by systematic end-to-end process optimization including conversion of cell-free DNA fragments into digital sequences, improvements in the Illumina next generation sequencing process itself, followed by bioinformatics algorithms which enable ctDNA to be measured as a quantitative percentage of total cell-free DNA.** **Two 10mls of whole blood are collected in Streck Cell-Free DNA Blood Collection (Streck) tubes, which contain a proprietary formaldehyde-free preservative in that stabilizes white blood cells, preventing the release of genomic DNA and allowing shipping and stability for seven days without need for refrigeration, cold bricks or preliminary centrifugation prior to shipping.** **After digital libraries are produced, the sample is sequenced and post-sequencing data is processed using bioinformatics algorithms to quantify the absolute number of unique DNA fragments at a given nucleotide position. This proprietary process is referred to as Digital Sequencing™ and enables**

reporting of the fractional concentration (mutant allele frequency) of a given SNV. Circulating cell-free DNA is mostly derived from leukocyte lysis (germline) and generally a much smaller amount of tumor DNA is derived from cancer cell apoptosis/necrosis. All of the cell-free DNA fragments, including leukocyte-derived and tumor-derived, are simultaneously sequenced with up to single molecule sensitivity. In other words, both tumor DNA and "normal"/germline DNA are sequenced and measured in the same sequencing assay. The fractional concentration or mutant allele frequency for a given mutation is calculated as the fraction of circulating tumor DNA harboring that mutation in a background of wild-type cell-free DNA fragments. The analytic sensitivity reaches detection of 1-2 single mutant cell-free DNA molecules from a 10 ml blood sample.

Gene list and genomic alterations in the Guardant360 Panel



Guardant360 Panel 2015

All NCCN Somatic Genomic Targets in a Single Test

POINT MUTATIONS - ***Complete*** or Critical Exon Coverage in 70 Genes*

AKT1	*ALK*	***APC***	***AR***	*ARAF*	***ARID1A***	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCDN1	***CCND2***	***CCNE1***	*CDH1*	***CDK4***	***CDK6***	***CDKN2A***	***CDKN2B***	*CTNNB1*	***EGFR***
ERBB2	*ESR1*	*EZH2*	*FBXW7*	***FGFR1***	***FGFR2***	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1*
MAP2K2	***MET***	*MLH1*	*MPL*	***MYC***	***NF1***	*NFE2L2*	*NOTCH1*	*NPM1*	***NRAS***
NTRK1	***PDGFRA***	***PIK3CA***	***PTEN***	*PTPN11*	***RAF1***	***RB1***	*RET*	*RHEB*	*RHOA*
RIT1	*ROS1*	*SMAD4*	*SMO*	*SRC*	*STK11*	*TERT*	***TP53***	*TSC1*	*VHL*

AMPLIFICATIONS

AR	*BRAF*	*CCNE1*	*CDK4*	*CDK6*	*EGFR*	*ERBB2*	*FGFR1*
FGFR2	*KIT*	*KRAS*	*MET*	*MYC*	*PDGFRA*	*PIK3CA*	*RAF1*

FUSIONS

ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

INDELS

EGFR exons 19/20	*ERBB2* exons 19/20	*MET* exon 14 skipping

GUARDANT HEALTH

RATIONALE: Added Guardant Health Assay language to describe the addition of cfDNA on the residual blood sample to optimize correlate information.

4.	References 88 - 95	**82**	**OLD TEXT:** N/A **NEW TEXT:** 88. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. PloS One. 2015;10: e0140712. doi:10.1371/journal.pone.0140712

			89. Zill OA, Greene C, Sebisanovic D, Siew L, Leng J, Vu M, et al. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. Cancer Discov. 2015; doi:10.1158/2159-8290.CD-15-0274 90. Ko AH, Bekaii-Saab T, van Ziffle J, Mirzoeva OK, Joseph N, Talasaz A, et al. A Multicenter, Open-Label Phase II Clinical Trial of Combined MEK plus EGFR Inhibition for Chemotherapy-Refractory Advanced Pancreatic Adenocarcinoma. Clin Cancer Res Off J Am Assoc Cancer Res. 2015; doi:10.1158/1078-0432.CCR-15-0979 91. Kim ST, Lee W-S, Lanman RB, Mortimer S, Zill OA, Kim K-M, et al. Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients. Oncotarget. 2015; online ahead of print 92. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Toward complete and accurate reporting of studies of diagnostic accuracy. The STARD initiative. Am J Clin Pathol. 2003;119: 18–22. doi:10.1309/8exc-cm6y-r1th-ubaf 93. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol. 2005;23: 9067–72. doi:10.1200/JCO.2004.01.0454 94. Teutsch SM, Bradley LA, Palomaki GE, Haddow JE, Piper M, Calonge N, et al. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative: methods of the EGAPP Working Group. Genet Med. 2009;11: 3–14. doi:10.1097/GIM.0b013e318184137c 95. Gargis AS, Kalman L, Berry MW, Bick DP, Dimmock DP, Hambuch T, et al. Assuring the quality of next-generation sequencing in clinical laboratory practice. Nat Biotechnol. 2012;30: 1033–6. doi:10.1038/nbt.2403 **RATIONALE:** Added references to correspond with Description of the Guardant Health Assay
5.	Protocol Type / Version #	**2**	**OLD TEXT:** ~~Amendment # 4/Version Date: March 2, 2016~~ **NEW TEXT:** **Amendment # 5/Version Date: April 12, 2016** **RATIONALE:** Updated Amendment date
6.	Headers	**1**	**OLD TEXT:** ~~March 2, 2016~~ **NEW TEXT:** **April 12, 2016** **RATIONALE:** Updated header date

NCI Protocol #: 9673

Local Protocol #: **NCI9673**

ClinicalTrials.gov Identifier: TBD

TITLE: A MULTI-INSTITUTIONAL PHASE 2 STUDY OF NIVOLUMAB IN REFRACTORY METASTATIC SQUAMOUS CELL CARCINOMA OF THE ANAL CANAL

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Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
P2C-IL057 / University of Chicago Comprehensive Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-MN026 / Mayo Clinic Cancer Center LAO
LAO-NC010 / Duke University - Duke Cancer Institute LAO
LAO-NJ066 / Rutgers University - Cancer Institute of New Jersey LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO

LAO-PA015 / University of Pittsburgh Cancer Institute LAO
LAO-NCI/National Cancer Institute LAO

Non-Member Collaborators

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NCI-Supplied Agent: Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC #748726)

IND #: TBD
IND Sponsor: DCTD, NCI
ClinicalTrials.gov Registration: Pending

Protocol Type / Version # / Version Date: Original/ Version Date: October 30, 2014
Amendment #1/Version Date : December 31, 2014
Amendment # 2/Version Date: January 27, 2015
Amendment # 3/Version Date: March 23, 2015
Amendment # 4/Version Date: March 2, 2016
Amendment # 5/Version Date: April 12, 2016

SCHEMA

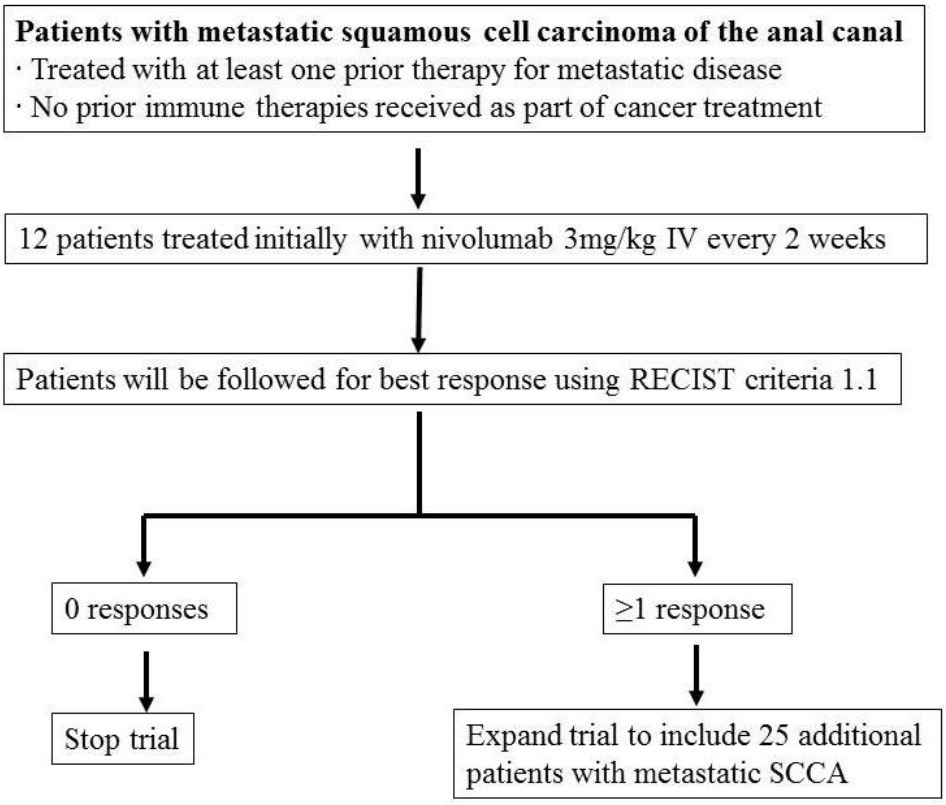

Patients with metastatic squamous cell carcinoma of the anal canal
· Treated with at least one prior therapy for metastatic disease
· No prior immune therapies received as part of cancer treatment
12 patients treated initially with nivolumab 3mg/kg IV every 2 weeks
Patients will be followed for best response using RECIST criteria 1.1
0 responses
≥1 response
Stop trial
Expand trial to include 25 additional patients with metastatic SCCA

TABLE OF CONTENTS

1 OBJECTIVES

1.1 Primary Objectives

To evaluate overall response rate (ORR) with nivolumab in patients with previously treated metastatic squamous cell carcinoma (SCCA) of the anal canal.

1.2 Secondary Objectives

1.2.1 To evaluate progression-free survival (PFS) of nivolumab in patients with previously treated metastatic SCCA of the anal canal.

1.2.2 To evaluate overall survival (OS) in patients with previously treated metastatic SCCA of the anal canal treated with nivolumab.

1.2.3 To evaluate the grade 3 and 4 toxicity rate in patients with previously treated metastatic SCCA of the anal canal when treated with nivolumab.

1.3 Exploratory Objectives

1.3.1 To evaluate ORR, PFS, and OS based on expression of PD-L1, PD-1, peritumoral CD_8^+ tumor infiltrating lymphoctyes (TILs), peritumoral CD_4^+ TILs, and regulatory T cells as analyzed from tumor biopsies in previously treated patients with metastatic SCCA of the anal canal when treated with nivolumab.

1.3.2 To evaluate radiographic responses according to relative changes in proportions of anti-HPV specific CD_8^+ and CD_4^+ TILs and regulatory T cells in patients with previously treated metastatic SCCA of the anal canal following treatment with nivolumab, analyzed from serial peripheral blood samples.

2 BACKGROUND

2.1 Squamous Cell Carcinoma of the Anal Canal

Squamous cell carcinoma (SCCA) of the anal canal accounts for an estimated 2% of all gastrointestinal malignancies in the US[1]. However, the annual incidence has risen steadily over the past two decades, with more than 7,000 new cases expected in the United States in 2014[2]. Initially considered an orphan malignancy, the standard treatment paradigm of concurrent chemoradiation has largely remained unchanged in the US for greater than 3 decades[3,4]. Approximately 25% of patients initially treated with chemoradiation will develop locally advanced disease and/or distant metastases[5-8], and an additional 10% of patients will be diagnosed with stage IV disease at initial presentation[9]. There is currently no standard treatment

approach for patients diagnosed with metastatic SCCA of the anal canal. Historically, chemotherapy regimens utilized in the treatment of these patients have largely been extrapolated from data in more common metastatic squamous cell carcinomas[10-14] (e.g., head and neck cancer and cervical cancer). A phase II international study has recently been initiated [InterAACT: C Eng (US Lead PI)] and is globally supported by the IRCI/NCI/EORTC/ECOG to identify the best chemotherapy backbone in treatment-naïve (HIV+ and HIV-) patients with SCCA of the anal canal). To that end, large academic institutions serve as major referral sources for this disease given that outside providers are limited by the paucity of treatment options available to this population of patients.

2.1.1 Nivolumab

Nivolumab (BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor[15]. PD-1 is a negative regulatory molecule that is expressed transiently following T-cell activation and on chronically stimulated T cells characterized by an "exhausted" phenotype. Nivolumab binds to cynomolgus monkey PD-1 but not mouse, rat, or rabbit molecules. Clinical activity of nivolumab has been observed in patients with melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC). The combination of nivolumab and ipilimumab (anti-cytotoxic T lymphocyte associated antigen-4 [anti-CTLA-4]) in a phase 1/2 trial showed markedly enhanced clinical activity with an acceptable safety profile in melanoma patients[16].

The clinical use of monoclonal antibodies to T-cell inhibitory receptors has provided transformative information on the nature of the immune system and cancer. An emerging picture suggests that endogenous immune responses can mediate effective tumor regression and/or improved survival even in patients with large volume tumors resistant to other forms of therapy. Some of the unique features of this type of therapy, based largely on experience in advanced melanoma, include: improved overall survival (OS) with or without radiographic responses or improved progression-free survival (PFS); responses that may be delayed or occur after radiographic disease progression; combinations of immune modulators with enhanced or novel activities (in the example of ipilimumab and nivolumab); and toxicity that is almost exclusively immune or inflammatory in nature. It is not yet clear what factors determine responses and which components of the immune system are needed for this to occur. It seems likely that both memory helper and effector cells would be needed to sustain long-term responses. Increasing emphasis has been placed on understanding the relationships of the tumor, cellular infiltrate, and immunologic milieu surrounding each tumor.

PD-1, a 55-kDa type 1 transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors that include Ig super family member CD28, CTLA-4, inducible co-stimulator (ICOS), and B and T lymphocyte attenuator (BTLA)[15]. PD-1 is transiently but highly expressed on activated T cells functioning to limit immune effectors at the site of activation. Chronic stimulation may prevent the re-methylation of the PD-1 gene leading to continuous expression and characterizes a state of "exhausted" T cells that lose function and proliferative capacity while enhancing a suppressive tumor microenvironment. PD-1 may act together with other T-cell modulating molecules, including CTLA-4, TIM-3, lymphocyte-activation gene 3

(LAG-3) as well as indoleamine-pyrrole 2, 3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta (TGF-beta).

Two ligands specific for PD-1 have been identified: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs], and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). The interaction of PD-1 with PD-L1 and PD-L2 results in negative regulatory stimuli that down-modulate the activated T-cell immune response through SHP-1 phosphatase.

PD-1 knockout mice develop strain-specific lupus-like glomerulonephritis (C57BL/6) and cardiomyopathy (BALB/c). In transplantable tumor models that expressed PD-1 and LAG-3 on tumor-infiltrating $CD4^+$ and $CD8^+$ T cells dual anti-LAG-3/anti-PD-1 antibody treatment cured most mice of established tumors that were largely resistant to single antibody treatment[17]. Despite minimal immunopathologic sequelae in PD-1 and LAG-3 single knockout mice, dual knockout mice abrogated self-tolerance with resultant autoimmune infiltrates in multiple organs, leading to eventual lethality.

PD-L1 expression is found on a number of tumors, and is associated with poor prognoses based on OS in many tumors, including melanoma[18], renal[19-21], esophageal[22], gastric[23], ovarian[24], pancreatic[25], lung[26], and other cancers[15].

The PD-1/PD-L1 axis plays a role in human infections, particularly in hepatitis C virus (HCV) and human immunodeficiency virus (HIV). In these cases, high expression levels of PD-1 were found in viral-specific $CD8^+$ T cells that also display a non-responsive or exhausted phenotype. Non-responsive PD-1-high T cells were observed in simian immunodeficiency virus (SIV) infection in rhesus macaques. Treatment of SIV-infected macaques with an anti-PD-1 mAb (3 mg/kg x4) resulted in decreased viral loads and increased survival along with expanded T cells with increased T-cell functionality.

2.1.1.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated[15]. Combination studies have highlighted the potential for toxicity when combined with ipilimumab, MDX-1408, and BMS-986016. Nivolumab bound specifically to PD-1 (and not to related members of the CD28 family such as CD28, ICOS, CTLA-4, and BTLA) with a K_d = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

2.1.1.2 Clinical Development of Nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC,

hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications[15]. In addition, two investigator-sponsored trials (ISTs) of nivolumab in combination with a peptide vaccine in melanoma are being conducted in the adjuvant setting and advanced disease.

Seven nivolumab studies were conducted in Japan, including six studies in advanced solid tumors and recurrent or unresectable stage III/IV melanoma sponsored by Ono Pharmaceuticals Co. Ltd., and one IST in recurrent or advanced platinum-refractory ovarian cancer.

2.1.1.2.1. Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (C_{max}) and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), with low to moderate inter-subject variability observed at each dose level[15]. Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

2.1.1.2.2. Efficacy

In a phase 1 (1, 3, and 10 mg/kg nivolumab doses) dose-escalation study the 3 mg/kg dose was chosen for expanded cohorts. Among 236 patients, objective responses (ORs) (complete or partial responses [CR or PR]) were seen in NSCLC, melanoma, and RCC. ORs were observed at all doses[27]. Median OS was 16.8 months across doses and 20.3 months at the 3 mg/kg dose. Median OS across all dose cohorts was 9.2 months and 9.6 months for squamous and non-squamous NSCLC, respectively[28]. In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year[29].

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered IV every 3 weeks for 4 doses followed by nivolumab alone every 3 weeks for 4 doses (concurrent regimen) [16]. The combined treatment was subsequently administered every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent regimen (53 patients), 53% of patients had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced-regimen (33 patients), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC patients, 43 patients were treated with nivolumab + PT-doublet[30]. No dose-limiting toxicities (DLTs) were reported and total/confirmed ORRs were 43/33%, 40/33%, and 31/31% in nivolumab/gemcitabine/cisplatin, nivolumab/pemetrexed/cisplatin, and

nivolumab/carboplatin/paclitaxel arms, respectively.

2.1.1.2.3. Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined[31]. Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, and vasculitis have also been reported.). In combination with ipilimumab in the concurrent-regimen group[16], grade 3 or 4 treatment-related events were noted in 53% of patients. Skin rash represents the majority of these events.

2.1.1.2.4. Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count[16]. With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell $CD8^+$ are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

2.2 **Rationale**

The development of SCCA of the anal canal is often triggered by enduring infection with high-risk strands of human papillomavirus (HPV), most commonly HPV-16 and HPV-18[32-35]. Approximately 75% of all sexually active adults will become infected with HPV during their lifetimes[36]. While most will clear infection of HPV without any required intervention[37], the virus may persist in a fraction of patients and cause cancer when viral DNA becomes incorporated into the host genome. The oncoproteins E6 and E7, translated products of HPV DNA integrated into the host cell, promote oncogenesis in mucosal squamous cells of anal canal through multiple mechanisms. For example, E6 binds p53 to generate a complex which results in ubiquitin-mediated p53 degradation[38-40]. Likewise, the interaction between E7 and the retinoblastoma protein Rb triggers a phosphorylation of Rb and allows continuation of cell cycle progression through the loss of tumor suppressor function by Rb[41-43].

The presence of high-risk HPV has been detected in approximately 75-95% of all reported cases of SCCA of the anal canal[32-35]. In addition, this virus has also been correlated to the development of other malignancies like SCCAs of the head and neck (over 70% of which are HPV-positive tumors), cervix (> 90%), penis (>60%), vulva (~70%), and vagina (75%) [44-51]. A clear association between altered immunity and the development of SCCA is apparent, and a functional immune system may be responsible for clearing HPV infection. Immunosuppression from HIV/AIDS, use of immunosuppressive medications following organ transplantation, and coexisting autoimmune disease are all recognized risk factors for developing SCCA[32,52-55]. Furthermore, the HPV-generated oncoproteins E6 and E7 are immunogenic and trigger an anti-tumor host immune response via activation of tumor infiltrating lymphocytes (TILs) [56-60]. These neoantigens are examples of "non-self" molecules recognized by T cells which are produced following viral incorporation into the host genome. However, suppression of such anti-tumor activity may allow a tumor to evade an immune response and survive in HPV-related malignancies. This notion has been verified in invasive cervical cancer, in which HPV-specific CD8+ cytotoxic T cells and CD4+ helper T-cells are lost and inhibitory HPV-specific regulatory T cells[61-63] become activated.

In a preventative trial, vaccination with a quadrivalent HPV vaccine (covering HPV-16) showed fewer cases of precancerous high-grade anal intraepithelial neoplasia when compared to people who received placebo, and no cases of invasive carcinoma[64]. Likewise, similar results in preventing development of pre-cancerous cervical dysplasia have been described by vaccinating young women with the quadrivalent HPV vaccine[56]. Despite such clinical promise, recent studies have suggested that fewer than 30% of eligible females in the United States have received the complete series of HPV vaccinations, and this proportion is even lower in males[65]. In underdeveloped countries, where the prevalence of HPV infection and HPV-associated malignancies are higher than in developed nations, providing access to these preventative vaccinations are currently in development, so that at present the majority of citizens do not receive these vaccines. In addition, patients with HPV-associated anogenital malignancies are exposed to an increased risk of developing a second HPV-related cancer from the effects of field cancerization caused by HPV infection[66]. Therefore, for these reasons it is expected that the incidence of SCCA of the anal canal will continue to rise and will continue to worsen in global prevalence for several decades.

Tumor cells express the programmed death receptor ligand-1 (PD-L1) as a means to down regulate T cell activation and thwart the local anti-tumor immune response by binding the inhibitory receptor programmed death-1(PD-1) on the surface of T cells[20,67]. Expression of PD-L1 on resected oral squamous cell tumors demonstrated a negative correlation with intratumoral CD8+ TIL density[68]. Additionally, in one study of HPV+ tumors of the oropharynx, 14 of 20 specimens (70%) demonstrated increased PD-L1 expression by immunohistochemistry (IHC), whereas only 2 of 7 (29%) HPV- tumors stained positive for PD-L1[69]. These results support the notion that HPV+ tumors may utilize the PD-1: PD-L1 interaction between the tumor cell and nearby T cell in order to disrupt an antitumor host immune response.

Nivolumab is a monoclonal antibody against PD-1. In vitro, disruption of PD-1: PD-L1

communication by nivolumab perpetuates T-cell activity against tumor cells[70,71]. When translated to a phase I study of nivolumab as a single agent, objective responses have been observed in over 20% of patients with refractory metastatic melanoma, non-small cell lung cancer, and renal cell carcinoma[31]. Additionally, among tumor specimens for which PD-L1 expression could be evaluated by IHC, a positive correlation between PD-L1 expression and response to nivolumab was detected, and no tumors that lacked PD-L1 expression demonstrated a response to nivolumab.

The prognostic impact of HPV in patients with metastatic SCCA of the anal canal remains unreported. In a series of 72 patients with metastatic SCCA of the anal canal who had undergone biopsy and/or surgical resection of their tumors at MD Anderson for survival outcomes,[72] tumors were tested for the presence of HPV using in-situ hybridization and by immunohistochemistry with monoclonal antibodies against P16, a protein regarded as a surrogate biomarker for HPV infection[73] with upregulated expression following loss of E7-mediated Rb function[74,75]. HPV was detected in 68 of the 72 patients (95%) analyzed. Per Figure 1, no differences in survival were measured between the HPV-positive and HPV-negative cohorts, although a trend towards improved survival was seen in the HPV-positive patients. This result may be attributed to an underpowering to detect true differences because the overwhelming majority of patients with metastatic SCCA of the anal canal treated at M.D. Anderson Cancer Center have detectable HPV.

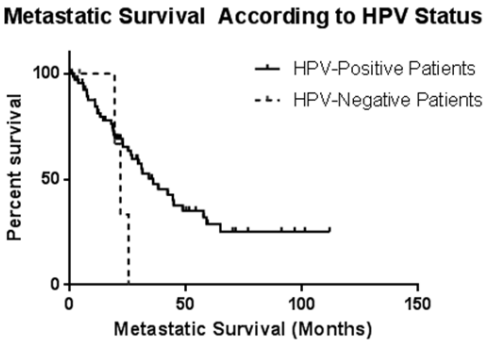


Figure 1: Median survival from the time of detection of metastatic disease was 35.9 months in the HPV-positive group and 27.1 months in the HPV-negative group (p-value = 0.11).

2.3 **Correlative Studies Background**

2.3.1 Role of PD-L1 and PD-1 in the response of SCCA of the anal canal to nivolumab

2.3.1.1 Rationale

While PD-L1 expression has been reported in a variety of solid tumors[20,76-79], no data exist describing the expression of this protein in SCCA of the anal canal.

2.3.1.2 Background

Unpublished data from a small cohort of surgically resected tumors from patients with metastatic SCCA of the anal canal was collected for PD-L1 expression using an assay at MD Anderson which had previously been optimized to detect successfully cell surface PD-L1 expression in HPV-positive cervical and penile cancers. Tumors were stained using a monoclonal antibody against PD-L1 (Epitomics; Burlingame, CA) and defined as "PD-L1 positive" if at least 5% of tumor cells demonstrated PD-L1 surface expression (based on criteria used in prior published studies with nivolumab)[31]. As exemplified in Figure 2, staining in PD-L1 was seen in two of the four (50%) tumors analyzed. However, all four tumors exhibited peritumoral lymphocytes, a

feature which has been associated with a more avid anti-tumor immune response in patients with other solid malignancies[80]. These data demonstrate that PD-L1 may be expressed in patients with HPV-positive metastatic SCCA of the anal canal.

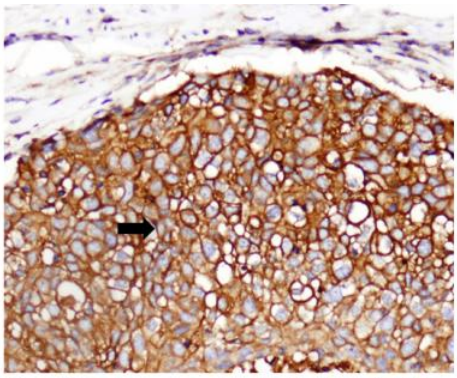


Figure 2: Using a monoclonal antibody against the PD-L1 protein, diffuse cell surface staining of PD-L1 (brown) is observed on the surfaces of tumors cells from a resected SCCA specimen. The arrow points to a representative single cell staining positive for PD-L1 around its entire membrane.

2.3.1.3 Hypothesis

Patients with metastatic SCCA of the anal canal will respond to therapy with nivolumab if their tumors stain positive for PD-L1 prior to treatment. Durable responses to therapy may be observed in patients whose tumors maintain PD-L1staining on immunohistochemistry after two cycles of therapy. Analogously, we hypothesize that the detection of PD-1, present on T-cells to interact with PD-L1, will also be associated with an anti-tumor response for patients treated with nivolumab.

2.3.2 Role of CD_8^+ Infiltrating T-cells in the response of SCCA of the anal canal to nivolumab

2.3.2.1 Rationale

The associations between the presence of CD_8^+ infiltrating T cells and (1) responses to nivolumab and (2) survival outcomes have never been described in a prospective study for patients with metastatic SCCA of the anal canal. This analysis would not only inform on the relevance of these immune cells in this particular disease for the first time, but could also be used in understanding further the role of CD_8^+ infiltrating T cells as a potential biomarker for immune checkpoint inhibitors like nivolumab across various HPV-related malignancies. The role of HPV-specific CD8+ T cells after nivolumab treatment may be of especial importance for clinical response. It will also be easy to assay since we already know their specificity (see 2.3.4. below).

2.3.2.2 Background

Tumor specimens from a series of 38 patients with locoregional, non-metastatic SCCA of the anal canal were reviewed retrospectively for a correlation between the presence of CD_8^+ infiltrating T cells[81]. No correlation between the higher numbers of these T cells and clinical outcomes were observed. However, patients with metastatic disease were not included in this analysis, and descriptions of an association between CD_8^+ infiltrating T cells, and not only overall survival in patients with metastatic SCCA of the anal canal but also responses to anti-PD-L1 therapy have not been described.

2.3.2.3 Hypothesis

Given the cytotoxic nature of CD_8^+ infiltrating T cells against tumors, we hypothesize that a positive correlation between CD_8^+ infiltrating T cells and response to nivolumab will be observed in this study. However, we recognize that the sample size for this phase II study is underpowered to detect a statistically relevant association, and therefore we plan to incorporate

these findings as the foundation for larger studies in the future.

2.3.3 Role of CD_4^+ Infiltrating T-cells in the response of SCCA of the anal canal to nivolumab

2.3.3.1 Rationale

The associations between the presence of CD_4^+ infiltrating T cells and (1) responses to nivolumab and (2) survival outcomes have never been described in a prospective study for patients with metastatic SCCA of the anal canal. This analysis would not only inform on the relevance of these immune cells in this particular disease for the first time, but could also be used in understanding further the role of CD_4^+ infiltrating T cells as a potential biomarker for immune checkpoint inhibitors like nivolumab across various HPV-related malignancies.

2.3.3.2 Background

A correlation between the presence of CD_4^+ infiltrating T-cells and improved clinical outcomes has been previously described in a cohort of patients with squamous cell carcinoma of the head and neck [82], an important finding given that a large proportion of these patients have tumors driven by HPV infection. However, whether or not an association may exist between the presence of CD_4^+ infiltrating T-cells and (1) response to nivolumab therapy and (2) survival outcomes remains unknown for patients with metastatic SCCA of the anal canal.

2.3.3.3 Hypothesis

Given that CD_4^+ infiltrating T-cells assist CD_8^+ infiltrating T-cells in maintaining an anti-tumor response in the tumor microenvironment, we hypothesize that patients with metastatic SCCA of the anal canal whose tumors contain CD_4^+ infiltrating T cells may respond to nivolumab in this study. However, we recognize that the sample size for this phase II study is underpowered to detect a statistically relevant association, and therefore we plan to incorporate these findings as the foundation for larger studies in the future. We will also study the potential of antigen-specific CD4+ T regulatory cells to decrease the response to nivolumab in this study (see 2.3.4., below).

2.3.4 Role of anti-HPV CD_8^+ TILs, CD_4^+ TILs and anti-HPV regulatory T cells in the response of SCCA of the anal canal to nivolumab

2.3.4.1 Rationale

While the presence of CD_8^+ and CD_4^+ infiltrating T-cells appear important in the anti-tumor response for HPV-associated malignancies, it is important to ensure that these immune cells are specific to tumor cells expressing immunogenic, viral neoepitopes introduced on the cell surface following incorporation of viral HPV DNA into the host cell genome.

2.3.4.2 Background

The presence of intratumoral anti-HPV T-cells has been described in other HPV-associated malignancies like cervical cancer and squamous cell carcinoma of the head and neck [63,82] and are associated with locoregional control of disease. However, any associations between the presence of immune cells specific to HPV-infected cells and clinical outcomes have not been described for patients with metastatic SCCA of the anal canal.

2.3.4.3 Hypothesis

We hypothesize that patients with metastatic SCCA of the anal canal whose blood samples show an increase in frequency of anti-HPV CD_8^+ and/or CD_4^+ infiltrating T-cells may respond to nivolumab in this study. Likewise, no change or a decrease in the frequency of anti-HPV CD_8^+ and/or CD_4^+ infiltrating T-cells may be associated with a lack of response to nivolumab for patients with previously treated metastatic SCCA of the anal canal. We also hypothesize that a negative correlation may exist between the presence of anti-HPV regulatory T cells and response to nivolumab. However, we recognize that the sample size for this phase II study is underpowered to detect a statistically relevant association, and therefore we plan to incorporate these findings as the foundation for larger studies in the future.

3 PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed previously treated metastatic squamous cell carcinoma of the anal canal.

3.1.2 Patients must have measurable disease according to the standard RECIST version 1.1. See Section 11 for the evaluation of measurable disease. CT scans or MRIs used to assess the measurable disease must have been completed within 28 days prior to study drug initiation.

3.1.3 Patients must have been treated with at least one prior systemic treatment for incurable advanced or metastatic SCCA of the anal canal. Prior treatment for metastatic disease is not required for patients who develop new metastatic lesions during or within 6 months of completion of chemoradiation for limited-stage disease. Patients who receive chemotherapy for incurable advanced or metastatic SCCA of the anal canal must wait a minimum $\geq$ 28 days (6 weeks for nitrosoureas or mitomycin C) after the date of completion of chemotherapy prior to initiating treatment with nivolumab on this study. Patients who undergo radiotherapy to a site of tumor must wait a minimum $\geq$ 3 months from the date of completion of radiotherapy prior to initiating treatment with nivolumab on this study.

3.1.4 Patients must be of age ≥18 years at the time of study registration. Because no dosing or adverse event data are currently available on the use of nivolumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.5 ECOG performance status 0 or 1 (Karnofsky ≥80%, see Appendix B).

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

- leukocytes ≥2,000/mcL
- absolute neutrophil count ≥1,500/mcL
- hemoglobin ≥ 9.0 gm/dL
- platelets ≥100,000/mcL
- total bilirubin ≤1.5× institutional upper limit of normal (ULN)
  (except patients with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)
- AST(SGOT)/ALT(SGPT) ≤ 2.5× ULN
- Serum creatinine ≤1.5× ULN
  OR
- creatinine clearance (CrCl) ≥50 mL/min (if using the Cockcroft-Gault formula below):

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

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

3.1.7 The effects of nivolumab on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab. Women must not be breastfeeding. Women who are not of childbearing potential (*i.e.*, who are postmenopausal or surgically sterile as well as azospermic men) do not require contraception.

Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes.

WOCBP receiving nivolumab will be instructed to adhere to contraception for a period of 23 weeks after the last dose of investigational product. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product.

These durations have been calculated using the upper limit of the half-life for nivolumab (25 days) and are based on the protocol requirement that WOCBP use contraception for 5 half-lives plus 30 days and men who are sexually active with WOCBP use contraception for 5 half-lives plus 90 days.

Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she (or the participating partner) should inform the treating physician immediately.

3.1.8 Ability to understand and the willingness to sign a written informed consent document.

3.1.9 Brain metastases are allowed if they have been adequately treated with radiotherapy or surgery and have been stable for at least three months prior to registration. Eligible subjects should be neurologically asymptomatic. There is no magnetic resonance imaging (MRI) evidence of progression for a minimum of 4 weeks after treatment is complete and within 28 days prior to the first dose of nivolumab administration. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (>10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.

3.1.10 All patients must be willing to undergo testing for HIV testing if not tested within the past 6 months.

3.1.11 If HIV+ positive, all patients infected with Human Immunodeficiency Virus (HIV) may be eligible for study provided that their CD4+ count $\geq 300/\mu L$; their viral load is undetectable; they are currently receiving Highly Active Antiretroviral Therapy (HAART).

3.1.12 All HIV+ patients will be under the care of an Infectious Diseases specialist. If a relationship with an Infectious Diseases specialist is not established, Infectious Disease specialist will be consulted. Records of all viral counts and peripheral T-cell counts must be sent to the Study Coordinator in order to follow these values over the course of treatment.

3.1.13 All patients must be willing to be tested for Hepatitis screening. Patients co-infected with hepatitis B virus and/or hepatitis C virus may be included in this study provided that their liver function tests remain within the limits listed above. Patients must be followed by a hepatologist during the course of this study.

3.2 **Exclusion Criteria**

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events (AEs) due to agents administered more than 4 weeks earlier (i.e., grade $\geq$ 2 AE present). Palliative (limited-field) radiation therapy is permitted, as long as the lesion being considered for palliative radiation is not a target lesion.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients should be excluded if they have had prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab.

3.2.5 History of severe hypersensitivity reaction to any monoclonal antibody.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including chronic prolonged systemic corticosteroids (defined as corticosteroid use of duration one month or greater), should be excluded. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or anti-phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease.

3.2.8 Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses ≤10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if ≤10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (*e.g.*, contrast dye allergy) or for treatment of non-autoimmune conditions (*e.g.*, delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

3.2.9 No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, *in situ* cervical cancer, adequately treated Stage I or II cancer from which the patient is currently in complete remission, or any other cancer from which the patient has been disease free for at least three years.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES)

4.1 Investigator and Research Associate Registration with CTEP

4.1.1 CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at http://ctep.cancer.gov/investigatorResources/investigator_registration.htm. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at pmbregpend@ctep.nci.nih.gov.

4.1.2 CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account is needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, and is critical to the conduct of this study, including document access, patient enrollment, and clinical data submission.

Additional information can be found on the CTEP website at http://ctep.cancer.gov/branches/pmb/associate_registration.htm. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at ctepreghelp@ctep.nci.nih.gov.

4.1.3 For Questions and Support

For questions about Investigator Registration, please contact the CTEP Investigator Registration Help Desk: pmbregpend@ctep.nci.nih.gov.

For questions about Associate Registration or CTEP-IAM Account Creation, please contact the CTEP Registration Help Desk: ctepreghelp@ctep.nci.nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain Institutional Review Board (IRB) approval for this protocol and submit all required regulatory documents (including any protocol specific documents) to the CTSU Regulatory Office before they can be approved to enroll patients.

The CTSU Regulatory Office tracks receipt of these documents in the CTSU Regulatory Support System (RSS), reviews for compliance, and transmits site approval data to CTEP.

Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not

required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing, or amendment review. However, sites must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB (via IRBManager) to indicate their intention to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office for compliance in the RSS. The Signatory site may be contacted by the CTSU Regulatory Office or asked to complete information verifying the participating institutions on the study. Other site registration requirements (*i.e.*, laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the NCI Protocol #9673 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the ETCTN link to expand, then select **Phase 2 Consortia**, followed by **P2C-TX035**, and **protocol #9673**.
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will automatically load to RSS.)

4.2.2 Submitting Regulatory Documents

Submit completed forms along with a copy of your IRB Approval to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-866-651-2878
Fax: 215-569-0206
E-mail: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

4.2.3 Checking **Site** Registration Status

Sites can check the status of their registration packets by querying the Site Registration subtab of the members' section of the CTSU Web site. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password.
- Click on the Regulatory tab at the top of your screen.
- Click on the Site Registration subtab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: If possible, please allow three working days for site registration approval before attempting to enroll your first patient.

4.3 Patient Registration

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

For trials with slot reservation requirements, OPEN will connect to IWRS at enrollment initiation to check slot availability. Registration staff should ensure that a slot is available and secured for the patient before completing an enrollment.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar.
- To approve slot reservations or access cohort management: Be identified to Theradex as the "Client Admin" for the study.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the CTSU web site as a tool to verify eligibility.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://theradex.com/CTMS/Downloads.aspx. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk: 609-619-7802 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5 TREATMENT PLAN

5.1 Treatment

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1.1 Nivolumab

There are no premedications recommended for nivolumab on the first dose. Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to Section 5.8.

5.1.2 Other Modalities or Procedures

N/A

5.2 Nivolumab Administration

Nivolumab will be given intravenously every two weeks (±3 days) at a dose of 3 mg/kg. Patients may be dosed no fewer than 12 days from the previous dose of drug.

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

Nivolumab is to be administered as a 60-minute IV infusion (a window of +/- 5 minutes is allowed), using a volumetric pump with a 0.2/1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be below 1 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

5.2.1 Other Modality(ies) or Procedures

N/A

5.3 General Concomitant Medication and Supportive Care Guidelines

Although there is not a potential for interaction of nivolumab with other concomitantly administered drugs through the cytochrome P450 system, the case report form must still capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) which include the following (see also section 6 and specific algorithms in Appendix E):
  - Any grade 4 events except as noted below.
  - Grade 3 drug-related autoimmune or inflammatory events including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation except as noted below:

:

- Any other grade 3 non-skin, drug-related AE lasting < 7 days including fatigue.

- Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, not associated with underlying organ pathology that does not require treatment except for electrolyte replacements **does not** require treatment discontinuation.

- Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation or clinical manifestations of pancreatitis and which decrease to ≤ Grade 2 within 1 week of onset **may** resume study treatment when resolved.

- Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.

- Patients requiring > two dose delays for the same type of event should go off protocol therapy.

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued study drug dosing

- Any dosing interruption lasting > 6 weeks, with the following exceptions:

  - Patients being tapered after high dose corticosteroids over one month followed by a two-week observation period will be allowed an additional two weeks to restart treatment (a maximum eight week interruption). Dosing interruptions >6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Principal Investigator must be consulted.

- Tumor assessments should continue as per protocol even if dosing is interrupted.

- Any patients who require additional immune suppressive treatment beyond steroids should go off study treatment

- Patient decides to withdraw from the study, or

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.5 Duration of Follow Up

All patients will be followed for adverse events for 100 days after last dose of nivolumab. Patients who discontinue treatment for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. If a patient stops treatment due to unacceptable adverse event(s) but has not demonstrated disease progression, then the patient will be followed with imaging studies every 6 weeks until the time of progression radiographically according to RECIST 1.1 criteria. In the event that a radiographic response is detected, then this event will be included as a response in the final analysis, and the time of progression used in calculation of the survival analysis.

Patients will be followed for survival status every 3 months for 2 years after treatment discontinuation or until death, whichever occurs first.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the applicable criteria, including progressive disease, adverse events, patient withdrawal or inability to follow study protocol as listed in Section 5.4. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.7 Criteria to Resume Treatment

Some patients may continue to benefit from treatment, maintaining or improving responses after progression including those treated with steroids.

Restarting applies only to grade 2 events and some grade 3 events (skin rash and thyroiditis).

For non-autoimmune or non-inflammatory events patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of Grade 2 fatigue
- Evaluation to exclude any additional immune mediated events endocrine, GI, and liver / pancreas function as clinically indicated must be made prior to restarting.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol the treatment should resume at the earliest convenient point that is within the six week delay period.

If treatment is delayed for >6 weeks, (>8 weeks for patients on a steroid taper), the patient must be permanently discontinued from study therapy, except as specified in Section 5.4 (Duration of

Therapy).

For patients treated with corticosteroids:

Grade 2 events must resolve to ≤ Grade 1 before considering retreatment.

All patients treated with steroids for grade ≥2 events should have nivolumab held until resolution to ≤ Grade 1 for at least 2 weeks following complete removal from steroid treatment except for maintenance replacement doses for adrenal insufficiency (preferably no greater than 10mg prednisone equivalent daily).

All patients treated with steroids for grade ≥3 events should have nivolumab discontinued. Patients with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Patients with hepatitis, pancreatitis, pneumonitis, and colitis are at risk for exacerbation with retreatment if there is residual inflammation and should resolve to Grade 0 or baseline before retreatment. Baseline can mean the initial grade *i.e.* grade <1 where permitted on study.

Patients with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses only of corticosteroids. Please note that grading and for hypophysitis with symptoms of headache, visual or neurologic changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.

New immune related events or exacerbation of existing events during steroid treatment or taper suggest the presence of ongoing immune activation and should require permanent discontinuation of nivolumab.

A patient who is treated with steroids, evaluated, and found to not have an autoimmune or inflammatory event requiring steroid treatment, may be restarted if asymptomatic off steroids for 2 weeks and other restarting criteria are met.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be drawn if clinically feasible to document baseline function and distinguish the pituitary from peripheral organ dysfunction and later from steroid (or thyroid) treatment associated ACTH (or TSH) suppression. Steroids should be started prior to obtaining results based on clinical indications.

5.8 Treatment 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, urticaria, angioedema, 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 version 4.0 guidelines.

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

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

Remain at bedside and monitor subject until recovery from symptoms. Infusion rate may be slowed. 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 patient closely.

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, slowing infusion rate as above.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [*e.g.*, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; close observation for recurrence and treatment medications may need to be continued for 24-48 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 patient 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 patient 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 patient 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 (acetaminophen) (or paracetamol) 325 to 1000 mg should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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

Grade 4 symptoms: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed.

Patient 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 patient until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (*e.g.*, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (*e.g.*, oral antihistamine, or corticosteroids).

Please note that late occurring events including isolated fever and fatigue may represent the presentation of systemic inflammation. Please evaluate accordingly.

5.9 Treatment Beyond Progression

A minority of subjects treated with immunotherapy may derive clinical benefit either delayed responses, stable disease, or increased overall survival despite initial evidence of progressive disease (PD) with nivolumab.

Patients may be permitted to continue treatment beyond initial RECIST 1.1-defined PD occurring during the initial treatment period (up to 12 weeks) as long as they meet the following criteria:

- No more than 4 new lesions, total sum of the longest diameter (SHORT diameter for LN) cannot exceed 40% of the initial sum including new lesions
- Patients must be clinically stable with no change in performance status due to disease progression
- No indication for immediate alternative treatment
- Patient [assessed by the investigator] is showing clinical benefit and tolerates study drug. The assessment of clinical benefit should take into account whether the subject is clinically stable or deteriorating and likely or unlikely to receive further benefit from continued treatment.
- The time of progression is noted from the first assessment that exceeds standard criteria

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase

in short axis to at least 15 mm).

Patients are allowed to continue treatment for 2-3 additional doses (4-6 weeks) and reassessed. Treatment may continue up to an additional 10% or 30% total single diameter increased over baseline. New measureable lesions are not permitted with this schema.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dosing Modifications

There will be no dose modifications allowed for management of toxicities.

6.2 Dosing Delays

ALL OTHER EVENTS*	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1 continue at investigator discretion
Grade 4	Off protocol therapy
* **Not agent related**, or agent related non-immunologically mediated	
Recommended management: As clinically indicated	
ALL OTHER EVENTS**	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline* When resolved < or following steroids resume at same dose level .
Grade 3	Off protocol therapy (exceptions noted in 5.4)
Grade 4	Off protocol therapy
* *immunologically mediated	
Recommended management: As clinically indicated	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
Grade 1-2	Refer to Skin Adverse Event management algorithm (Appendix D).
Grade 3	Delay nivolumab therapy. Refer to Skin Adverse Event management algorithm (Appendix D).
Grade 4	Off protocol therapy
*Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
Rule out non-inflammatory causes. If non-inflammatory cause, then treat accordingly and continue nivolumab therapy.	

Renal Function	**Management/Next Dose for Nivolumab**
Grade 1-2	Refer to Renal Adverse Event management algorithm (Appendix D).
Grade 3	Delay nivolumab therapy. Refer to Renal Adverse Event management algorithm (Appendix D).
Grade 4	Off protocol therapy
Liver Function (AST, ALT, total bilirubin)	**Management/Next Dose for Nivolumab**
Grade 1	Refer to Hepatic Adverse Event management algorithm (Appendix D).
Grade 2	Delay nivolumab therapy. Refer to Hepatic Adverse Event management algorithm (Appendix D).
Grade 3	Delay nivolumab therapy. Refer to Hepatic Adverse Event management algorithm (Appendix D).
Grade 4	Off protocol therapy.
Continued treatment of active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug to evaluate LFT changes and to rule out non-inflammatory causes. Early treatment is recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
Grade 1	Refer to GI Adverse Event management algorithm (Appendix D).
Grade 2	Delay nivolumab therapy. Refer to GI Adverse Event management algorithm (Appendix D).
Grade 3	Delay nivolumab therapy. Refer to GI Adverse Event management algorithm (Appendix D).
Grade 4	Off protocol therapy.
See GI AE Algorithm for management of symptomatic colitis. Patients with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: see GI AE management Algorithm	

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab**
Grade 1	Hold dose until grade 0

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab**
Grade 2	Hold dose until Grade 0. Resume at same dose level if asymptomatic.
Grade 3-4	Hold* dose until Grade 0. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or DM should be taken off treatment. *
*Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm	

Pneumonitis	**Management/Next Dose for Nivolumab**
Grade 1	Hold dose pending evaluation and resolution to Grade 0 or baseline including baseline pO2. Refer to Pulmonary Adverse Event management algorithm (appendix D).
Grade 2	Hold dose pending evaluation. Refer to Pulmonary Adverse Event management algorithm (appendix D).
Grade 3	Hold dose pending evaluation. Refer to Pulmonary Adverse Event management algorithm (appendix D).
Grade 4	Off protocol therapy.
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza killed vaccine for all patients.	
Recommended management: See Pulmonary Adverse Event Management Algorithm	

Other GI Nausea/Vomiting	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	Hold pending evaluation for gastritis duodenitis and other immune adverse events or other causes. Resume at same dose level after resolution to ≤ Grade 1.
Grade 3	Hold pending evaluation until ≤ Grade 1. Resume at same dose level. If symptoms do not resolve within 7 days with symptomatic treatment patients should go off protocol therapy
Grade 4	Off protocol therapy
Patients with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.	

Fatigue	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 2. Resume at same dose level
Grade 4	Off protocol therapy
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Neurologic events	**Management/Next Dose for Nivolumab**
Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose. * Refer to Neurological Adverse Event management algorithm (Appendix D).
Grade 2	Hold dose pending evaluation and observation.* Refer to Neurological Adverse Event management algorithm (Appendix D).
Grade 3-4	Off protocol therapy
*Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, or myasthenia gravis should be off study.	
Recommended management: See Neurologic Adverse Event Management Algorithm	

Endocrine Hypophysitis or Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
Grade 1-2	Asymptomatic TSH elevation * Hold pending evaluation, endocrine consult . Refer to Endocrinopathy Adverse Event management algorithm (Appendix D).
Grade 3-4	Off protocol therapy
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm	

Fever	**Management/Next Dose for Nivolumab**
≤ Grade 1	Continue nivolumab at same dose. If no improvement after 5-7 days or

Fever	**Management/Next Dose for Nivolumab**
	worsening, then treat as Grade 2.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	
See section 5. infusion reactions	

If treatment is delayed >6 weeks (>8 weeks for patients on high dose steroids) with recommended 4 weeks taper and 2 week observation, the patient must be permanently discontinued from study therapy, except as specified in Section 5.7 (Criteria to Resume Treatment.)

Patients requiring a delay of >6 weeks (>8 weeks for patients on high dose steroids with required 4 weeks minimal taper and 2 week observation), should go off protocol therapy.
Patients requiring > two dose delays for the same event should go off protocol therapy.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be obtained to document baseline.

Patients may be dose-delayed for evaluation and restarted depending on results.

Any patient started on corticosteroids initially who is determined to not require steroids treatment for an autoimmune adverse event may resume therapy after a 2 week observation period without further symptoms at the discretion of the PI or investigator.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously.

7.1.1 CAEPR for CTEP IND Agent Nivolumab

7.1.1.1 CAEPR for Nivolumab

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
BMS-936558 (Nivolumab, MDX-1106, NSC 748726)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2069 patients.* Below is the CAEPR for BMS-936558 (Nivolumab, MDX-1106).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.1, December 11, 2015[1]

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 4.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency		
	Endocrine disorders - Other (hypophysitis)		
	Hyperthyroidism		
	Hypothyroidism		
EYE DISORDERS			
		Eye disorders - Other (diplopia)	
		Eye disorders - Other (Graves ophthalmopathy)	

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 4.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Eye disorders - Other (optic neuritis retrobulbar)	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis		
		Colonic perforation	
	Diarrhea		***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
		Gastritis	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[3]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
		Infusion related reaction[4]	
	Injection site reaction		***Injection site reaction (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	
		Autoimmune disorder[5]	
		Cytokine release syndrome[6]	
		Immune system disorders - Other (sarcoid granuloma)[5]	
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
		Musculoskeletal and connective tissue disorder - Other (polymyositis)	

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 4.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Musculoskeletal and connective tissue disorder - Other (rhabdomyolysis)	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy	
		Facial nerve disorder[5]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (Guillain-Barre syndrome)[5]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)	
		Nervous system disorders - Other (myasthenia gravis)[5]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion		
	Pneumonitis		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	
	Pruritus		***Pruritus (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Skin hypopigmentation		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail

[2]Pericardial tamponade may be related to possible inflammatory reaction at tumor site.

[3]Pancreatitis may result in increased serum amylase and/or more frequently lipase.

[4]Infusion reactions, including high-grade hypersensitivity reactions which have been observed following administration of nivolumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of nivolumab.

[5]BMS-936558 (Nivolumab, MDX-1106) being a member of class of agents involved in the inhibition of "immune checkpoints", may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[6]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopen

Adverse events reported on BMS-936558 (Nivolumab, MDX-1106) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that BMS-936558 (Nivolumab, MDX-1106) caused the adverse event:

CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Heart failure; Pericarditis; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Vestibular disorder
ENDOCRINE DISORDERS - Endocrine disorders - Other (autoimmune thyroiditis); Endocrine disorders - Other (hypopituitarism)
EYE DISORDERS - Eye disorders - Other (iridocyclitis); Optic nerve disorder
GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Enterocolitis; Flatulence; Gastrointestinal disorders - Other (mouth sores); Mucositis oral; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Pain
HEPATOBILIARY DISORDERS - Bile duct stenosis; Hepatobiliary disorders - Other (autoimmune hepatitis)
IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (limbic encephalitis)
INFECTIONS AND INFESTATIONS - Bronchial infection; Encephalitis infection; Lung infection; Sepsis; Upper respiratory infection
INVESTIGATIONS - Alkaline phosphatase increased; CPK increased; GGT increased; Investigations - Other (blood LDH increased); Investigations - Other (CRP increased); Investigations - Other (eosinophil count increased); Investigations - Other (protein total decreased); Investigations - Other (thyroxine free increased); Investigations - Other (tri-iodothyronine free decreased); Investigations - Other (WBC count increased); Lymphocyte count increased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Musculoskeletal and connective tissue disorder - Other (musculoskeletal pain); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (histiocytic necrotizing lymphadenitis)
NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage; Nervous system disorders - Other (autoimmune neuropathy); Stroke
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (nephritis); Renal and urinary disorders - Other (tubulointerstitial nephritis)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (interstitial lung disease); Respiratory, thoracic and mediastinal disorders - Other (lung infiltration); Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pain of skin; Periorbital edema; Photosensitivity; Rash acneiform; Skin and subcutaneous tissue disorders - Other (rosacea);

Toxic epidermal necrolysis
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Vasculitis

Note: BMS-936558 (Nivolumab, MDX-1106) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **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.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The Coordinating Center of the Corresponding Organization is responsible for submitting to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days

Not resulting in Hospitalization ≥ 24 hrs	Not required	10 Calendar Days	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

N/A

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol. For this trial the Adverse Event CRF is used for routine AE reporting in Rave. Submission of the pathology report is not required.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

7.7 Safety Monitoring Plan

All participants will be carefully followed for safety. Participants are seen by their study doctor and research nurse before each dose of nivolumab (every 2 weeks). Safety evaluations at this time include a physical exam, vital signs, performance status assessment, and safety laboratory tests. The study team will continuously monitor participants for treatment side effects. Participants are instructed to inform their study doctor right away if they notice or feel anything different so the study doctor can check for side effects. The study doctor may be able to provide treatment for side effects. The study doctor may temporarily hold the study drug to reduce side effects. The study doctor will permanently stop the study drug if side effects are too severe and/or long lasting. All participants will be followed for side effects for 100 days from their last dose of nivolumab. Participants with ongoing side effects will continue to be followed until resolution or stabilization of the side effects. Because it is not known if nivolumab will be effective against anal cancer, to enrollment will stop after 12 participants are treated with nivolumab if none of them have their tumors shrink. Study team conferences will be held monthly or more frequently if needed.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent

8.1.1 Nivolumab (NSC 748726)

Amino Acid Sequence: 4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

Other Names: BMS-936558, MDX1106

Classification: Anti-PD-1MAb

M.W.: 146,221 daltons

Mode of Action: Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), pH 6.0.

How Supplied: Nivolumab is supplied by Bristol-Myers Squibb and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as 100 mg vials (10 mg/mL) with a 0.7 mL overfill. It is supplied in 10 mL type I flint glass vials, with butyl rubber stoppers and aluminum seals.

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 1 mg/mL. Vial contents from different lots should not be mixed in the same infusion.

Storage: Vials of nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing, and shaking.

Stability: Shelf-life surveillance of the intact vials is ongoing.

The administration of undiluted and diluted solutions of nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 4-hour period under room temperature and room light conditions includes the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after

initial entry.

Route of Administration: Intravenous infusion. Do not administer as an IV push or bolus injection.

Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter.

Potential Drug Interactions: No incompatibilities between nivolumab injection and polyvinyl chloride (PVC), non-PVC/non-DEHP (di[2-ethylhexyl]phthalate) IV components, or glass bottles have been observed.

Availability

Nivolumab is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Nivolumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Integral Laboratory or Imaging Studies

Not applicable

9.2 Integrated Correlative Studies

Not applicable

9.3 Exploratory Correlative Studies

9.3.1 Exploratory Studies – Methodology

Providers must offer and encourage optional tissue biopsies for tumor tissue for conduction of exploratory studies to all patients on this trial. Blood collections for correlative studies will be mandatory. Tumor tissue and blood samples will be collected for immune monitoring as previously published[83-86], under the supervision of the Immunotherapy Platform at MD Anderson. In tumor tissues, immunohistochemical studies will be performed to evaluate CD_4^+ and CD_8^+ T cells and regulatory T cells. In peripheral blood, we will also evaluate T cell populations including, but not limited to, CD4 cells, CD_8^+ cells, and regulatory T cells in pre- and post-therapy samples.

Peripheral blood
Up to 100 mL (within 24 hours) of peripheral blood will be collected for testing of biomarkers described in this clinical protocol at the following time points:

- At screening (any time prior to first dose of nivolumab)
- Before doses 2, 4, and 6
- At treatment discontinuation

For those patients who come off study due to reasons other than progression while on treatment (e.g., excessive toxicity, prolonged treatment break, withdrawal of consent), blood at the time of treatment discontinuation will not be required. The patient's hemoglobin concentration must be ≥ 10.0 g/dL in order for the blood sample to be collected. The treating physician or designee will have the option to cancel the laboratory protocol collection for patient safety without protocol deviation.

Please refer to Appendix E For additional details regarding sample analyses.

9.3.2 Blood Processing and Specimen Storage Management

The MD Anderson Immunotherapy Platform (IMT) has worked on a number of methods testing different factors affecting the yield and quality of peripheral blood mononuclear cells (PBMCs) from blood samples of cancer patients. We have arrived at an optimal method that involves collecting blood in heparinized "Green Top" tubes, processing the blood within 24 hours of the blood draw and diluting the blood 5 times with D-PBS. All samples are maintained at ambient temperature (room temperature) during transport and processing up until freezing or cell

cryopreservation. This method ensures maximum PBMC recovery, especially with patients who are lymphopenic.

An important aspect of our PBMC isolation and manipulation of cells, after thawing for experiments, is the use of an automated viable cell counting instrument (Cellometer; Nexcelom Bioscience, USA) instead of a hemocytometer. This instrument has been shown to be essential for consistent cell counting. We have also tracked the viability of the PBMC after thawing samples processed and frozen from melanoma patients to verify the quality of our cryopreservation techniques. The viabilities have been consistently good (80%-98%) after thawing. At the same time, we have also monitored the time periods between blood draws and processing in the lab and found that shipping cells at room temperature (25^{o}C) greatly improves cell recovery when processing is carried out within 24 hours.

We have on hand an interactive web-based and powerful software system developed with Aptia Systems (Houston, TX) to track all the cryopreserved cell and serum samples in this trial. The software, called the "Visual Specimen Manager" or "VSM" for short, has its own independent server, backed up hourly, and allows each authorized user to place in or take out vials of samples in a Windows-based interface. It is CFR 21 Part 11 compliant, with only specific users allowed access with passwords. The VSM system is capable of storing all relevant information on each sample stored by simply moving over it with a mouse. In addition, it allows rapid vial labeling and identification of samples by automatically generating a unique bar code. It can also print the specimen information on a cryolabel as the user enters it in a set template. The bar code system then allows for quick identification and localization of the specimens. The system works for liquid nitrogen (LN) tanks as well as -80 freezers.

Immune monitoring screening for blood specimens is mandatory for this study and must occur:
(1) Pre-Treatment: Within 14 days prior to treatment start.
(2) During Treatment: Before nivolumab treatment doses 2, 4, and 6.
(3) At study discontinuation (for patients who come off study due to disease progression while on treatment).

Kits for the immune monitoring part of the protocol will be shipped by the MD Anderson Immunotherapy Platform (IMT) to the enrolling institution prior to enrollment. A total of 2 kits will be sent. All kits are for blood draws as per the protocol and are interchangeable from patient to patient. Samples must be processed as per the immune-specific processing instructions below. The collection, processing, and shipping conditions must be followed.

NOTE: Samples must be shipped to the IMT during the weekdays (Monday-Thursday morning) by overnight priority shipping/courier at room/ambient temperature (20-30 ^{o}C). Blood and serum should not be collected from Thursday afternoon through Friday to avoid an over-the-weekend delay in receipt by the IMT that will compromise sample quality. **Under no circumstances can immune monitoring blood products collected at any site be frozen or placed in any fixative reagent.**

9.3.3 Due to the limitations of fresh tissue sampling in this high impact study, residual blood samples from MDACC and from our ETCTN collaborators will be considered for a

noninvasive approach in mutation analysis to optimize all available information we have from all collaborators. For the purpose of this analysis, all correlative blood work is exploratory and will not be used for the purpose of treatment decision-making.

9.3.4 **Description of the Guardant Health Assay**

Guardant360 is a next-generation sequencing (NGS) panel of 70 clinically actionable onco- and tumor suppressor genes utilizing digital sequencing of cell-free circulating tumor DNA (cfDNA) isolated from a simple, non-invasive blood draw. It is medically indicated for the prevention of a repeat invasive biopsy in advanced cancer patients when the initial biopsy is insufficient (QNS) or unavailable/unobtainable as well as when cancer has progressed or recurred despite treatment. The test detects single nucleotide variants via complete exon sequencing in 70 genes, copy number amplifications in 16 genes, small indels in EGFR, ERBB2 and MET exon 14 skipping, and fusions in ALK, FGFR2, FGFR3, RET, ROS1 and NTRK1. The genes are selected because mutations in these genes have FDA-approved matched therapies or are eligible for late phase clinical trials, as well as non-druggable genes with high prevalence alterations that may be helpful in monitoring for molecular response/non-response such as TP53. The panel also includes genomic markers of acquired resistance that may require a change in pharmacotherapy, e.g. EGFR T790M, ALK or ESR1 mutations.

Guardant360 is an advanced diagnostic laboratory test (ADLT) offered by a sole source laboratory certified by the Clinical Laboratory Improvement Amendments (CLIA) for high complexity (molecular pathology) testing and accredited by the College of American Pathology (CAP). Due to high rates of false positives with traditional NGS assays when tumor DNA is in low concentrations, the majority of "liquid biopsy" methods interrogating cell-free DNA have been limited to hotspot analyses. In contrast, the ultra-high specificity (> 99.9999%) of the digital sequencing method enables the sequencing of long, targeted regions (146,000 base pairs) without false positives. Complete exons are sequenced for all exons in 30 genes and the critical exons (those reported as having a somatic mutation in COSMIC) in 40 additional genes. Thus, its key differentiating characteristic from other "liquid biopsy" methods is the ability to sequence complete exons in many genes, in contrast to gene hotspot testing.

Advantages of the Guardant360 cell-free DNA (cfDNA) NGS methodology versus solid tumor tissue-based NGS are:

1. An invasive needle or surgical biopsy is avoided with cfDNA, reducing costs and complications.
2. CfDNA provides a quantitative measure (concentration or mutant allele frequency) of mutations present whereas solid tumor biopsy typically provides a qualitative result (mutation either present or not detected). The quantitative cfDNA result may be followed over time to monitor response to treatment and evolution of acquired resistance.
3. CfDNA sequencing identifies both germline and somatic mutations in the same sample.
4. The assay failure rate is for cfDNA is less than 0.5% (in the first 9,000+ samples) compared to 15%-25% failure rates of tissue-based NGS related to insufficient quantity of tissue (QNS).

Guardant360 utilizes algorithmic methods to encode and ultimately decode inputs and outputs from massively parallel deep sequencing analysis. By leveraging signal transduction processing technology where voice or image data is digitally encoded before transmission and then decoded post-transmission, this NGS method, known as Digital SequencingTM, enables signal interference to be reduced by two orders of magnitude or more [88]. Four validation studies have been published with concordance to tissue biopsy-based genomic testing [88-91]. With high enough sensitivity and specificity to robustly quantitate ctDNA from blood, this approach has the potential to evaluate the multiple genomic targets required in NCCN guidelines, to act as a "summary" of the different tumor clones in patients with intra-tumor and inter-tumor heterogeneity, and to prevent the time delays, costs and complications inherent in invasive biopsiesThe analytical and clinical validation of Guardant360 is conducted in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy (STARD), REporting of tumor MARKer Studies (REMARK), Evaluation of Genomic Applications in Practice and Prevention (EGAPP), and the recent Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) biomarker guidelines [92-95].

Methodology
The gene panel was selected to focus on those genomic alterations that are currently actionable defined as being targets of sensitivity or resistance to an FDA-approved matched therapy and/or a targeted therapy in clinical trials. The test simultaneously sequences the 70 cancer-related genes to an average depth of coverage of greater than 8,000X. To summarize, cell-free DNA is extracted from plasma and genomic alterations are analyzed by massively parallel paired end synthesis-by-sequencing of amplified target genes utilizing an Illumina Next-Seq platform complemented by systematic end-to-end process optimization including conversion of cell-free DNA fragments into digital sequences, improvements in the Illumina next generation sequencing process itself, followed by bioinformatics algorithms which enable ctDNA to be measured as a quantitative percentage of total cell-free DNA.

Two 10mls of whole blood are collected in Streck Cell-Free DNA Blood Collection (Streck) tubes, which contain a proprietary formaldehyde-free preservative in that stabilizes white blood cells, preventing the release of genomic DNA and allowing shipping and stability for seven days without need for refrigeration, cold bricks or preliminary centrifugation prior to shipping.

After digital libraries are produced, the sample is sequenced and post-sequencing data is processed using bioinformatics algorithms to quantify the absolute number of unique DNA fragments at a given nucleotide position. This proprietary process is referred to as Digital SequencingTM and enables reporting of the fractional concentration (mutant allele frequency) of a given SNV. Circulating cell-free DNA is mostly derived from leukocyte lysis (germline) and generally a much smaller amount of tumor DNA is derived from cancer cell apoptosis/necrosis. All of the cell-free DNA fragments, including leukocyte-derived and tumor-derived, are simultaneously sequenced with up to single molecule sensitivity. In other words, both tumor DNA and "normal"/germline DNA are sequenced and measured in the same sequencing assay. The fractional concentration or mutant allele frequency for a given mutation is calculated as the fraction of circulating tumor DNA harboring that mutation in a background of wild-type cell-free DNA fragments. The analytic sensitivity reaches detection of 1-2 single mutant cell-free DNA molecules from a 10 ml blood sample.

Gene list and genomic alterations in the Guardant360 Panel

Guardant360 Panel 2015

All NCCN Somatic Genomic Targets in a Single Test

POINT MUTATIONS - ***Complete*** or Critical Exon Coverage in 70 Genes*

AKT1	*ALK*	***APC***	***AR***	*ARAF*	***ARID1A***	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCDN1	***CCND2***	***CCNE1***	*CDH1*	***CDK4***	***CDK6***	***CDKN2A***	***CDKN2B***	*CTNNB1*	***EGFR***
ERBB2	*ESR1*	*EZH2*	*FBXW7*	***FGFR1***	***FGFR2***	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1*
MAP2K2	***MET***	*MLH1*	*MPL*	***MYC***	***NF1***	*NFE2L2*	*NOTCH1*	*NPM1*	***NRAS***
NTRK1	***PDGFRA***	***PIK3CA***	***PTEN***	*PTPN11*	***RAF1***	***RB1***	*RET*	*RHEB*	*RHOA*
RIT1	*ROS1*	*SMAD4*	*SMO*	*SRC*	*STK11*	*TERT*	***TP53***	*TSC1*	*VHL*

AMPLIFICATIONS

AR	*BRAF*	*CCNE1*	*CDK4*	*CDK6*	*EGFR*	*ERBB2*	*FGFR1*
FGFR2	*KIT*	*KRAS*	*MET*	*MYC*	*PDGFRA*	*PIK3CA*	*RAF1*

FUSIONS

ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

INDELS

EGFR exons 19/20	*ERBB2* exons 19/20	*MET* exon 14 skipping

GUARDANT HEALTH

Serum: 1 Red Top Tube (10 ml)

1. Label tube with Protocol number, collection date/time, protocol time-point collected (e.g. pretreatment, post-treatment), and clearly mark specimen as "serum".
2. Allow one red top tube to clot for 30 minutes at room temperature.
3. Spin in a standard clinical centrifuge at ~2500 RPM for 10 minutes at 4 ^{o}C (preferred). If sites are unable to process samples at 4 ^{o}C then spinning at room temperature is acceptable if done within 2 hours of draw but must be noted.
4. Place tube into Styrofoam container (together with the Green top tubes mentioned below), then into a biohazard bag and then place the bag into the Cardboard shipping (outer) box.
5. Ship to the address below the same day using overnight courier (FedEx), early morning delivery option.

Under no circumstances are collected blood specimens to be frozen or put in any sort of fixative.

PLEASE MAKE SURE THAT EVERY SPECIMEN IS LABELED and include collection time point on the Specimen Transmittal Form (STF).

Whole Blood: 8-9 Green Top Tubes (90 ml)

1. After collection, invert tube(s) multiple times to ensure adequate mixing of Sodium Heparin.
2. Label tube with protocol number, collection date/time, protocol time-point collected (e.g. pretreatment, post-treatment), and clearly mark specimens as "PBMC."
3. Place tubes into Styrofoam container (together with the Red top tube mentioned above), then into a biohazard bag and then place the bag into the Cardboard shipping (outer) box.
4. Ship to the address below the same day using overnight courier (FedEx), early morning delivery option.

Under no circumstances are collected blood specimens to be frozen or put in any sort of fixative.

PLEASE MAKE SURE THAT EVERY SPECIMEN IS LABELED and include collection time point on the STF.

9.3.3 Multicolor Flow Cytometry-Based Lymphocyte Phenotyping

To support the flow cytometry assays in this clinical trial, the IMT has developed robust multicolor flow cytometry (FACS) staining protocols for analyzing lymphocyte subsets in peripheral blood and in tumor isolates. The IMT has developed a standard research protocol (SRP) that details a comprehensive flow cytometry staining panel using validated antibodies (a copy of this SRP can be supplied on request).

Multicolor flow cytometry-based lymphocyte phenotyping will be carried out at: 1) Before nivolumab treatment and 2) Prior to the 2nd, 4th, 6th administration of nivolumab and at progression/discontinuation (if the patient receives as many doses prior to discontinuation).

9.4 Immunohistochemical (IHC) Staining and Analysis for PD-L1, PD-1, CD_8^+, and CD_4^+ Tumor-Infiltrating Lymphocytes

Tissue biopsies for exploratory correlative studies are to be offered to each patient on study, yet are optional for enrollment and treatment. Patients who develop thrombocytopenia (platelet count < 50,000/ µL) or are otherwise deemed to be at high risk for bleeding from biopsy by the investigator may have the second biopsy delayed by two additional weeks/ rescheduled to prior to receiving the next dose of nivolumab. If the patient is still deemed to be at too high risk for bleeding, then they may proceed with additional treatment without undergoing the second biopsy. Patients who choose not to undergo tissue biopsy will still be permitted to remain on study, as will patients who are unable to complete a second tissue biopsy due to high risk for bleeding.

Core biopsies under radiographic (ultrasound, CT, or MRI) – guided biopsy will be performed using a lumen no smaller than an 18-gauge needle pretreatment and 2 weeks following initiation of treatment with nivolumab (up to 3 days prior to 3rd dose of nivolumab). 5-8 core samples should ideally be taken, and stored as fresh frozen tissue and as paraffin-embedded tissue, alternating between the two with each successive tissue core biopsy.

Detection of the selected markers for this study will be performed using immunohistochemistry with Dako kits (Agilent Tehcnologies) provided by BMS. These kits will included all necessary reagents including, but not limited to, primary and secondary antibodies, needed in order to perform exploratory biomarker studies. After the staining, the slides will be digitally scanned in an Aperio AT system (Aperio™, Leica Biosystems™) to convert the IHC slides into digital pathology files for posterior analysis. IHC expression analyses of markers consider a thorough staining pattern evaluation including distribution (percentage of positive cells) and intensity in the form of H-score, and evaluating the IHC expression in the proper subcellular location (i.e. membrane, cytoplasm or nucleus). IHC analysis and scoring will be performed by a certified pathologist at MD Anderson using an image analysis software (Image Toolbox, Aperio™).

Outcome from the analysis will be calculated according to the H-score. The H-score ranges from 0 to 300, and it considers both intensity of the IHC (from 0 to 3) and distribution (percentage of the target cells positive, from 0 to 100). The scoring is the addition of the percentages of cells with intensity 0 + intensity 2 + intensity 3, thus the addition of the final percentage is 100% and the scoring will range from 0 to 300. Hence, the H score will incorporate both percentage of positive cells and intensity of marker expression.

9.5 Specimen Submission Summary

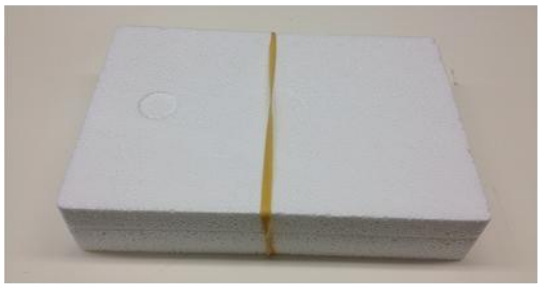
9.5.1 Submission of Blood Specimens

9.5.1.1 Handling Instructions

Specimens for Immune Monitoring (required) for shipment to MD Anderson—Immunotherapy Platform: See Section 9.4			
Specimens taken from patient:	**Collected when:**	**Submitted as:**	**Shipped: Same day as Blood draw.**
SERUM: 10 mL of whole blood in 1 red-top tube and centrifuge	Prior to nivolumab treatment start -at screening or any time prior to first dose of nivolumab), before doses 2,4, 6, and at study end/disease progression	Whole blood samples in red top tubes	Sent at room temperature via overnight carrier to MD Anderson Immunotherapy Platform
WHOLE BLOOD: 90 mL of whole blood in sodium heparin tubes	Prior to nivolumab treatment start -at screening (or any time prior to first dose of nivolumab), before doses 2, 4, 6,, and at study end/disease progression	Whole blood samples in 5 green top tubes (Na Heparin)	Sent at room temperature via overnight carrier to MD Anderson Immune Monitoring Lab

*If the patient discontinues therapy prior to dose 6, then only the blood draws prior to treatment administration and at progression/discontinuation will be required.

9.5.1.2 Shipping Instructions for Blood Specimens



1. Place the blood tubes (red top, green top and PAXgene) in the Styrofoam container as shown in the figure. This figure shows only the positioning of the tubes: the color top does not depict what you will have.

2. Place the rubber band around the Styrofoam container as shown in this figure (this is only for added stability)

3. Place the Styrofoam container into the cardboard shipping (outer) box, and then place the cardboard box into a biohazard bag together with the Absorbent shipping materials.

4. Place everything into the FedEx shipping back and send to the address below (blood spesimens only):
   Immunotherapy Platform
   MD Anderson Cancer Center
   Attention: Karen Millerchip/Jaimol Peedikayil
   SCR3.3208
   7455 Fannin St.
   Houston, TX 77054-1901

9.5.2 Submission of Slides for IHC Analysis

9.5.2.1 Handling Instructions

Specimens for Central Pathology Review
for shipment to MD Anderson—Jorge Blando: See Section 9.5.2.2

Specimens taken from patient:	**Collected when:**	**Submitted as:**	**Shipped: <u>after paraffin embedding/slice cutting.</u>**
6-8 unstained tumor slides per biopsy or time point	Two separate biopsies: one prior to treatment initiation and another after 2 doses of treatment with nivolumab (i.e., up to 3 days prior to 3rd dose of nivolumab).	Paraffin-embedded unstained tumor slides	Overnight mail to Dr. Jorge Blando

9.5.2.2 Shipping address for IHC samples only:

Immunopathology Laboratory
Immunotherapy Platform (IMT)
UT MD Anderson Cancer Center,
Life Science Plaza Building
2130 W. Holcombe Blvd,
Unit 2951, Houston, TX 77030

9.6 Special Studies - Analysis for the Presence of HPV

When available, HPV testing, if not previously performed, will be conducted on archival tissue for each patient at the participating site treating each particular patient. The availability of remaining tissue for HPV testing is not required for participation on this study. Testing will include detection of HPV DNA by in-situ hybridization and/or detection of p16 by immunohistochemistry. Formalin-fixed, paraffin-embedded samples will be deparaffinized in

preparation for DNA extraction. To determine whether or not HPV had been incorporated into the host genome, in-situ hybridization will be utilized to assess for positive hybridization in the tumor cell nuclei using probes for various oncogenic subtypes of HPV. To determine whether or not p16 is expressed in tumor cells, IHC analysis using monoclonal antibodies against the p16 protein will performed. Tumors will considered to express p16 if 5% or greater of tumor cells demonstrate p16 staining on IHC. Patients will be considered to have HPV-positive tumors if HPV DNA is detected by in-situ hybridization and/or p16 is detected in the tumor cells by IHC. Patients will be deemed to have HPV-negative tumors if HPV DNA is absent and p16 is not detected.

9.7 **Optional Banking of Residual Tissue and Blood Samples for Future Research**

All patients who sign consent for this study also will be asked to consider participation in this optional part of the study. In consenting patients, any remaining blood and tissue samples not used for biomarker assessment will be banked for potential future research. The samples will be banked in the Immunotherapy Platform Laboratory, The University of Texas MD Anderson Cancer Center, South Campus Research Bldg SCR3.3208, 7455 Fannin Street, Houston, TX 77054.

IRB approval will be obtained prior to the use of the banked samples for any research not described in this protocol. The samples will be given a code number. No identifying information will be directly linked to the samples. Only the research team in charge of the bank will have access to the code numbers and be able to link the samples to the subject. If a patient withdraws his/her consent for banking of samples, the banked samples will be destroyed. However, if any samples were previously used for research prior to the withdrawal of consent, the samples will not be able to be destroyed.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. Unless otherwise noted, a standard window of -1 day to + 2 days will be considered acceptable for all testing and evaluations (will not be considered study deviation). In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next dose of therapy.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11[h]	Wk 12	Treatment Discontinuation Visit[i]	28 Days from Last Dose of Nivolumab	Follow-up
Nivolumab		A		A		A		A		A		A				
Informed consent	X															
Demographics	X															
Medical history	X															
Concurrent medications	X	X[g]		X		X		X		X		X				
Physical exam	X	X[g]		X		X		X		X		X		X	X	
Vital signs	X	X[g]		X		X		X		X		X		X	X	
Height	X															
Weight	X	X		X		X		X		X		X		X	X	
Performance status	X	X[g]		X		X		X		X		X		X	X	
CBC w/diff, plts	X	X[g]		X		X		X		X		X		X	X	
Serum chemistry[a]	X	X[g]		X		X		X		X		X		X	X	
Thyroid stimulating hormone[b]	X													X		
HIV Antibody[c]	X															
HIV Viral Load[d]	X			X				X				X		X	X	
Hepatitis B Panel[e]	X															
Hepatitis C Viral Antibody	X															

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11[h]	Wk 12	Treatment Discontinuation Visit[i]	28 Days from Last Dose of Nivolumab	Follow-up
EKG (as indicated)	X															
Adverse event evaluation		X----------											----------X	X	X	X[j]
Tumor measurements	X	Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X		
Radiologic evaluation: CT or MRI of Chest, Abdomen, Pelvis & other known or suspected sites of disease	X	Radiologic measurements should be performed every 6 weeks.												X		
B-HCG	X[f]	Serum or urine pregnancy test must be performed every 6 weeks													X[f]	X[f]
HPV testing	X[o]															
Blood for Correlative Studies[m]	X			X				X				X		X		
Tumor Biopsies for Correlative Studies[n]	X					X[l]										
Survival status																X[k]

A: Nivolumab IV 3 mg/kg given every 2 weeks +/- 3 days.
a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Patients with TSH > 10.0 U/mL or TSH < 0.5 U/mL must be referred to an endrocrinologist with correction in a range between 0.5-10.0 U/mL prior to treatment initiation.
c: If not tested within past 6 months.
d: Only for patients with HIV infection as documented by the presence of a positive HIV antibody test.
e: Hepatitis B surface antibody, Hepatitis B surface antigen, hepatitis B core antibody
f: Serum or urine pregnancy test (women of childbearing potential) must be performed with 24 hours prior to start of nivolumab, then every 6 weeks. After discontinuation from nivolumab these should be repeated at approximately 30 days and approximately 70 days.
g. Tests/evaluations do not need to be repeated prior to first nivolumab dose if performed within past 7 days.
h. Nivolumab may continue to be given every 2 weeks until any of the criteria outlined in section 5 require it be stopped.
i: Off-treatment evaluation, only for patients who discontinue treatment due to disease progression.
j. All patients will be followed for adverse events for 100 days from last dose of nivolumab. Patients with ongoing adverse event(s) will continue to be followed until resolution or stabilization of the adverse event. Patients who discontinue therapy due to unacceptable toxicity but have not demonstrated radiographic disease progression will continue to be followed on schedule every 6 weeks with imaging studies until disease progression is documented.
k. Patients will be followed for survival status every 3 months for 2 years after treatment discontinuation or until death, whichever occurs first. Follow up may be accomplished by clinic visit, medical record review, phone contact or email.
l. 2 weeks after initiation of treatment with nivolumab (up to 3 days prior to 3rd dose of nivolumab).
m. Peripheral blood will be collected as described in section 9.3 at screening (any time prior to first dose of nivolumab) and before doses 2, 4, 6, and at treatment discontinuation (for patients who progress while on nivolumab). Blood should not be collected if the hemoglobin concentration is < 10.0 g/dL.
n. Optional, but not required, for participation on study.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11[h]	Wk 12	Treatment Discontinuation Visit[i]	28 Days from Last Dose of Nivolumab	Follow-up
o. If not previously performed, DNA in-situ hybridization and/or p16 by IHC testing if sufficient archival tissue is available will be performed at enrolling site .																

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks following initial documentation of objective response.

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)[87]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

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

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one dose of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression after the first dose will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one dose of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (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.

11.1.3 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 (≥1 cm) 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.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

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 (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), 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.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response.

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria:

11.1.4.1 Evaluation of Target Lesions

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 (<1 cm).

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 (0.5 cm). (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.

11.1.4.2 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 [<1 cm] short axis).

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).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Responses will be assessed using CT scans or magnetic resonance imaging according to standard RECIST 1.1 criteria in order to assess disease progression. These criteria will also allow for patients who experience an initial disease flare, and as some patients who will have a delayed response may experience an initial disease flare, we

will allow patients with radiographic disease progression to continue on the trial provided that they have a stable ECOG performance status, no need for immediate alternative treatment, and progression of no more than 40% with three or fewer new lesions. If, at the next evaluation, these patients demonstrate further disease progression, then they will be labeled as non-responders to therapy. However, if they do meet the criteria for response according to RECIST 1.1, then they will be deemed as a responder and counted accordingly in the tabulations for determining whether or not to proceed to a second stage or achieving the study end point.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is

increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented. [87]

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

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

11.1.7 Response Review

All responses will be reviewed by an expert independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

11.2 Other Response Parameters

11.2.1 Overall Survival (OS)

OS is defined as the duration of time from start of treatment to time of death.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site

user must have an active CTEP IAM account (https://eapps-ctep.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read-Only, or Site Investigator) on either the Corresponding Organization or Participating Organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.1.1 Method

CTMS Routine Monitoring: This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data is to be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: http://www.theradex.com/CTMS. On-site audits will be conducted on a 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at ctms@theradex.com for additional support with Rave and completion of CRFs.

12.1.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

See Section 12.1.1 for details on CDUS reporting. As the data management center for this trial, Theradex is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

N/A

12.3 Collaborative Agreements Language

The agent supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company

(hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

13.1.1 Primary Endpoint

Overall response rate is the primary endpoint for this study.

13.1.2 Study Design

This study is a two-staged, Simon Optimal phase II clinical trial of nivolumab as a single agent for the treatment of patients with locally advanced/unresectable or metastatic squamous cell carcinoma of the anal canal who have progressed through at least one prior line of therapy. Responses will be assessed using CT scans or magnetic resonance imaging according to standard RECIST 1.1 criteria in order to assess disease progression. These criteria will also allow for patients who experience an initial disease flare, and as some patients who will have a delayed response may experience an initial disease flare, we will allow patients with radiographic disease progression to continue on the trial provided that they have a stable ECOG performance status, no need for immediate alternative treatment, and progression of no more than 40% with three or fewer new lesions. If, at the next evaluation, these patients demonstrate further disease progression, then they will be labeled as non-responders to therapy. However, if they do meet the

criteria for response according to RECIST 1.1, then they will be deemed as a responder and counted accordingly in the tabulations for determining whether or not to proceed to a second stage or achieving the study end point. If no response is observed among the 12 patients treated in the first stage at six months, then the trial will be stopped for futility. However, if one or more responses are observed, then the trial will be expanded to include an additional 25 patients, with 4 or more responses needed among the 37 total participants to declare an efficacy of this single-agent therapy.

13.1.3 Null/Alternative Hypotheses

For this Simon Optimal, two-stage phase II study, we propose a null hypothesis H_o: $p \leq 0.05$ and an alternative hypothesis H_a: $p \geq 0.20$, where p represents the percentage of patients with metastatic SCCA of the anal canal demonstrating either a partial response or a complete response according to RECIST 1.1 criteria when treated with nivolumab. If the null hypothesis is rejected and the alternative hypothesis is accepted based on the results of this study, then a phase III trial comparing nivolumab to best supportive care in patients with refractory metastatic squamous cell carcinoma of the anal canal will be proposed.

13.2 Sample Size/Accrual Rate

Using an $\alpha = 0.10$ and a $\beta = 0.10$, we estimate that at least twelve patients, and no more than 37 patients, will be enrolled onto this trial.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	2	0	0	0	**2**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	3	1	0	0	**4**
White	20	7	3	1	**31**
	0	0	0	0	**0**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
Total	25	8	3	1	37

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015) OMB No. 0925-0001/0002

13.3 Stratification Factors

N/A

13.4 Analysis of Secondary Endpoints

13.4.1 Progression-free survival

Kaplan-Meier analysis will be performed to estimate the median progression-free survival with a 90% confidence interval. Progression-free survival will be calculated as the time from initiation of treatment with nivolumab until the time of disease progression according to RECIST version 1.1 criteria.

13.4.2 Overall survival

Kaplan-Meier analysis will be performed to estimate the median overall survival with a 90% confidence interval. Overall survival will be calculated as the time from initiation of treatment with nivolumab until death.

13.4.3 Toxicity

Adverse events will be monitored and recorded according to grade for each patient from the time of treatment initiation until 30 days after the time of study drug discontinuation. The frequency of each serious adverse event will be measured relative to the total number of patients treated. Toxicities will be tabulated by type and grade.

13.5 Analyses of Exploratory Endpoints:

13.5.1 Descriptive Statistics of IHC and Blood Sample Studies.

Descriptive statistics including plots, mean, median and standard deviations will be used to summarize data. For continuous outcomes, t-test and ANOVA will be used to compare outcome measures across patient characteristics. Dunnett's and Tukey's test that properly adjust for multiplicity in multiple tests will be implemented. Pair-wise comparisons will be

performed using pre- and post-therapy samples from each patient . The chi-square (c2) test or Fisher's exact test will be used to test the association between two categorical variables such as disease state and performance status. Both univariate and multivariate logistic regressions will be performed to model prognostic factors.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with nivolumab.

13.6.2 Evaluation of Response

All eligible patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The overall response (CR and PR) rate along with the 95% confidence interval will be estimated.

14. STUDY STATUS UPDATES AND STUDY CLOSURE

14.1 Definitions of Study Status Changes

14.1.1 Temporarily Closed to Accrual

The study status is Temporarily Closed to Accrual when no patient slots are currently available, but there is the possibility that the trial will re-open for accrual (patient slots become available). Sites are not permitted to accrue additional patients until CTEP is notified of Re-Activation.

Study status will need to be changed to Temporarily Closed to Accrual when any of the following criteria are met:

- Sites are notified by CTEP (via Request for Rapid Amendment [RRA]) of changes in the risk/benefit ratio that necessitate changes to the patient Informed Consent document. Requested changes will be specified in the RRA and must be reviewed by the study's IRB.
- CTEP and the lead investigator agree that unacceptable toxicities necessitate a discussion to change the dosing/regimen.
- A protocol-defined benchmark has been achieved (such as an interim analysis before proceeding to the next stage).

14.1.2 Closed to Accrual

The study status is (permanently) Closed to Accrual when no more patient enrollment slots are available, and at least one patient is still actively receiving the study treatment. Sites are no longer permitted to enroll additional patients.

Patient slots are no longer available when the following criteria are met:

- The pre-specified number of evaluable patients has been successfully enrolled, treated, and evaluated.
- The study treatment has failed to meet the pre-specified efficacy goal at the stage 1 interim analysis.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment.

14.1.3 Closed to Accrual and Treatment

The study status is Closed to Accrual and Treatment when no more patient enrollment slots are available and no patients are currently receiving the study treatment. Patients may still be enrolled on the protocol only for the purposes of follow-up.

Patient accrual and treatment will be permanently halted when any of the following criteria are met:

- Enrollment was previously closed (study status of "Closed to Accrual"), and no patients are receiving the study treatment.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment. In this case, CTEP and the investigators must collaborate to alter the regimen or to halt the study treatment altogether as soon as it can be safely done

for patients currently receiving treatment.

CTEP and Theradex **must be notified** when patients are no longer receiving treatment [*i.e.*, when the last patient(s) to be receiving treatment is/are no longer receiving the study regimen for any reason].

14.1.4 Closed to Follow-Up

The study is considered Closed to Follow-Up when all protocol-defined follow-up procedures have been completed for all patients who have not been removed from the study for other reasons. That is, there are no outstanding follow-up procedures to be performed as mandated by the protocol.

CTEP does **not** need to be notified of a status change to "Closed to Follow Up."

14.1.5 Complete

Study is considered Complete if it has been at least thirty (30) days since the last patient follow-up evaluation.

A citation to a final study report (manuscript, meeting abstract, etc.) is required with the submission of the Protocol Status Update Form to CTEP PIO.

14.2 Responsibility for Filing Protocol Status Update Forms

CTEP must be notified of all study status changes in Section 14.1 (except for Closed to Follow-Up) by the Corresponding Organization via Protocol Status Update Form, available from the CTEP website at http://ctep.cancer.gov/protocolDevelopment/default.htm#amendments.

Theradex must be notified as soon as all patients are off treatment (*i.e.*, when study status changes to Closed to Accrual and Treatment). Theradex will produce a report within 90 days of this notification.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B CTEP MULTICENTER GUIDELINES FOR NON-ETCTN TRIALS

N/A

APPENDIX C BIOASSAY TEMPLATES

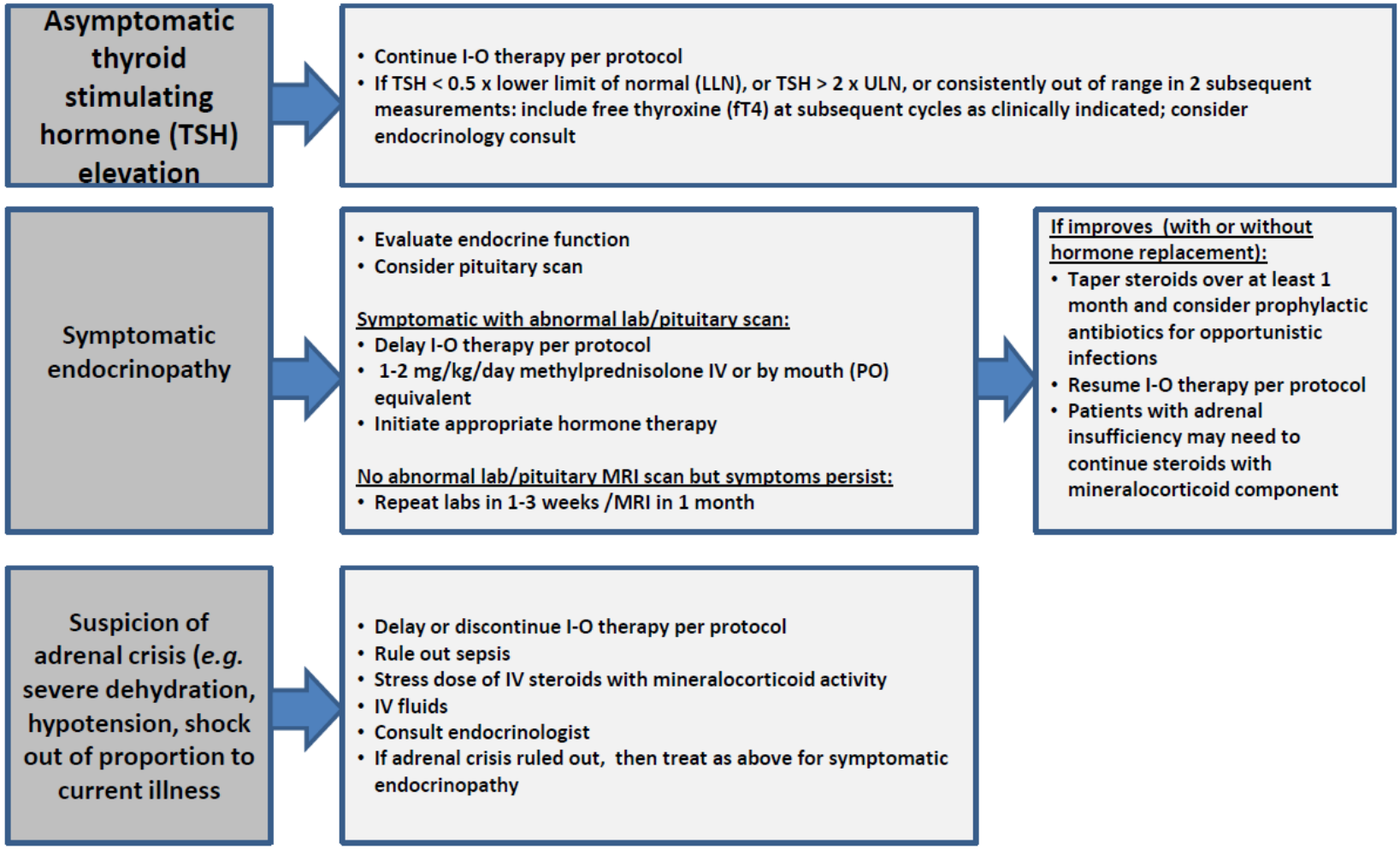
No integrated or integral biomarkers will be used for this study.

APPENDIX D MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

Please note that the abbreviation “I-O” in the following pages stands for “Immunotherapy-Oncology”

Endocrinopathy Management Algorithm

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



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

GI Adverse Event Management Algorithm

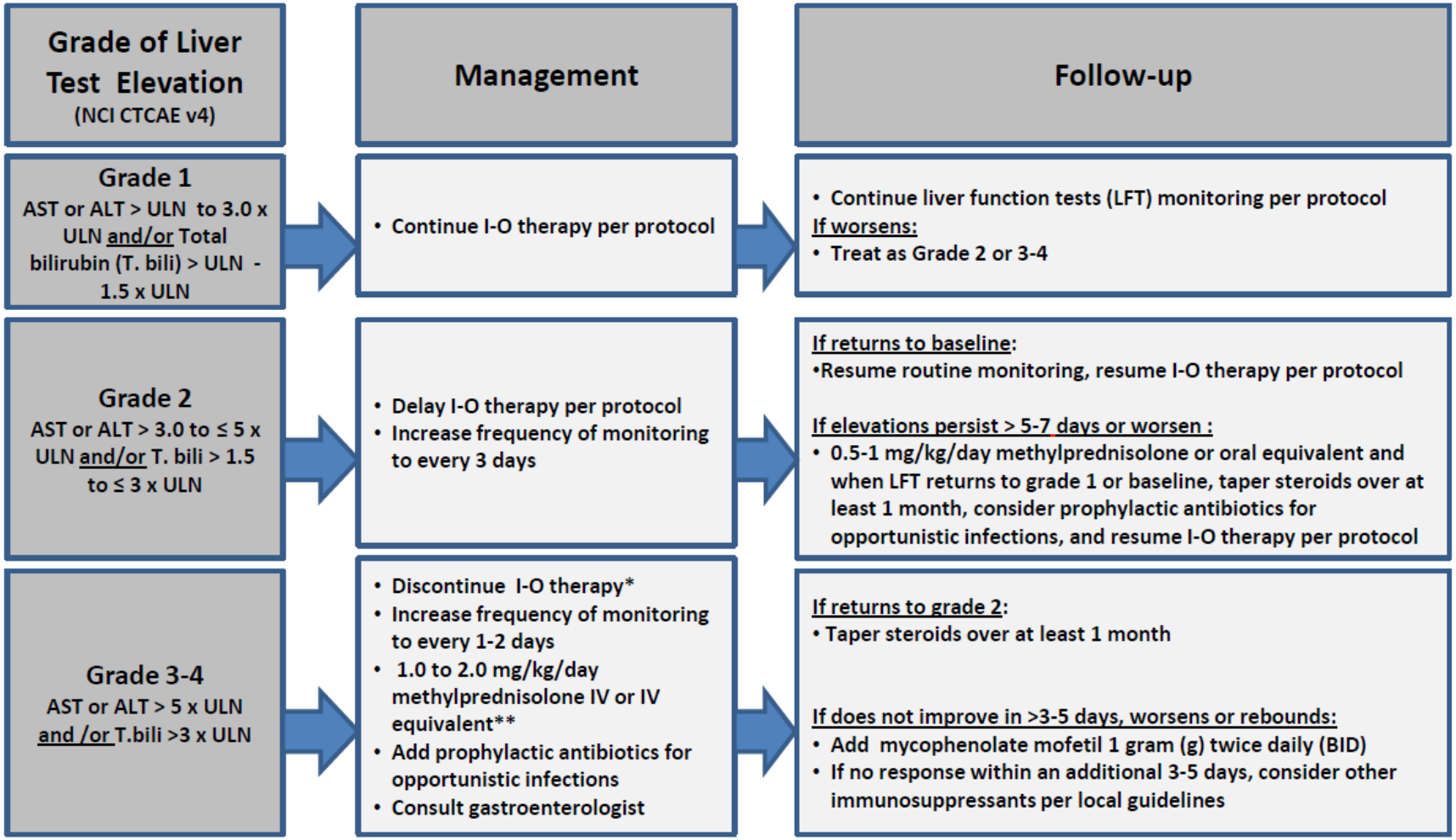
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

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

Hepatic Adverse Event Management Algorithm

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



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

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

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

Neurological Adverse Event Management Algorithm

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

Grade of Neurological Toxicity (NCI CTCAE v4)	Management	Follow-up
Grade 1* Asymptomatic or mild symptoms; Intervention not indicated	• Continue I-O therapy per protocol	Continue to monitor the patient. If worsens: • Treat as Grade 2 or 3-4
Grade 2* Moderate symptoms; Limiting instrumental ADL	• Delay I-O therapy per protocol • Treat symptoms per local guidelines • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent	If improves to baseline: •Resume I-O therapy per protocol when improved to baseline If worsens: • Treat as Grade 3-4
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	• Discontinue I-O therapy per protocol • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: • Taper steroids over at least 1 month If worsens or atypical presentation: • Consider IVIG or other immunosuppressive therapies per local guidelines

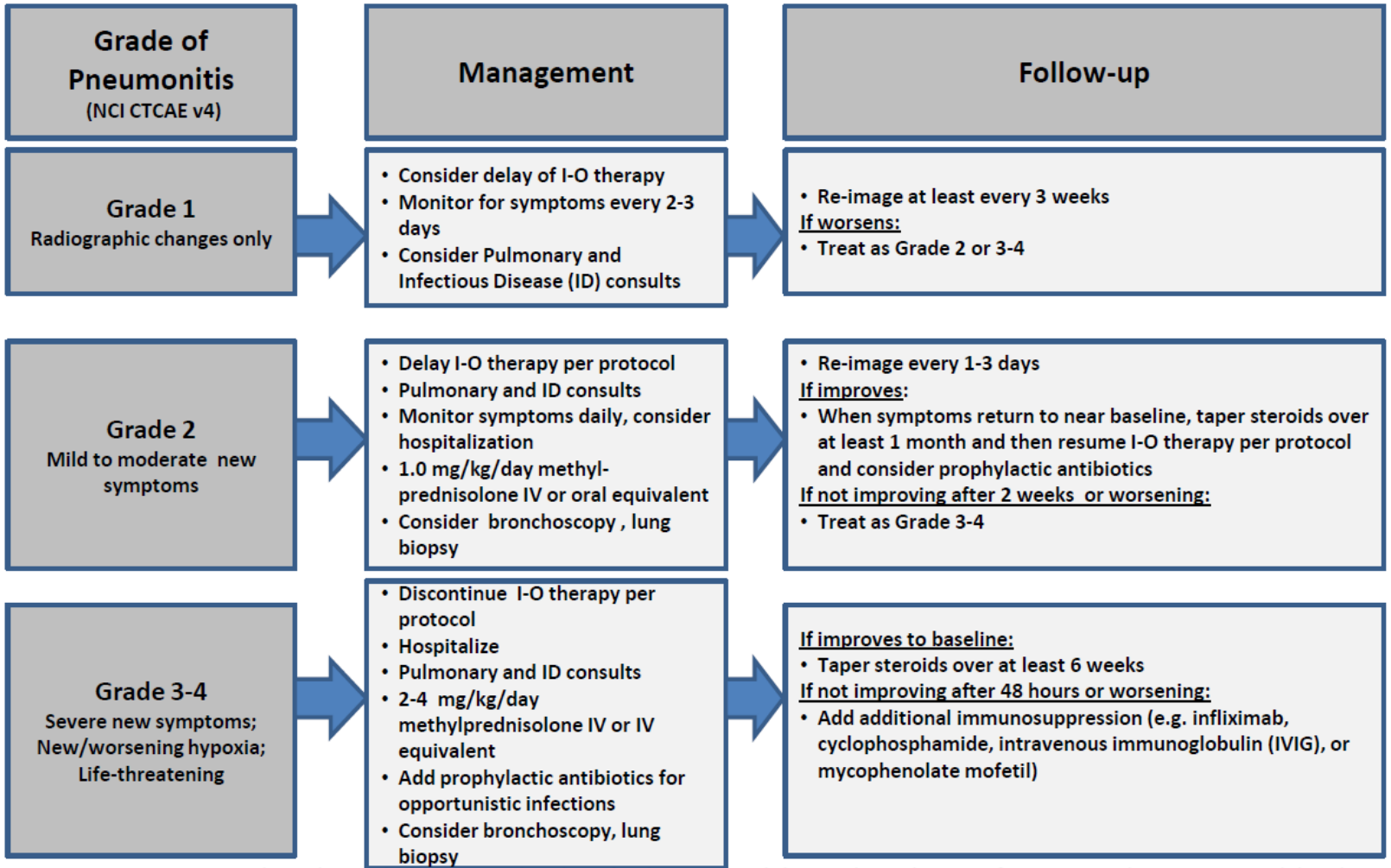
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

*Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, or myasthenia gravis should be off study.

nts

Pulmonary Adverse Event Management Algorithm

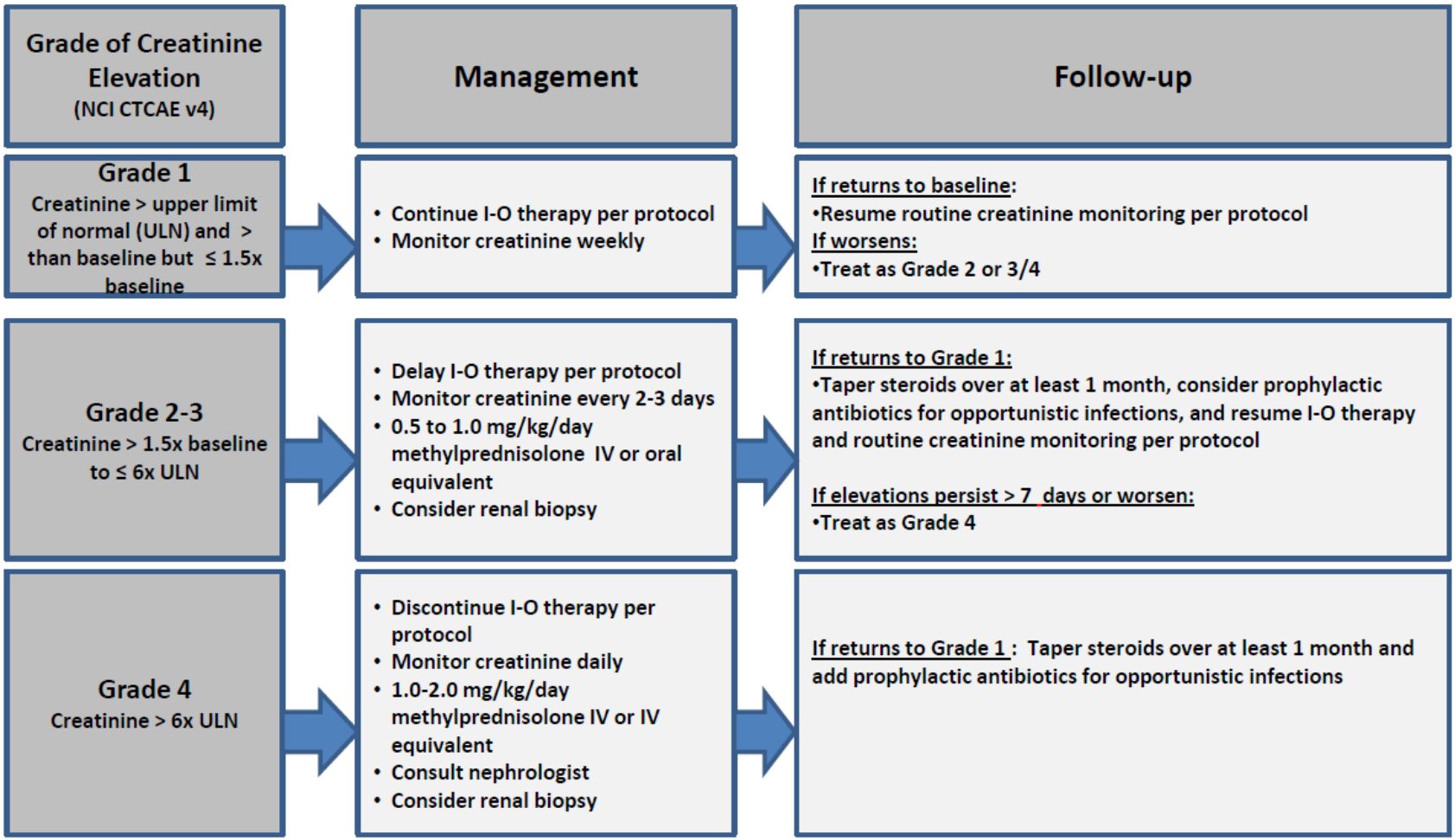
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Renal Adverse Event Management Algorithm

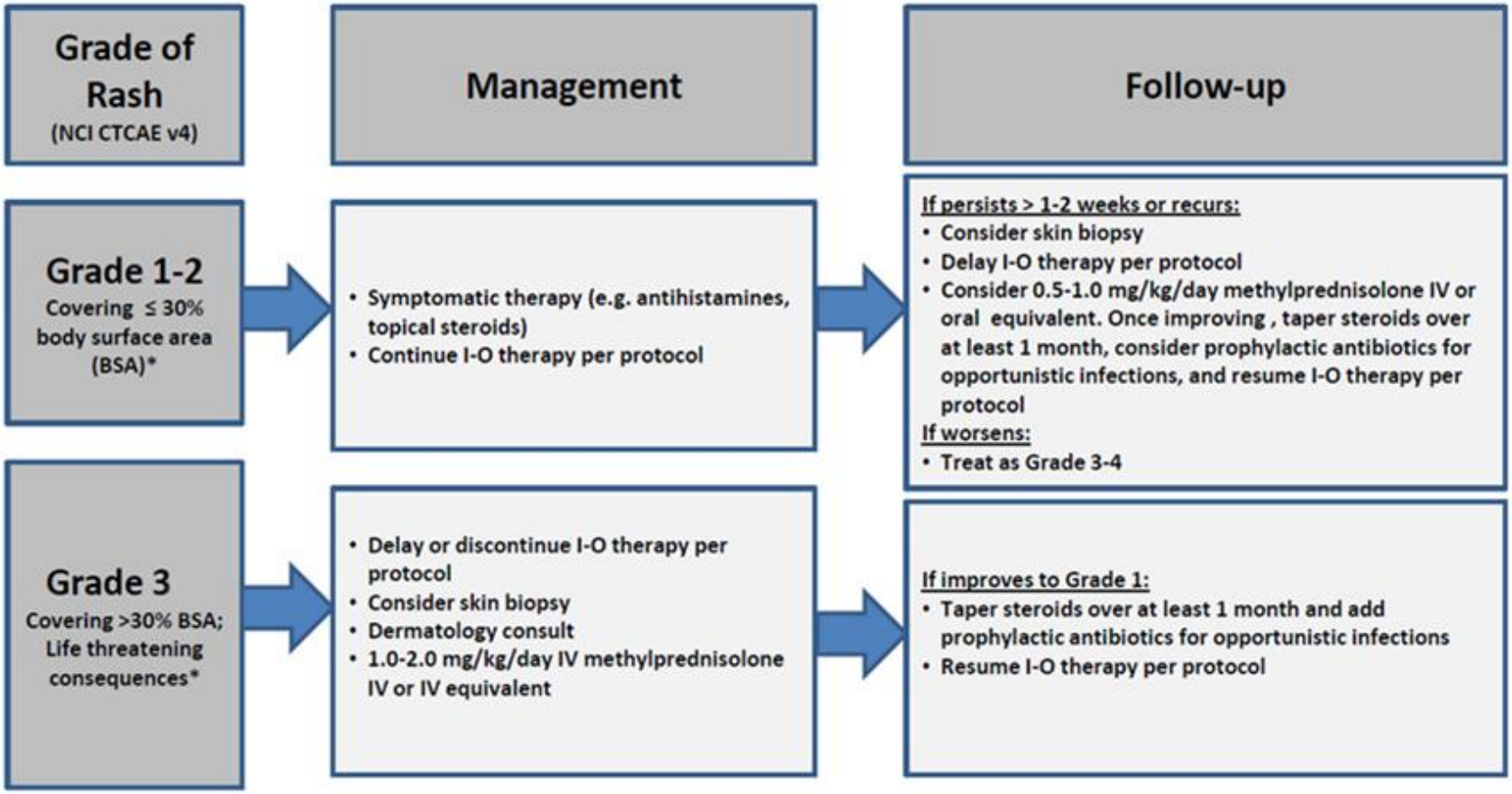
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Skin Adverse Event Management Algorithm

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



GRADE 4 ADVERSE EVENT: I-O therapy should be discontinued and patient should come off study.

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

APPENDIX E ANALYSES OF SAMPLES FOR EXPLORATORY STUDIES

Fresh frozen tumor samples, if available, will be analyzed by RT-PCR to assess for mRNA expression of genes that may contribute to anti-tumor immune responses, e.g. IFN-γ and other cytokines. Tumor tissues will also be analyzed by immunohistochemistry (IHC) for markers that may include, but are not limited to, CD4, CD8, FoxP3, and Granzyme.

Fresh tumor samples, if available and assessed by physician adequate for selection, from routine surgical procedures, will be used for flow cytometry analysis of tumor infiltrating lymphocytes (TILs) when possible.

Lymphocytes from collected blood or from tumor infiltrating cells will be analyzed using 17 core panels by flow cytometry to identify sub-populations of T-cells (for example, CD4+ helper T-cells, CD8+ cytotoxic T-cells, CD4+, FoxP3+ regulatory T-cells). These studies require 20 cc of blood per blood draw.

Lymphocytes from collected blood will be analyzed by ELISPOT analyses, if possible, to identify T-cells that are functionally reactive against aHPV-specific antigens E6 and E7. For example, in an ELISPOT assay that uses HPV as the target antigen, a patient's T-cell will be analyzed to determine if a cytokine, such as interferon- gamma, is produced by the T-cell, in response to recognition of the HPV antigen in the context of an antigen-presenting cell. These studies require a minimum of 80 cc of blood per blood draw.

Funding for these tests will be provided by MD Anderson Cancer Center institutional sources and philanthropic donations.

APPENDIX F PARTICIPANT WALLET CARD

INFORMATION FOR PATIENTS AND PRESCRIBERS

You are enrolled on a clinical trial using the experimental agent nivolumab. This clinical trial is sponsored by the NCI. It is very important to:

- Take this card with you to the emergency room or any healthcare provider other than your study doctor. Tell all healthcare providers that you are being treated with nivolumab and SHOW THEM THIS CARD. Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. Do not take over-the-counter medications, dietary supplements, or prescription medications without the approval of your study doctor.

- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial.

- See your study doctor for help managing symptoms. Doctors who are not familiar with nivolumab may not be aware of the appropriate management or side effects.

- If you have any of these signs or symptoms, call your study doctor or nurse right away: new or worsening shortness of breath, cough, or wheezing; diarrhea or any increase in the amount or number of bowel movements above normal, blood in stool or dark, tarry, sticky stools; stomach pain/cramps; yellowing of the white part of the eye(s); blurry vision, double vision, or other vision problems; eye pain or redness; severe rash, itching, or peeling; yellowing of the skin; fever; decreased strength or energy; dizziness; headaches; muscle weakness; numbness or tingling in the hands or feet; changes in behavior; dark urine; palpitations, or fast or irregular heartbeats.

- Before prescribing new medicines, your regular prescribers should contact your study doctor.

- Your study doctor's name is _____

  and can be contacted at _____.