NCI 9742 / MC1471: Multicenter Phase II Study of Nivolumab in Previously Treated Patients with Recurrent and Metastatic Nasopharyngeal Carcinoma

Amendment 4

<table>
<tr><th>#</th><th>Section</th><th>Revision</th></tr>
<tr><td>1.</td><td>Throughout protocol</td><td>The title page and headers of the document have been updated to the new NCI version date.</td></tr>
<tr><td>2.</td><td>6.2</td><td>Addition of more specific guidelines for cardiac toxicities including the stipulations listed below :

o Add on study evaluation of cardiac function including EKG and ECHO cardiogram for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated.
o For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, ECHO cardiogram.
o Drug modification table for cardiomyopathy myocarditis should be included in the appropriate section
▪ Drug will be held for grade 2 cardiac dysfunction pending evaluation
▪ Drug will be permanently discontinued for grade 3 or 4 cardiac dysfunction and grade 2 events that do not recover to baseline or that reoccur
▪ Treatment with steroids as clinically indicated
▪ Add the table as follows in the treatment modification and AE management section

<table>
<tr><th>Cardiac *</th><th>Management/Next Dose for BMS-936558 (Nivolumab) + Ipilimumab Cardiac Toxicities</th></tr>
<tr><td>≤ Grade 1</td><td>Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation</td></tr>
<tr><td>Grade ≥2 with suspected</td><td>Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy.</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><td></td><td></td><td>
<table>
<tr><td>myocarditis</td><td>Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Consult algorithm for more details. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.</td></tr>
<tr><td>Grade ≥2 with confirmed myocarditis</td><td>Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone
Add ATG or tacrolimus if no improvement. Off treatment.</td></tr>
<tr><td colspan="2">*Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin
**Patients with evidence of myositis without myocarditis may be treated according as “other event”
Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.</td></tr>
</table>
</td></tr>
<tr><td>3.</td><td>7.1</td><td>The CAEPR and corresponding Condensed Risk List for Nivolumab is updated to version 2.2 dated November 15, 2016 from version 2.1 dated December 11, 2015. The revisions are as follow:

• Added New Risk:
  • Less Likely: Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)
  • Rare but Serious: Immune system disorders - Other (GVHD in the setting of allotransplant); Myositis; Nervous system disorders - Other (encephalitis)
  • Also Reported on BMS-936558 Trials But With Insufficient Evidence for Attribution: Immune system disorders - Other (autoimmune thrombotic microangiopathy)

• Increase in Risk Attribution:
  • Changed to Less Likely from Rare But Serious: Infusion related reaction
  • Changed to Rare but Serious from Also Reported on BMS-936558 Trials But With Insufficient Evidence for Attribution: Pericarditis

• Deleted Risk:
  • Also Reported on BMS-936558 Trials But With Insufficient Evidence for Attribution: Alkaline phosphatase increased; Arthritis; CPK increased; Encephalitis infection; Endocrine disorders - Other (autoimmune thyroiditis); Endocrine disorders - Other (hypopituitarism); Enterocolitis;</td></tr>
</table>

		Hepatobiliary disorders - Other (autoimmune hepatitis); Investigations - Other (CRP increased); Investigations - Other (eosinophil count increased); Investigations - Other (thyroxine free increased); Investigations - Other (tri-iodothyronine free decreased); Nervous system disorders - Other (autoimmune neuropathy); Renal and urinary disorders - Other (nephritis); Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (interstitial lung disease); Respiratory, thoracic and mediastinal disorders - Other (lung infiltration); Stroke; Wheezing; White blood cell decreased • <u>Provided Further Clarification:</u> • The following footnote #7 was added: "Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving BMS-936558 (Nivolumab, MDX-1106). These complications may occur despite intervening therapy between receiving BMS-936558 (Nivolumab, MDX-1106) and allo-SCT."

TITLE: Multicenter Phase II Study of Nivolumab in Previously Treated Patients with Recurrent and Metastatic Nasopharyngeal Carcinoma

Corresponding Organization: P2C-MN026 / Mayo Clinic Cancer Center P2C

Principal Investigator: Brigette B.Y. Ma
Department of Clinical Oncology
Prince of Wales Hospital
Shatin, NT, Hong Kong SAR
Telephone: (852) 26321042
Telefax: (852) 26487097
Email: brigette@clo.cuhk.edu.hk

Participating "Rostered" Organizations

P2C-IL057 / University of Chicago Comprehensive Cancer Center P2C
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
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-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

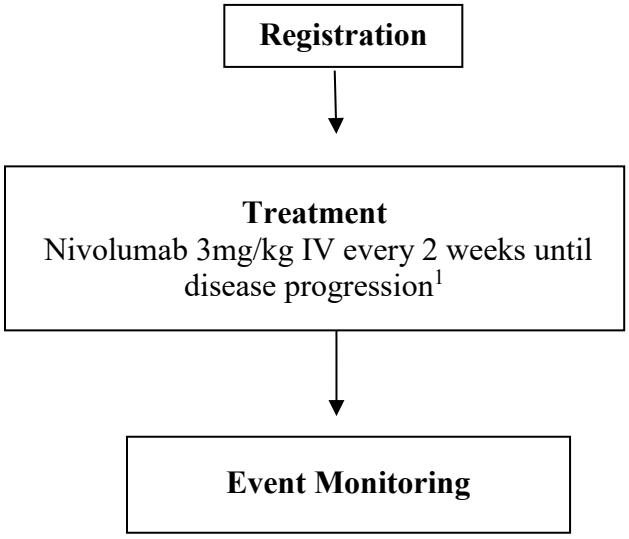
Statistican: Nathan R. Foster
Mayo Clinic Cancer Center
Telephone: (507) 284-5051
Email: foster.nathan@mayo.edu

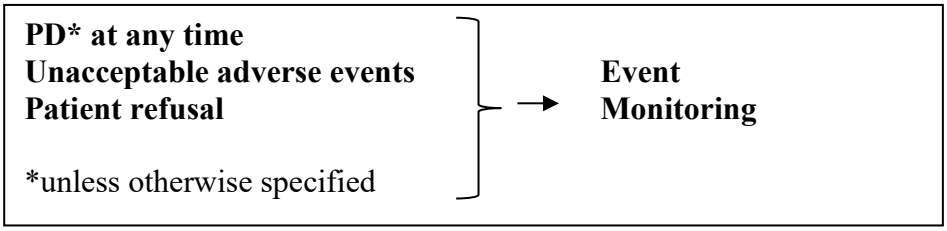
NCI-Supplied Agent: Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC #748726)

IND #: 126146
IND Sponsor: DCTD, NCI

Protocol Type: / Version / Version Date:
Original / Version 1 / December 8, 2014
Resubmission / Version 2 / March 17, 2015
Resubmission / Version 3/ April 10, 2015
Resubmission / Version 4/ June 2, 2015
Resubmission / Version 5 / November 19, 2015
Resubmission / Version 6 / Amendment 1 / December 22, 2015
Resubmission / Version 7 / Amendment 2 / March 14, 2016
Resubmission / Version 8 / Amendment 3 / March 18, 2016
Resubmission / Version 9 / Amendment 4 / January 6, 2017

SCHEMA





[1] Cycle length= 4 weeks

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Objective tumor response to nivolumab in patients with previously treated recurrent and/or metastatic nasopharyngeal carcinoma (NPC) based on RECIST criteria version 1.1.

1.2 Secondary Objectives

1.2.1 Tumor response to nivolumab based on immune-related response criteria (IRC).

1.2.2 Duration of response.

1.2.3 Progression-free survival and overall survival.

1.2.4 Safety and tolerability.

1.3 Correlative Objectives

1.3.1 To investigate the effect of nivolumab on tumor burden by analyzing the clearance of plasma Epstein-Barr virus (EBV) DNA during the first 4-6 weeks of treatment.

1.3.2 To investigate the association between treatment outcome and immunological markers:

a) (Mandatory) Intratumoral expression of PD-1 and PD-L1 in archived NPC tissues

b) (Mandatory) Serum absolute lymphocyte count at baseline and post-treatment

c) Plasma cytokine levels at baseline and serially during the first 8 weeks of treatment.

d) (Optional) Expression of PD-1 in CD8+ T cells in tumor infiltrating lymphocytes (TIL) at baseline.

1.3.3 To investigate functional MRI sequences as an early predictor of response to nivolumab. (Optional only at CUHK)

2. BACKGROUND

2.1 Nasopharyngeal Carcinoma and Programmed Cell Death Protein 1 Signaling

Non-keratinizing nasopharyngeal carcinoma (NPC) is a common cancer in Southeast Asia where the incidence reaches its peak in regions such as Hong Kong, Guangzhou province of China and Singapore. It is the 7th most common cancer in Hong Kong and in 2008, over 84,000 incident cases were diagnosed worldwide. Despite the high rate of cure of AJCC stage I-II NPC, overall 50,000 people died of NPC in 2002 alone worldwide. This is because 20% of patients who present with metastatic disease have a median overall survival (OS) of only 12 to 15 months

despite chemotherapy. For the 50% of patients who present with locoregionally advanced (stage III-IVB) disease, 30% will develop distant metastases following modern chemoradiotherapy.

Immunotherapy for EBV associated NPC – our experience: EBV is ubiquitous in undifferentiated NPC in endemic regions, and EBV latent infection plays a vital role in NPC tumorigenesis. Expression of the oncogenic EBV latent proteins has been shown to modulate the tumor inflammatory microenvironment and abolish host immune response [1]. In order to overcome the immune-escape mechanisms of the EBV-associated NPC, strategies such as adoptive EBV-specific cytotoxic T-cell therapy and active immunization with EBV vaccines have been evaluated in patients with recurrent NPC [2-4]. Significant tumor regressions have been reported in a few patients. However, the labor-intensive and costly nature of these technologies has limited the broader application of these therapies in the clinics. Based on the collaborative effort by some of our investigators from the Chinese University of Hong Kong (CUHK) with the University of Birmingham, a new, recombinant modified vaccinia Ankara-based vaccine that encodes EBV-specific tumor antigens has been developed and evaluated in a phase 1 study in NPC patients in Hong Kong [5]. Our work underscores our capability in and commitment to research in immunotherapy for NPC.

Targeting programmed cell death protein 1 (PD-1) signaling in cancer: In a healthy host, PD-1 signaling in T cells regulates immune responses to minimize damage to bystander tissue and prevents the development of autoimmunity by promoting tolerance to self-antigens [6]. PD-1 is a trans-membranous protein receptor member that is highly expressed on activated T cells. It has 2 known ligands, PD-ligand 1 (B7-H1 or CD274, which is expressed on tumor, antigen-presenting cells APCs and dendritic cells) and PD-L2 (B7-DC or CD273). PD-L1 is overexpressed in many cancers and has been linked to poor prognosis [6]. Membranous PD-L1 is thought to be more relevant prognostically than cytosolic PD-L1. Inhibition of PD-1 signaling can induce tumor regression in vivo, increase effector T-cell function and cytokine production theoretically by reactivating PD-1 expressing TILs.

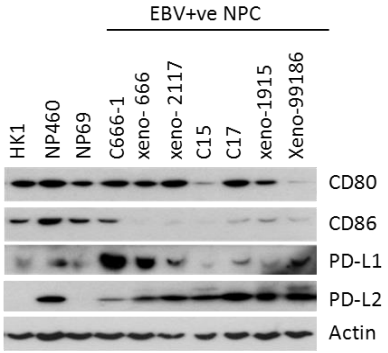
PD-1 signaling pathway in NPC: To provide a proof-of-concept for the current study, our Prof K.W. Lo and his team from the Chinese University of Hong Kong (unpublished confidential data) has generated some preliminary data on PD-1 signaling in NPC:

Figure 1, a and b: Expression of PD-L1 and PD-L2 in NPC models. PD-L1 is up-regulated in EBV-positive NPC cell lines, and expressed in over 70% of the 20 NPC samples analyzed.

Figure 2 & 3 a and b: A significant number of PD-1+ cells in tumor infiltrating lymphocytes and also circulating CD8+ T cells can be found in NPC patients. By IHC staining, we also detected PD-1-expressing infiltrating lymphocytes in NPC primary tumors. PD-L2 is also overexpressed in some NPC tumors (data not shown) but its significance is uncertain.

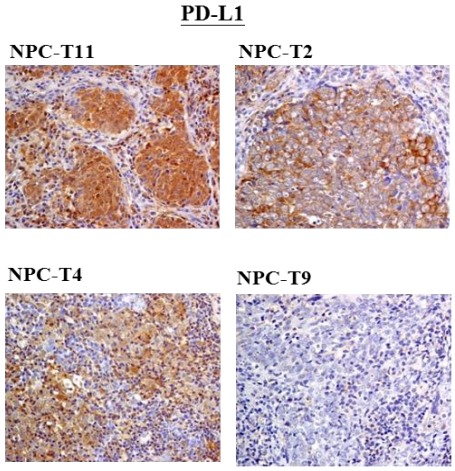
Figure 1 (unpublished confidential data, Prof KW Lo, CUHK)

a.



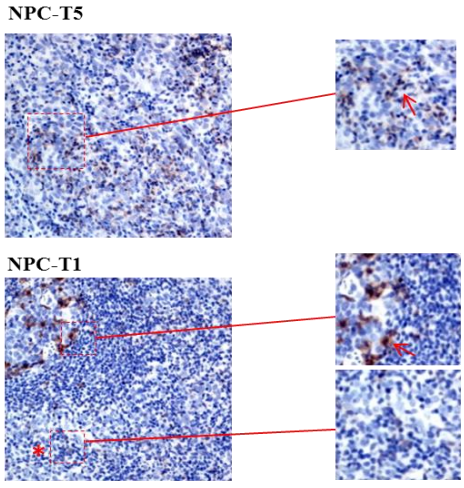
Expression of co-inhibitory molecules (CD80, CD86, PD-L1, and PD-L2) in EBV-positive NPC tumor lines were determined by Western blotting. Upregulation of PD-L1 expression was observed in C666-1, xeno-666 and xeno-2117. An EBV-negative NPC cell line HK1 and the immortalized NP cell lines NP69 and NP460 was included as controls.

b.



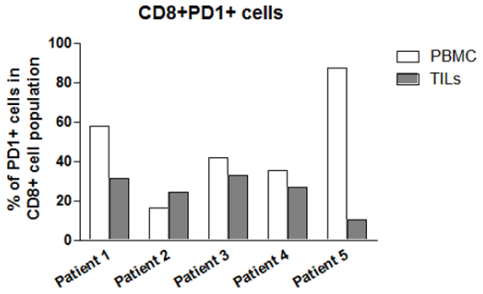
Overexpression of PD-L1 was detected in 15/20 primary NPCs by IHC staining. Intensive PD-L1 expression was shown in the cases NPC-T11, -T2, and -T4. No PD-L1 expression was found in case NPC-T9..

Figure 2: Unpublished confidential data
Courtesy Prof KW Lo, CUHK

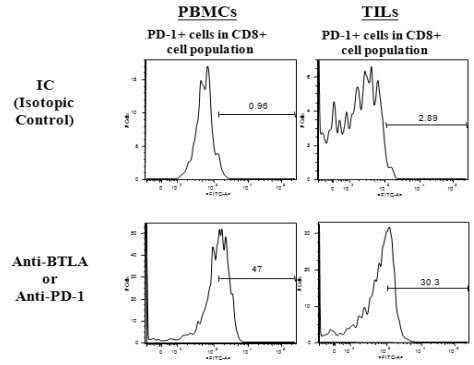


Significant number of PD-1+ lymphocytes were detected in primary NPC tumors by IHC staining (e.g. NPC-T5). In NPC-T1, PD-1+ cells (red arrow) were found within the germinal centre, but not the tumor nest (*).

a. Figure 3: unpublished data (KW Lo)



b.



(a) Detection of PD-1+ CD8+ T cells from TILs and PBMCs of NPC patients by flow cytometry. PD-1+ CD8+ T cells were observed in both PBMCs and TILs of 5 NPC patients (b) Representative example of PD-1 expression in CD8+ T cells from TILs and PBMCs of NPC patients .

2.2 Nivolumab

2.2.1 Mechanism of Action

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 [7]. 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 [8].

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) [7]. 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 [9]. 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[10], renal [11-13], esophageal [14], gastric [15], ovarian [16], pancreatic [17], lung [18], and other cancers [7].

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.

2.2.2 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated [7]. 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.

2.2.3 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 [7]. 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.

2.2.4 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 [7]. 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.

2.2.5 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 [19]. 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 [20]. In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥ 1 year [21].

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) [8]. 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 [22]. 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.

2.2.6 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined [23]. 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 [8], grade 3 or 4 treatment-related events were noted in 53% of patients. Skin rash represents the majority of these events.

2.2.7 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 [8]. 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.

2.3 Rationale

Hypothesis for current study:

a) Based on our preliminary preclinical data as generated from NPC cell lines and patient's tissues (see above), we hypothesize that NPC inhibits the activity and proliferation of tumor-specific CD8+ T cells via PD-L1/PD-1 inhibitory receptor pathways, thereby contributing to the evasion of host's immune-surveillance in EBV-positive NPC.

b) Inhibition of PD-1 signaling may thus reactivate PD-1 expressing tumor-infiltrating lymphocytes and induce tumor regression.

2.4 Correlative Studies

Rationale for using plasma EBV DNA as a surrogate marker of tumor burden in NPC

Investigators at the CUHK pioneered the use of quantitative real-time-PCR to quantitate the level of plasma EBV DNA [24, 25]. It is a powerful prognostic marker which closely reflects tumor burden in NPC [26, 27]. Our investigators have also reported the kinetics of plasma EBV DNA during radiotherapy and surgery for NPC. An initial surge followed by a rapid drop in plasma EBV DNA level can be observed, with an estimated half-life of around 3.8 days (range 2.2-4.4 days) [25, 28]. Wang *et al.* found that the plasma EBV DNA clearance during the 1st 4 weeks of chemotherapy was prognostic, such that patients with a half-life of < 8 days were more likely to respond than those with longer half-life values [29]. Our experience with plasma EBV DNA half-life in a phase II study of a AKT inhibitor, MK-2206, suggested that patients with a drop in plasma EBV DNA levels were more likely to remain on treatment for 6 months or more, compared with those who did not experience with a drop in level [30].

In a recently published study which compared the detection capability of this marker across different laboratories in Stanford University (USA), Taiwan and the CUHK, the Hong Kong site

was found to have one of the highest detection sensitivity (93%) [31]. This study also serves to harmonize the plasma EBV DNA assay in preparation for an international NRG study (NRG-HN001, NCT02135042). This study aims to individualize adjuvant chemotherapy using plasma EBV DNA in locoregionally advanced NPC, and involves investigators from the NRG, Chinese University of Hong Kong and Taiwan.

Rationale for measuring PD-L1 expression in tumor and immune cells

Topalian *et al.* found that high level of tumoral expression of PD-L1 was associated with higher response duration in nivolumab-treated patients [23]. But this was not observed in a nivolumab-ipilimumab study in melanoma [8]. Our preliminary work has shown that 15/20 NPC tumors (75%) expressed PD-L1, thus we plan to validate this marker in this study. The dynamic nature of tumoral PD-L1 expression may be dependent upon changes in the tumor microenvironment, and is closely correlated with the degree of immune infiltrate in tumors [32]. More recently in a phase 1b study of another PD-1 inhibitor, MK-3475 in recurrent head and neck squamous carcinoma, patients with tumors that overexpressed PD-L1 above an arbitrary cut-off point (Roche/Genentech assay used, defined as PD-L1 expression in $\geq$1% of both tumor and adjacent immune cells) had higher tumor response rate (45.5%) than those who did not (11.4%) [33]. Based on these data, therefore, PD-L1 expression in the TILs in tumors will also be measured in this study. A recently published study has shown that the EBV oncogenic protein, latent membrane protein-1 (LMP-1) could up-regulate PD-L1 through STAT3, AP-1 and NF-κB pathways in NPC cell lines. IFN-γ could also up-regulate PD-L1 independent of but synergetic with LMP1 in vitro [42].

Rationale for measuring absolute lymphocyte count

A study on ipilimumab has shown that an absolute lymphocyte count (ALC) of > 1,000/mL and an increase in ALC around 2 months after treatment may predict clinical benefit [12]. But this was not observed in a nivolumab-ipilimumab study [8]. Further validation in this study is warranted.

Rationale for the functional MRI study
Discussed in section 9.2.2.1.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Age ≥ 18 years old

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

3.1.3 Histologically or cytologically confirmed non-keratinizing nasopharyngeal carcinoma (NPC) that has recurred at locoregional and/or distant sites. **Note:** Patients with local recurrence at the bony skull-base as the only site of index disease are excluded since such lesions are difficult to measure radiologically. Patients with locoregional recurrence without distant metastases must have undergone radical radiotherapy previously.

3.1.4 Measurable disease according to the RECIST criteria (version 1.1), as defined in section 11 for the evaluation of measurable disease.

3.1.5 Received one or more lines of chemotherapy, which must include prior treatment with a platinum agent and must not be amenable to potentially curative radiotherapy or surgery.

3.1.6 Archived or fresh tumor sample available. Willingness to donate blood and tissue for mandatory correlative research studies. (see Section 9).

3.1.7 ECOG performance status of 0, 1 or 2 (see Appendix A).

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

• Absolute neutrophil count	≥ 1.5 x 10^9/L
• Platelets	≥ 100 x 10^9/L
• Hemoglobin	≥ 8.0 g/dL
• Serum alanine aminotransferase (ALT; serum glutamate-pyruvate transferase, [SGPT]), or Serum aspartate aminotransferase (AST) where available at the center)	< 2.5 x upper limit of normal (ULN), OR < 5 x ULN in the presence of liver metastases.
• Serum total bilirubin	< 2 x ULN (except patients with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)
• Serum creatinine	< 1.5 x ULN

3.2 Exclusion Criteria

3.2.1 Any of the following:

- Chemotherapy ≤ 4 weeks prior to registration
- Radiotherapy ≤ 4 weeks prior to registration
- Nitrosoureas or Mitomycin C ≤ 6 weeks prior to registration
- Those who have not recovered from adverse events (to grade ≤ 1 in severity) due to agents administered more than 4 weeks earlier. Prior palliative radiotherapy to bone metastases ≤ 2 weeks prior to registration (i.e., prior palliative radiotherapy to bone metastases is allowed if it is performed > 2 weeks prior to registration.)

3.2.2 Prior investigational agents ≤ 4 weeks prior to registration.

3.2.3 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 Known brain metastases or leptomeningeal metastases. **Note:** symptomatic, and/or if they require immunosuppressive doses of corticosteroids (e.g., >10 mg/day prednisone or equivalents) for at least 2 weeks prior to study drug administration. Patients with treated brain metastases, who are deemed clinically stable and without radiological progression on PET, MRI or CT scan performed ≤ 8 weeks of study entry, are not excluded. **Note**: Primary nasopharyngeal cancers that directly invade the skull base and extend into the infratemporal fossa (e) are not regarded as brain metastases and are not excluded.

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

3.2.6 History of severe hypersensitivity reaction to any monoclonal antibody.

3.2.7 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.

3.2.8 Any of the following:

- Pregnant women
- Nursing women
- Men or women of childbearing potential who are unwilling to employ adequate contraception

Note: Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab, breastfeeding should be discontinued if the mother is treated with nivolumab. Women of childbearing potential and men must use two forms of contraception (hormonal or barrier method of birth control; abstinence) prior to

study entry and for the duration of study participation. They must adhere to contraception for a period of 31 weeks after the last dose of nivolumab."

3.2.9 For patients with unknown HIV status at the time of enrollment, HIV serology must be tested during screening. Patients who are tested positive for HIV could be included if there is an adequate CD4 count (>350/μl) on a stable regimen of HAART with no detectable or minimal viral burden, and no active infections.

3.2.10 For patients with unknown hepatitis B virus surface antigen (HbsAg) status, they must be tested during study screening. Patients who are tested positive test for HbsAg are excluded if they have inadequately controlled hepatitis B and/or Child-Pugh Class B or C cirrhosis. However, patients with adequately controlled hepatitis are not excluded from the study if they satisfy all of the following criteria: (i) must be receiving a nucleoside analog anti-viral drug for 3 or more months, and (ii) have a serum HBV DNA level of less than 100 IU/ml via polymerase chain reaction quantification assays prior to enrollment.

3.2.11 For patients with unknown hepatitis C virus ribonucleic acid (HCV antibody) status, they must be tested during study screening. Patients who are tested positive for HCV antibody are excluded from the study if they have inadequately controlled hepatitis C and/or Child-Pugh Class B or C cirrhosis. Patients with adequately controlled hepatitis are not excluded from the study as defined by having undetectable level of serum HCV antibody level prior to enrollment. Patients who are currently on interferon or other anti-HCV therapy may be at risk of developing anemia and neutropenia from such therapy, therefore will be excluded from study.

3.2.12 Active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. **Note:** 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, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease. Patients with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjögren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

3.2.13 Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger (precipitating event).

3.2.14 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.

3.2.15 Evidence of active or acute (i.e. current, or recent within 4 weeks prior to registration) diverticulitis, intra-abdominal abscess, GI obstruction and abdominal carcinomatosis which are known risk factors for bowel perforation. Patients with abdominal carcinomatosis, a history of non-recent intra-abdominal abscess, or a history of non-recent GI obstruction should be evaluated for the potential need for additional treatment before coming on study.

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 (ROSTERED PROTOCOL MODEL)

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 #9742 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-MN026], and protocol #9742
- 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 sub tab of the members' section of the CTSU Web site. (Note: Sites <u>will not</u> 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 sub tab.
- 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 Screening and General Guidelines

Following registration, patients should begin protocol treatment ≤ 21 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. After the consent form is signed, refer to section 10 'Study Calendar' for screening procedures. Standard contrast CT will be done for assessing non-local recurrences. MRI will be used for assessing local recurrence. If dual PET and contrast CT is used to assess non-local recurrences at baseline, all subsequent radiological assessments must include a PET-CT.

5. TREATMENT PLAN

5.1 Agent Administration

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

Nivolumab is given every 2 weeks on day 1 and day 15 (± 1 days), in a **4-week** cycle, at a dose of **3mg/kg** intravenously.

Patients may be dosed no less than **13 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 to the nearest milligram. Dose rounding based on institutional guidelines is not preferred. There will be no dose modifications allowed.

Nivolumab is to be administered as a 60-minute IV infusion (± 10 minutes), 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 0.35 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 General Concomitant Medication and Supportive Care Guidelines

Nivolumab is not expected to have any effect on cytochrome P450 or other drug metabolizing enzymes in terms of inhibition or induction, and is, therefore, not expected to induce these types of PK-based drug interactions [7].

Nivolumab is a full human monoclonal antibody and infusion reactions, including high-grade hypersensitivity reactions, following administration of nivolumab are uncommon [7]. Pre-medications are not routinely given.

Growth factors (e.g. granulocyte colony stimulating factor, erythropoietin) are not allowed during the study.

Supportive measures such as anti-emetics (e.g., metoclopramide, tropisetron, granisetron) and other pre-medications which have been mentioned in this protocol will be allowed.

5.3 Duration of Therapy and Criteria for Study Discontinuation

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

- Disease progression (unless the investigator judges that the patient is eligible for continuing beyond progression as stated in section 5.7),
- Intercurrent illness that prevents further administration of 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.
- Unacceptable adverse event(s) which include the following (see also section 6 and specific algorithms in Appendix C):

    a) Any grade 4 events except as noted in item **'h'** below.

    b) 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 in item **'h'** below:

    c) 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.

d) Patients requiring > two dose delays for the same type of event (of grade 2 severity or above) should go off protocol therapy, unless the study doctor thinks that it is in the patient's best interest to remain in the study. The study doctor should also consult the Principal Investigator before continuing study treatment.

e) 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

f) 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

g) 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.

h) The following exceptions do NOT require permanent study discontinuation:
- Any other grade 3 non-skin, drug-related AE (other than grade 3 immune mediated event) 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 clinical manifestations of pancreatitis and which decrease to 0 Grade 0 **may** resume study treatment when resolved.

5.4 Duration of Follow Up

Patients will be followed for a maximum of 3 years from study entry or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. The frequency and procedure of follow-up is determined at the discretion of the doctor, or according to institutional practice.

5.5 Criteria to Resume Treatment

5.5.1 General Criteria for Restarting Nivolumab:

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). The section should emphasize stopping treatment and starting steroids earlier to obtain resolution with the possibility for restarting rather than waiting for higher grade events.

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 5.3 (Duration of Therapy).

It should be emphasized that if adverse event occurs which requires treatment with steroids, it is preferable to withhold nivolumab and start steroids earlier to obtain resolution with the possibility for restarting nivolumab, rather than just waiting for higher grade events.

5.5.2 Restarting Nivolumab in 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 withheld 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.

5.6 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.

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

5.6.1 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.

5.6.2 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.

5.6.3 For Grade 3 or Grade 4 Symptoms
(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 ventilator 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.

5.7 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 or combination treatment.

Patients may be permitted to continue treatment beyond initial RECIST 1.1-defined PD occurring during the initial induction period (up to 12 weeks), as long as they meet all of the following criteria (see section 5.7.1 below). Patients are allowed to continue treatment for one additional cycle (4 weeks) and then be reassessed.

5.7.1 Criteria for Continuing Treatment Beyond Progression

1) No more than 4 new lesions (excluding new brain metastases), with the total sum of the longest diameter (SHORT diameter for LN) cannot exceed 40% of the initial sum including new lesions
2) Patients must be clinically stable with no change in performance status due to disease progression
3) No indication for immediate alternative treatment (such as radiotherapy).
4) 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.
5) 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).

All decisions to continue treatment beyond initial progression must be documented in the study record and are a part of an agreement with DCTD, NCI.

For statistical analyses: Subjects who continue treatment beyond first progression (as determined by the initial investigator-assessed, RECIST 1.1-defined progression) will be considered to have investigator-assessed ‘progressive disease’ at the time of the initial

progression event. However, if they subsequently experience a CR or PR they may be designated as a 'delayed response' for the purposes of determining ORR and thus, in the final analysis, be counted as a responder for the two-stage design.

6. DOSING DELAYS/DOSE MODIFICATIONS

No dose modifications are allowed in this protocol. Nivolumab can be 'continued', 'delayed', 'withheld' or 'permanently discontinued'.

See Appendix C to the protocol for toxicity management algorithms which include specific treatment guidelines. These algorithms should be followed unless there are specific clinical circumstances which the treating physician indicates variations or alternative treatment is needed.

6.1 Guideline for Nivolumab Related Adverse Events in General

ALL NON-IMMUNOLOGIC 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 but non-immunologically mediated**	
Recommended management: As clinically indicated	
ALL IMMUNOLOGIC 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 5.3)
Grade 4	Off protocol therapy
Recommended management: As clinically indicated	

6.2 Guideline for Specific Nivolumab Related Adverse Events

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose *
Grade 2	Hold* until ≤ 1 Grade resolved. Consider steroid treatment if > 7 days. Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Consider steroid treatment. Resume at same level at investigator discretion
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/pemphigoid. 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.	
Recommended management: AE management guidelines	

Liver Function: ALT, AST, total bilirubin	**Management/Next Dose for Nivolumab**
≤ Grade 1	Continue nivolumab and monitor liver enzymes as per protocol.
Grade 2	Hold until ULN or baseline. Resume at same dose level. Consider steroid treatment > 7 days
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Continuation of nivolumab during active immune-mediated hepatitis may exacerbate the inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis /pancreatitis.	
Recommended management: see Hepatic AE management algorithm (Appendix C)	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold until Grade 0 or baseline. No change in dose
Grade 2	Hold until Grade 0 or baseline. Consider steroid treatment if lasting > 7 days, if condition does not improve within a reasonable time frame, the patient should be withdrawn from study if this is in the best interest of the patient as judged by the investigator.
Grade 3	Off protocol therapy.
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. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes C. diff, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and inflammatory bowel disease.	
Recommended management: see GI AE management Algorithm (Appendix C)	

Pancreatitis Amylase/Lipase*	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose until grade 0
Grade 2	Hold until Grade 0. Resume at same dose level if asymptomatic
Grade 3**	Hold until Grade 0. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or diabetes mellitus should be taken off treatment
Grade 4**	Patients who develop symptomatic pancreatitis or diabetes mellitus should be taken off treatment
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as diabetes mellitus and diabetic ketoacidosis. 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 (Appendix C). * In sites where lipase analysis is not readily available, monitoring with amylase level will suffice. **Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation clinical manifestations of pancreatitis and which decrease to Grade 0 may resume study treatment when resolved.	

Pneumonitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and resolution to ≤ Grade 0 or baseline. Resume no change in dose. Consultation with pulmonary and/or infectious disease (ID) specialist is recommended.
Grade 2	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded. Off study if steroids are required.
Grade 3	Off protocol therapy
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 (Appendix C)	

Other GI, Nausea-Vomiting	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level after resolution to ≤ Grade 1. If recurs after resuming nivolumab, evaluate for gastritis duodenitis and other immune adverse events or other causes
Grade 3	Hold pending evaluation until ≤ Grade 1. Evaluate for gastritis duodenitis and other immune adverse events or other causes. 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 3 (or recurrent grade 2) nausea-vomiting 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.*
Grade 2	Hold dose pending evaluation and observation. Hold until ≤ Grade 1.* Off protocol therapy if treatment with steroids is required. Resume at same dose level for peripheral isolated n. VII (Bell's palsy).
Grade 3	Off protocol therapy
Grade 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), Guillain Barre syndrome, myasthenia gravis should be off study.	
Recommended management: See Neurologic Adverse Event Management Algorithm (Appendix C)	

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
≤ Grade 1	Asymptomatic TSH elevation * Hold pending evaluation, consider endocrine consultation.
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	Off study treatment.
Grade 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 (Appendix C)	

Fever	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose if patient is clinically stable*.
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 5.6 for management of infusion reactions	

Cardiac *	**Management/Next Dose for BMS-936558 (Nivolumab) + Ipilimumab Cardiac Toxicities**
≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation
Grade ≥2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Consult algorithm for more details. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
Grade ≥2 with confirmed myocarditis	Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone Add ATG or tacrolimus if no improvement. Off treatment.
*Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin **Patients with evidence of myositis without myocarditis may be treated according as "other event" Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.	

6.3 Guideline for Steroid Use

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 5.5 (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 weeks 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)

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 2.2, November 15, 2016[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]	
		Pericarditis	
ENDOCRINE DISORDERS			
	Adrenal insufficiency		
	Endocrine disorders - Other (hypophysitis)		
	Hyperthyroidism		
	Hypothyroidism		
EYE DISORDERS			
		Eye disorders - Other (diplopia)	
		Eye disorders - Other (Graves ophthalmopathy)	
		Eye disorders - Other (optic neuritis retrobulbar)	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***

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%)**	
	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 (GVHD in the setting of allotransplant)[7]	
		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)	
		Musculoskeletal and connective tissue disorder - Other (rhabdomyolysis)	

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%)**	
		Myositis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy	
		Facial nerve disorder[5]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)	
		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		
	Skin and subcutaneous disorders - Other (Sweet's Syndrome)		

[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 pancytopenia.

[7]Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving BMS-936558 (Nivolumab, MDX-1106). These complications may occur despite intervening therapy between receiving BMS-936558 (Nivolumab, MDX-1106) and allo-SCT.

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; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Vestibular disorder
EYE DISORDERS - Eye disorders - Other (iridocyclitis); Optic nerve disorder
GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; 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
IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (autoimmune thrombotic microangiopathy); Immune system disorders - Other (limbic encephalitis)
INFECTIONS AND INFESTATIONS - Bronchial infection; Lung infection; Sepsis; Upper respiratory infection
INVESTIGATIONS - GGT increased; Investigations - Other (blood LDH increased); Investigations - Other (protein total decreased); Investigations - Other (WBC count increased); Lymphocyte count increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - 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
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (tubulointerstitial nephritis)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia
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) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

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 Distribution of Adverse Event Reports

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 <u>both</u> 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.

Phase 1 and Early Phase 2 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 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

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 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[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

None.

7.4 Routine Adverse Event Reporting

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

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.

Indicate form for reporting in Rave, timeframes, and if loading of the pathology report is 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.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent(s)

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 0.35 mg/mL.

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. NOTE REF TO DOSING SCHEDULE

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 Correlative Laboratory Studies

9.1.1 Immunohistochemistry (IHC) in NPC tissues: PD-L1, PD-L2, PD-1 and PD-1 expression

9.1.1.1 Hypothesis, rationale and preliminary data:
See sections 2.1 and 2.4. For information on sample processing and analysis, see Appendix B 'Immunohistochemical (IHC) Marker Template for Integral Markers'. In essence, for this clinical trial, archival tumor will be tested using the BMS provided DAKO kits in addition to testing done at Dr. Lo's laboratory. Slides will also be sent to a BMS reference laboratory. Positive tumor expression will be defined as the presence of ≥ 1% of NPC tumor cells AND ≥ 1% Tumor infiltrative lymphocytes (TIL) which are positively stained [33].

9.1.1.2 Collection of Specimen(s):
Mandatory collection of archived, paraffin-embedded NPC tissue (derived from primary or metastatic tumors) will apply to all enrolled subjects.

9.1.1.3 Handling of Specimens(s):
For archived samples: Samples can be sent as tumor blocks, or as 8-10 unstained slides, 4μm thick tissue sections. The sections should be labelled with the subject's initials, study code and protocol number (NCI# 9742). Each sample should be accompanied by a laboratory form containing the following information: site where subject was enrolled (e.g., Prince of Wales Hospital, Hong Kong), anatomical origin of the specimen (e.g. NPC primary tumor, or liver metastasis).

9.1.1.4 Shipping of Specimen(s):

For archived specimens: samples can be sent in room temperature. Specimens should be sent preferably on a working weekday (Monday to Wednesday) so that the samples will not arrive on a non-working day. Shipment can be done in batches to

Professor Kwok-Wai Lo
Nasopharyngeal Carcinoma Laboratory,
Rm 321, 3/F Sir YK Pao Centre for Cancer,
Prince of Wales Hospital,
The Chinese University of Hong Kong, Shatin, N.T.,
Hong Kong SAR
Telephone (852) 26322178 or (852) 26321130
Telefax: (852) 26376274
Email: kwlo@cuhk.edu.hk

9.1.1.5 Site Performing Correlative Study
Analysis of the archived sections will be performed in the research laboratory of Prof. Kwok Wai Lo. Ph.D. at Chinese University of Hong Kong.

9.1.2 Mandatory Baseline Serum Lymphocyte Count
The baseline absolute lymphocyte count result will be collected during routine blood sampling for differential white cell count. This value will be correlated with response.

9.2 Exploratory/Ancillary Correlative Studies

9.2.1 Plasma EBV DNA Clearance

9.2.1.1 Hypothesis: We hypothesize that plasma EBV DNA half-life during the first 6 weeks of treatment may be associated with subsequent RECIST-response to nivolumab.

9.2.1.2 Collection of Specimen(s):
This test is **mandatory** for all enrolled subjects. 3-5ml of EDTA blood sample (x 1 tube) will be taken at baseline (within 7 days of starting the first dose of study drug), and then at weekly interval: week 1 (day 7 +/- 2 days), week 3 (day 21 +/-2 days), week 4 (day 28 +/- 2 days), week 5 (day 35 +/- 2 days) and week 6 (day 42, +/- 2 days). Week 2 sampling is omitted given the possibility of a surge in level. Consecutive samples can only be taken with no more than 5 to 9 days apart.

9.2.1.3 Handling of Specimens

After blood sampling, the EDTA blood samples should be centrifuged as soon as possible (within 30 minutes to 1 hour) at 1600 x g for 10 minutes at 4degrees Celsius temperature. The plasma will be transferred to a fresh tube (1.5 ml) and then centrifuged at 16000 x g for 10 minutes at 4 degree Celsius. The centrifuged plasma will be transferred to a fresh tube without disturbing the pellet at the bottom of the tube, and then stored at –70 to -80 degree until analysis. Analysis will be performed using the real-time quantitative PCR technique as previously described [25, 28]. Each sample will be labeled by the patient's study code, study protocol number and date of sample collection. Each blood tube should be accompanied by a laboratory form containing the following information: date of blood collection, patient's study code, type of sample (e.g. 'baseline', 'week 1', 'week 2', 'week3', 'week 4').

9.2.1.4 Shipping of Specimens

Samples should be shipped at -70 to -80 degree Celsius temperature (or shipped frozen on dry ice) in batches, preferably on a working weekday (Monday to Wednesday) so that the samples will not arrive on a non-working day. Samples can be shipped in batched to the following address:

Professor Brigette Ma (c/o Dr Charles Chan)
Tumor Marker Laboratory
4th floor Sir Y.K. Pao Cancer Center
Prince of Wales Hospital
Shatin, New Territories, Hong Kong SAR
Email: brigette@clo.cuhk.edu.hk
Telefax: (852) 26487097
Telephone: (852) 26322118

9.2.1.5 Site Performing Correlative Study

Plasma EBV DBA analysis using real-time quantitative PCR assays will be performed at the Prince of Wales Hospital, Hong Kong, through collaboration with the Department of Chemical Pathology, Chinese University of Hong Kong. This is one of the reference laboratories for determining this biomarker in a NRG study (31).

9.2.2 Functional Magnetic Resonance Imaging (For Prince of Wales Hospital only)

9.2.2.1 Hypothesis and rationale

Anatomical imaging (CT and MRI) and functional imaging (FDG- PET) are the current standards for monitoring cancer treatment response. However, research into functional MRI techniques has also shown potential value for predicting and monitoring treatment response (41). Dynamic contrast enhanced MRI (DCE-MRI) (which uses the same standard contrast agent that is used for conventional anatomic imaging) and T2* mapping assess the vascularity of tumours, while diffusion weighted imaging (DWI) provides ADC parameters that can reflect cell density, cell swelling and membranes. Using the ADC values obtained at low b values by an intatravoxel incoherent motion (IVIM) DWI technique the vascularity can be assessed also without the need for intravenous contrast.

An early (around 2-3 weeks) intratreatment increase in ADC values (as cells shrink and die) has been shown to predict a favourable treatment response to chemoradiation in head and neck tumours (44, 45), but there are conflicting results for other regions of the body where it is known that results are influenced by tumour types and treatment modalities. It has also been reported that head and neck tumours which, after an initial ADC rise early intratreatment, later in the course of treatment show a fall in ADC (presumed to represent repopulation by tumour cells so increasing cell density) are also associated with an unfavourable outcome (45).

The effect of Nivolumab treatment on functional imaging parameters in NPC is unknown but the use of PD-1 inhibitor maybe associated with an increased level of tumor infiltration by T-cells and inflammatory cells. We hypothesize that this early inflammatory response will increase vascularity and cell density and decrease the extracellular space and so will be reflected as an early increase in vascular parameters on DCE and T* and decease in ADC, before a reversal in these parameters later in the course of treatment.

The first step is to document these changes in functional imaging parameters over the course of treatment and correlate with changes in tumour volume. Subsequently, if the sample size is sufficient, we plan to correlate the functional imaging parameters with treatment outcome based on objective response (RECIST) and survival.

9.2.2.2 Data collection and analysis

MRI will be performed on a 3T MRI scanner (Achieva, Philips Healthcare, Best, The

Netherlands) using a 16 channel head and neck SENSE coil. Anatomical and functional data will be obtained to document changes in the target lesions during and after treatment. Target lesions will include: Primary or nodal metastases in the head and neck, distant metastatic sites. MRI scanning will be performed at the Department of Diagnostic Radiology and Interventional Radiology at the Prince of Wales Hospital (PWH), Hong Kong. Only subjects enrolled from the PWH will be invited to participate in this optional correlative study.

MRI Sequences

a) Conventional axial The MRI examination (1.5 or 3T) will cover the skull base down to the lower neck below the suprasternal notch using section thickness = 4mm with no intersection gap. Conventional MRI consists of following images; axial T1W, axial T2W with fat suppression; sagittal T1W; contrast-enhanced axial T1W post contrast with and without fat suppression; coronal T1W post contrast. The T1W and T2W images will be obtained before the functional images and the T1+C will be performed after the DCE-MRI). Tumour volume will be calculated (summation of areas X slice thickness).

b) Functional sequences

- Diffusion weighted imaging (DWI). Single-shot echo-planar imaging technique with b-values from 0-1000 s/mm2. Assessment of the apparent diffusion coefficient (ADC), diffusion coefficient (D), pseudo-diffusion (perfusion) coefficient (D*), and fraction of pseudo-diffusion (f). Whole body Diffusion weighted MRI (DWIBS) will be performed. T2* Mapping: T2* mapping is derived from data acquired using a multiecho T2* sequence based on a multishot fast-field echo technique. T2* mapping will be obtained by exponentially fitting the six images pixel by pixel with different TEs for each slice to obtain the T2* measurements.

- Dynamic contrast enhanced MRI (DCE-MRI), a pre-contrast T1 map is derived using two different flip angles [2º, 15º]. The dynamic sequence is obtained using a short 3D T1-weighted spoiled gradient echo sequence. Contrast injection is commenced 6 seconds after the start of the dynamic MRI acquisitions, given in the form of an intravenous bolus injection of a standard MRI contrast agent (gadopenetate dimeglumine (Dotarem, Guerbet, France) at a concentration of 0.1 mmol/kg of body weight. In addition, immediately after the DCE-MRI scan, post-contrast enhanced anatomical T1-weighted images will be obtained. Assessment of the Ktrans, Kep and Ve of tumors pre-treatment.

9.2.2.3 Imaging timepoints (see also section 10, Study Calendar):

- **Routine MRI scan points as part of response assessments (mandatory)**: Anatomical based MRI examination (T2W, T1W, T1W post contrast) for conventional assessment of treatment response based on size, plus addition of

functional MRI (DCE, DWI/DWIBS, T2* mapping) performed within the same week as the scheduled mandatory contrast MRI and/or CT scans, at the following time-points: baseline, after cycle 4 (on week 8) and after cycle 8 (on week 16).

- **Additional MRI scans points (optional).**
  (a) Early intra-treatment Week 3 (after the 2nd dose of nivolumab), Anatomical and functional MRI will be repeated
  (b) Intra-treatment Week 6- short MRI protocol without IV contrast (T1W, T2W, DWI and T2*)

Table Summarizing the Time Points for MRI Scans

	Baseline	**Week 3**	**Week 6**	**Week 8**	**Week 16**
Routine (Mandatory)	Yes (Contrast, anatomical MRI)	No	No	Yes (Contrast, anatomical MRI)	Yes (Contrast, anatomical MRI)
MRI sub-study (Optional)	Yes (Functional MRI)	Yes (Functional MRI)	Yes (Plain MRI)	Yes (Functional MRI)	Yes (Functional MRI)

9.2.3 Monitoring of Inflammatory Cytokines during Nivolumab Therapy

9.2.3.1 Hypothesis: Nivolumab may affect the level of inflammatory cytokines (e.g. IFN-γ, TNF-α, IL-6) and immune cell levels in nasopharyngeal carcinoma.

9.2.3.2 Sample collection: This blood sampling is mandatory for all patients enrolled into this study. 5 ml of EDTA blood will be collected at baseline, and just prior to nivolumab dosing on week 3 (± 2 days), week 5 (± 2 days) and week 7 (± 2 days). The blood sampling schedules should coincide with the plasma EBV DNA blood samples (section 9.2.1) or routine bloods (see section 10), where applicable.

9.2.3.3 Sample processing: same as section 9.2.1.3.

9.2.3.4 Shipment: same as section 9.2.1.4

10. STUDY CALENDAR

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11, etc	Wk 16	Off Study[c]
Nivolumab		A		A		A		A		A		A		
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X----------X												
Physical exam	X	X				X				X		Wk 13 onwards: every 4 weeks		X
Vital signs	X	X				X				X				X
Height	X													
Weight	X	X				X				X				X
Performance status	X	X		X		X		X		X		X		X
CBC w/diff, plts	X*	X *		X [d]		X [d]		X [d]		X [d]		X [d]		X
Serum chemistry	X [a*]	X *		X [d]		X [d,e]		X [d]		X [d,e]		X [d]		X
EKG (as indicated)	X													
Adverse event evaluation		X----------X												X
Tumor measurements/Radiologic Evaluation [i]	X	Tumor assessments will be done at week 8 after the first dose of nivolumab, and then at week 16 and week 24, and then every 12 weeks thereafter. Starting at week 16, imaging may be performed within ± 7 days of the scheduled visit. Documentation (radiologic) must be provided for patients removed from study for progressive disease. Patients who have not progressed at study discontinuation are encouraged to undergo radiological assessment every 8 to 12 weeks until progression, at the discretion of the study doctor.												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, etc	Wk 16	Off Study[c]
B-HCG	X [b]													
Archived tumor retrieval or fresh tissue collected by biopsy (Mandatory) [R]	X [f]													
Plasma EBV DNA blood sampling (Mandatory) [h,R]	X*	X*		X	X	X	X							
Plasma cytokine level [h, R]	X			X		X		X						
MRI study (Optional for Hong Kong site only) [R]	X [g]			X			X		X				X	
HIV, hepatitis B and C serology [j]	X													

Baseline blood tests are to be conducted within 1 week prior to start of protocol therapy except for the HIV, hepatitis B and C serology which may be done within 4 weeks prior to starting study treatment. Scans and x-rays must be done < 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All visits may be held within ± 2 days of scheduled visit.

A: Administration schedule of nivolumab

a: Baseline chemistry: Albumin, alkaline phosphatase, total bilirubin, urea, calcium, creatinine, fasting glucose, LDH, phosphorus, potassium, total protein, SGPT [ALT] (or AST if available at the center), sodium, TSH (with reflexive Free T4 and Free T3), amylase. (* The tests done at 'pre-study' period can be used as 'week 1' bloods as long as they are done within 7 days of the 1st dose. Baseline serum absolute lymphocyte count will be collected.)

b: A serum or urine pregnancy testing is required within 24 hrs of study enrollment.

c: Off-study evaluation should be performed within 30 days of coming off study. Should reinforce criteria for follow up for 100 days for AE assessment.

d: Laboratory tests to be done within 72 hrs prior to re-dosing: CBC w/ differential, LFTs, serum urea and creatinine, Ca, Mg, Na, K, LDH, random glucose, amylase.

e: Laboratory tests to be done every 4 weeks: TSH (with reflexive Free T4 and Free T3)

f: Retrieval of archived tumor block/ unstained slides is mandatory. Each site should retrieve the specimen within 4 weeks of study registration. If a patient does not have archived tissue available, a fresh tissue sample must be obtained by biopsy at this time.

g: MRI study (section 9.2.3): This optional study applies only to the Hong Kong site and will be performed at baseline, weeks 3, 6, 8 and 16. For the patient's convenience, the contrast-enhanced sessions of the MRI scans that are required at baseline, week 8 and 16, should be scheduled on the same day (if possible) as the mandatory radiological assessments (e.g. CT or MRI). For the other time points that happen to fall on nivolumab administration days (i.e. week 3 and 6), the MRI scan should be scheduled after nivolumab has been given and not on the same day. An interval of at least 5 days should be allowed between consecutive scans.

h: Plasma EBV DNA sampling time intervals – see section 9.2.1.2. Plasma cytokine level: see section 9.2.3.

i: Standard contrast CT will be done for assessing non-local recurrences. MRI will be used for assessing local recurrence. If dual PET and contrast CT is used to assess non-local recurrences at baseline, all subsequent radiological assessments must include a PET-CT. Starting at Week 16, imaging may be performed within ± 7 days

j: HIV and hepatitis B and C serology: See section 3.2.9-3.2.11. If the HIV and/or hepatitis B and C status is unknown at the time of study registration, these tests must be performed during screening and may be performed within 4 weeks prior to starting study treatment.

R: Research, not standard of care (SOC)

11. MEASUREMENT OF EFFECT

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) guidelines (version 1.1). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline. [42]

11.1 Schedule of Evaluations

For the purposes of this study, patients should be re-evaluated for objective tumor response at week 8 after the first dose of nivolumab, and then at week 16 and week 24, and then every 12 weeks thereafter. In addition to a baseline scan, confirmatory scans should also be obtained within 8 weeks (not less than 4) following initial documentation of objective response.

11.2 Definitions of Measurable and Non-Measurable Disease

11.2.1 Measurable Disease

A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥ 2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

A superficial non-nodal lesion is measurable if its longest diameter is ≥ 1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

A malignant lymph node is considered measurable if its short axis is ≥1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

Note: Tumor lesions in a previously irradiated area are not considered measurable disease.

11.2.2 Non-Measurable Disease

All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: '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. In addition, lymph nodes that have a short axis <1.0 cm are considered non-pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.3.1 Measurement Methods

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.
- Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

11.3.2 Acceptable Modalities for Measurable Disease

- Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

- 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. The lesions should be measured on the same pulse sequence. 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: 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.

- 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 scans are preferable.

- Physical Examination: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. In the case of skin lesions,

documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

- FDG-PET: FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A 'positive' FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered 'negative.' 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:

        i. If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

        ii. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal PDG-PET scan.

        iii 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, it is not classified as PD.

11.3.3 Measurement at Follow-up Evaluation

A subsequent scan must be obtained at least 4 weeks (within 8 weeks but not less than 4 weeks apart) following initial documentation of an objective status of either complete response (CR) or partial response (PR).

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.

Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain.)

11.4 Measurement of Effect

11.4.1 Target Lesions and Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.

- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.

- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.4.2 Non-Target Lesions and Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with 11.433.

11.4.3 Response Criteria

All target lesions and target lymph nodes followed by CT/MRI/PET-CT/Chest X-ray/physical examination must be measured on re-evaluation at evaluation times specified in Section 11.1. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

Evaluation of Target Lesions

- Complete Response (CR): All of the following must be true:
  a. Disappearance of all target lesions.
  b. Each target lymph node must have reduction in short axis to < 1.0 cm.

- Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (see Section 11.41).

- Progression (PD): At least one of the following must be true:
  a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
  b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.
  c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

Evaluation of Non-Target Lesions and Non-target Lymph Nodes

- Complete Response (CR): All of the following must be true:

  a. Disappearance of all non-target lesions.
  b. Each non-target lymph node must have a reduction in short axis to < 1.0 cm.

- Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.

- Progression (PD): At least one of the following must be true:

  a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
  b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (Note: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)
  c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

11.44 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following table:

Target Lesions and Target Lymph Nodes	**Non-Target Lesions and Non-Target Lymph Nodes**	**New Sites of Disease**	**Overall Objective Status**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration: Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. A patient is classified as having PD due to "symptomatic deterioration" if any of the following occur that are not either related to study treatment or other medical conditions:

- Weight loss >10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

11.5 Antitumor Effect – Immune Response Criteria

Wolchok et al [34] described the Immune Response Criteria (IRC) which was used to evaluate response to nivolumab and ipilimumab in melanoma. The IRC is derived from the WHO criteria and is characterized by the following features:

- Objective response is based on the total measurable tumor burden: For the irRC, only index and measurable new lesions are taken into account.

- At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (≥5 × 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumor burden:

$$\text{Tumor Burden} = \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new, measurable lesions}}$$

- Complete response: Disappearance of all lesions in two consecutive observations not less than 4 weeks apart.
- Partial response: ≥ 50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart (within 8 weeks and not less than 4 weeks apart).
- Stable disease: 50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir.
- Progressive disease: At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart.
- At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out PD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening).

Measurable lesions	Non-measurable Lesions		Overall Response
Index and new, measurable lesions (tumor burden),* %	Non-index lesions	New, non-measurable lesions	
↓100	Absent	Absent	CR†
↓100	Stable	Any	PR†
↓100	Unequivocal progression	Any	PR†
↓≥50	Absent/ Stable	Any	PR†
↓≥50	Unequivocal progression	Any	PR†
↓<50 to <25↑	Absent/ Stable	Any	SD
↓<50 to <25↑	Unequivocal progression	Any	SD
≥ 25 ?	Any	Any	PD

*Decreases assessed relative to baseline, including measurable lesions only (> 5 × 5 mm).
†Assuming response (CR) and progression (PD) are confirmed by a second, consecutive assessment at least 8 weeks apart in this protocol.

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

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will 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 an 18-36 month basis as part of routine cancer center site visits for U.S. and Canadian sites. CTRG sites will be audited under the previously established procedures for Mayo P2C CTRG participation. 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 Collaborative Agreements Language

The agent(s) 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(ies) (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 Background/Overview

In a study of nivolumab alone, the response rates were 18% in lung cancer, 28% in melanoma and 27% in renal-cell cancer (RCC). Over 60% of responders have durable responses lasting > 1 year [23], the PFS at 24 weeks was 41% for melanoma, 26% for lung cancer and 56% for RCC. In the phase 1 study, the median duration of therapy was 12 weeks (range, 2 to 111), and the objective response rates were: 17% in melanoma, 11% in RCC, 10% in lung cancer and 5% in ovarian cancer. Responses > 1 year were reported in 50% of patients [35]. In general, there is a paucity of data on PFS at 24 weeks, SD rate and other survival data from studies involving multiply-treated NPC patients. Data from phase II 2nd line studies of cytotoxic agents such as a gemcitabine and capecitabine, reported the SD rate to be around 30-49%, median TTP of 3.6-5 months, and *PFS at 6 months of around 30-40% (*estimated from the published curves) [36-38]. The overall response rate (ORR) for cytotoxic agents in patients who failed only 1 prior line of chemotherapy is estimated to be around 20%, but the response is usually not durable [36-38]. The problem of using such data for this proposal is that nearly all patients from these studies had just 1 prior line of therapy, while the current proposal (and also published studies on nivolumab) enrolled patients who were more heavily pre-treated.

Therefore, we have decided to use the data from two of our prior phase II studies of targeted agents, namely, pazopanib and cetuximab (the latter in combination with carboplatin) for calculating the sample size in this proposal because the cohort in these studies are similar to the current proposal. In the pazopanib study, patients had a median of 3 lines of therapy, and clinical benefit rate (CBR, PR + SD + CR) at 12 weeks was used to calculate the sample size. A CBR of 35% was estimated to be clinically meaningful, and the actual rate reported was > 50% [39]. The ORR was 6.1%, median TTP was 4.4 months (95% CI: 3.9–5.8 months) and the PFS-6 month was around 30-35% (read from the curve). In the cetuximab study [40], 70% of patients had 1 prior line of chemotherapy only. Although the ORR was 11.7% and the CBR rate was 60% (which is higher than the pazopanib study), the median TTP was quite short at 2.7 months, and the PFS-6 months was around 20-30% (read off the curve) [40]. Therefore, the average PFS at 24 weeks (6 months) is around 25-30% a benchmark for heavily-pretreated NPC patients, with a ORR between 6% to 20%.

These prior studies have shown that the response rate is generally around 5% in this disease setting [39-40]. If Nivolumab can increase the response rate to 20% or more, that would be exciting and worth further study. This protocol will assess the confirmed response rate as the primary endpoint. In addition this study will assess multiple secondary endpoints, including adverse events, tumor response to nivolumab based on immune-related response criteria (IRC), duration of response, progression-free survival (PFS), overall survival (OS), and laboratory correlates.

13.1.2 Primary Endpoint

The primary endpoint of this trial is the confirmed response rate using RECIST 1.1 criteria. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be considered evaluable for the primary endpoint. A confirmed tumor response is defined to be a CR or PR noted as the objective status on 2 consecutive evaluations at least 4 weeks apart (within 8 weeks and not less than 4 weeks apart).

- For late responders to nivolumab (see section 5.7.1): Subjects who continue treatment beyond first progression (as determined by the initial investigator-assessed, RECIST 1.1-defined progression) will be considered to have investigator-assessed 'progressive disease' at the time of the initial progression event. However, if they subsequently experience a CR or PR, they may be designated as a 'delayed responder' for the purposes of determining ORR and thus, in the final analysis, be counted as a 'responder' for the study.

13.1.3 Statistical Design Overview

A patient that has a confirmed response is considered a treatment "success". The following one-stage design with an interim analysis requires a maximum of 35 evaluable patients, and simultaneously discriminates between tumor response rates of 5% vs. 20%.

13.1.4 Final Analysis Decision Rule

Enroll 35 evaluable patients. If at least 4 patients have a confirmed tumor response (at least 11%) among the 35 evaluable patients, this agent would be considered worthy of further testing in this disease. If 3 or fewer patients have a confirmed tumor response, this agent will be considered negative and no further study would be warranted.

Interim Analysis Decision Rule**:** Enter 20 evaluable patients. If 2 or more of these 20 evaluable patients have a confirmed tumor response, the study will continue to full accrual. Otherwise, if fewer than 2 confirmed responses are observed, the study will be stopped early and the treatment will be considered ineffective in this patient population. Accrual will continue through the interim analysis phase, unless undue toxicity is observed.

13.1.5 Power and Significance Level

This design has 90% power to detect a true confirmed response rate of 20%, with a significance level of 0.09 if the true confirmed response rate is 5%. The likelihood of stopping at stage 1 of enrollment is 64.5% and 7% if the true confirmed response rate is 5% and 20%, respectively.

13.1.6 Over Accrual

If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making process.

13.1.7 Other Considerations

Toxicity, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries or changes in standard care will be taken into account in any decision to terminate the study.

13.2 Sample Size/Accrual Rate

13.2.1 Sample Size

A total of 35 evaluable patients will be accrued per study design, unless the study is permanently closed at interim analysis or undue toxicity is observed. We anticipate accruing an additional 5 patients in order to account for ineligibility, cancellation, major treatment violation, or other reasons. Therefore, maximum accrual is 40 patients for this trial.

13.2.2 Accrual Time and Study Duration

We expect to accrue about 2 patients per month to this trial. Therefore, the accrual period for this Phase II study is expected to be about 20 months. The final analysis can begin approximately 26 months after the trial begins, i.e. as soon as the last patient has been observed for at least 6 months.

13.3 Stratification Factors and Subset Analyses for Primary Endpoint

Since the study is not randomized, there are no stratification factors for randomization purposes. In an exploratory analyses, response rates will also be summarized in the following subsets: PD-L1, PD-L2, PD-1 and PD-1 IHC expression levels (positive vs. negative expression), number of lines of prior chemotherapy for metastatic or recurrent NPC (1 or > 1 line), and prior exposure to EBV vaccines.

13.4 Analysis of Secondary Endpoints

This study will assess multiple secondary endpoints, including adverse events, tumor response to nivolumab based on immune-related response criteria (IRC), duration of response, progression-free survival (PFS), overall survival (OS), and laboratory correlates. The analysis population for these endpoints will be the same as that for the primary endpoint – i.e., all patients meeting the eligibility criteria who have signed a consent form and have begun treatment.

13.4.1 Adverse Events

All patients that have initiated treatment will be considered evaluable for assessing adverse events. The maximum grade for each type of adverse event will be recorded for each patient, and frequency tables will be reviewed to determine adverse event patterns. Only the severe or worse adverse events will be assessed, regardless of relationship to the study treatment. In addition, the following adverse events will be captured and followed over time: pneumonitis, endocrinopathy, infusion-related reactions, diarrhea, skin events [23].

13.4.2 Immune-Related Response

Tumor response to nivolumab based on immune-related response criteria (IRC) will also be assessed. Responses will be summarized descriptively using frequencies and percentages. See section 11 for details on IRC response.

13.4.3 Duration of Response

Defined for all evaluable patients who have achieved an objective response as the date at which the patient's objective status is first noted to be either a CR or PR to the date progression is documented for both response criteria (RECIST 1.1 and IRC).

13.4.4 Progression-Free Survival (PFS)

Defined as the time from registration to the first of either death due to any cause or progression. The distribution of PFS will be estimated using the method of Kaplan-Meier [46].

13.4.5 Overall Survival

Defined as the time from registration to death due to any cause. The distribution of survival time will be estimated using the method of Kaplan-Meier [46Special attention will be paid to any registered patient who dies early (i.e., within 60 days) of initiating their treatment. Circumstances surrounding such early deaths will be classified as being either due to malignant disease, toxicity, other causes, or of unknown reasons.

13.4.6 Laboratory Correlative Studies

All analyses with respect to the translational component of this study are intended to be hypothesis-generating and descriptive in manner. For this study, we will investigate the effect of nivolumab on tumor burden by analyzing the clearance of plasma Epstein-Barr virus (EBV) DNA during the first 4-6 weeks of treatment. In addition, we will investigate the association between treatment outcome and the following immunological markers: Intratumoral expression of PD-1 and PD-L1 in archived NPC tissues, serum absolute lymphocyte count at baseline and post-treatment, expression of PD-1 in CD8+ T cells in tumor infiltrating lymphocytes (TIL) at baseline. Plasma EBV DNA half-life during the first 6 weeks of treatment will be correlated with RECIST-response to nivolumab.
The Chi-Square (or Fisher's Exact test) will be used to assess the association of categorical clinical data with categorical biomarker data. Time-to-event clinical data (PFS, OS) will be correlated with biomarker data using Kaplan-Meier methodology and Cox regression models. Logistic regression models will also be used to predict binary clinical data with baseline biomarker data. Finally, graphical methods and descriptive statistics will be used to summarize the data as well. Two-sided p-values < 0.05 will be considered statistically significant. For the functional MRI parameters, graphical methods and descriptive statistics will be used to assess the changes in these parameters over time. In addition, we will determine the associations between the MRI parameters and clinical endpoints like response and survival.

13.5 Adverse Event Stopping Rule

13.5.1 Monitoring

The principal investigator and the study statistician will review the study periodically (every 4-6 months, or more frequently if clinically indicated) to identify accrual, toxicity, and any endpoint problems that might be developing.

13.5.2 Adverse Event Stopping Rule

Based on prior studies using nivolumab at the dose and schedule to be used in this study, we expect approximately 10% of patients to experience grade 4+ adverse events (at least possibly related to study medication). In this study, we will suspend accrual to allow for a full review of the data, if any of the following occur:

(1) If at any time, 4 of the initial 20 patients (or 20% of all patients when accrual is greater than 20) have experienced any grade 4 adverse event (at least possibly related to the study medication).

(2) If at any time, 2 of the initial 20 patients (or 10% of all patients when accrual is greater than 20) have experienced any grade 5 adverse event (at least possibly related to the study medication). Of course, we will also closely monitor each grade 5 event on a case-by-case basis, and may immediately suspend accrual after one grade 5 event if we feel it is best for patient safety.

After consideration by the study team [ie, Study Chair(s), Statistician, P2C Operations Office, etc] and consultation with representatives at the Cancer Therapy Evaluation Program (CTEP) and the NCI Central IRB (CIRB) , a decision will be made as to whether and how the study will proceed.

13.6 Inclusion of Women and Minorities

Non-keratinizing NPC has a unique epidemiological predilection for southern China, Southeast Asia, the Arctic, and the Middle East/North Africa. Furthermore, there is a male predominance.

Racial Categories (U.S.A sites)	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	1	0	0	1
Asian	0	1	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More Than One Race	0	0	0	0	0
Total	0	2	0	0	2

Racial Categories (Outside U.S.A)	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	9	28	0	0	37
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More Than One Race	0	1	0	0	1
Total	9	29	0	0	38

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino – all other ethnicities not listed above

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

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).
- Investigators encounter any of the stopping criteria described in Section 5.3.

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.
- Investigators encounter any of the stopping criteria described in Section 5.3.

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.

REFERENCES

1. Lo, K.W., G.T. Chung, and K.F. To, Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Semin Cancer Biol, 2012. **22**(2): p. 79-86.
2. Comoli, P., et al., Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. J Clin Oncol, 2005. **23**(35): p. 8942-9.
3. Lin, X., et al., CD4 and CD8 T cell responses to tumour-associated Epstein-Barr virus antigens in nasopharyngeal carcinoma patients. Cancer Immunol Immunother, 2008. **57**(7): p. 963-75.
4. Straathof, K.C., et al., Treatment of nasopharyngeal carcinoma with Epstein-Barr virus--specific T lymphocytes. Blood, 2005. **105**(5): p. 1898-904.
5. Hui, E.P., et al., Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer Res, 2012. **73**(6): p. 1676-88.
6. Sznol, M. and L. Chen, Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res, 2013. **19**(5): p. 1021-34.
7. Investigator Brochure, Nivolumab, BMS-936558, MDX1106. . 2014.
8. Wolchok, J.D., et al., Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med, 2013. **369**(2): p. 122-33.
9. Woo, S.R., et al., Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res, 2012. **72**(4): p. 917-27.
10. Taube, J.M., et al., Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med, 2012. **4**(127): p. 127ra37.
11. Thompson, R.H., et al., Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res, 2006. **66**(7): p. 3381-5.
12. Thompson, R.H., et al., Costimulatory molecule B7-H1 in primary and metastatic clear cell renal

cell carcinoma. Cancer, 2005. **104**(10): p. 2084-91.

13. Thompson, R.H., et al., Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A, 2004. **101**(49): p. 17174-9.
14. Ohigashi, Y., et al., Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res, 2005. **11**(8): p. 2947-53.
15. Wu, C., et al., Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem, 2006. **108**(1): p. 19-24.
16. Dong, H. and L. Chen, B7-H1 pathway and its role in the evasion of tumor immunity. J Mol Med (Berl), 2003. **81**(5): p. 281-7.
17. Nomi T, et al., Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res, 2007. **13**(7): p. 2151-7.
18. Zitvogel, L., A. Tesniere, and G. Kroemer, Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol, 2006. **6**(10): p. 715-27.
19. Sznol M, Kluger H, Hodi F, et al. Survival and long-term follow-up of safety and response in patients (pts) with advanced melanoma (MEL) in a phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538) in ASCO. J Clin Oncol. 31:ACRA9006.2013.
20. Brahmer JR, L. Horn, S.J. Antonia, et al. Survival and long-term follow-up of the phase I trial of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with previously treated advanced non-small cell lung cancer (NSCLC) in ASCO. J Clin Oncol. 31:A8030. 2013.
21. Drake CG, D.F. McDermott, M. Sznol, et al. . Survival, safety, and response duration results of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in a phase I trial in patients with previously treated metastatic renal cell carcinoma (mRCC): Long-term patient follow-up. in ASCO J Clin Oncol. 31:A4514. 2013.
22. Rizvi NA, SJ Antonia, Chow L, et al. A phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive non-small cell lung cancer (NSCLC) patients (pts). in ASCO. J Clin Oncol. 31:A8072. 2013.
23. Topalian, S.L., et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012. **366**(26): p. 2443-54.
24. Lo, Y.M., Chan AT, Chan LY, et al. Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA. Cancer Res, 2000. **60**(24): p. 6878-6881.
25. Lo, Y.M., Leung SF, Chan LY, et al. Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. Cancer Res, 2000. **60**(9): p. 2351-2355.
26. Chan, A.T., Lo YM, Zee B, et al. Plasma Epstein-Barr Virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. J Natl Cancer Inst, 2002. **94**(21): p. 1614-1619.

27. Ma BB, Kam M, Lo YM, et al. Relationship between pretreatment level of plasma Epstein-Barr virus DNA, tumor burden, and metabolic activity in advanced nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys, 2006. **66**(3): p. 714-20.

28. To, E.W., et al., Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. Clin Cancer Res, 2003. **9**(9): p. 3254-9.

29. Wang, W.Y., et al., Plasma EBV DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. Clin Cancer Res, 2010. **16**(3): p. 1016.

30. Ma B, Boon C, Wan Teck Lim, et al. Multicenter Phase II study of MK-2206 in previously treated patients (pts) with recurrent and metastatic nasopharyngeal carcinoma (NPC): Mayo Clinic Phase II Consortium (Protocol: MC1079). Mol Cancer Ther November 2013 12; B273, 2013. **Abstract B273:**.

31. Le, Q.T., et al., An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. Clin Cancer Res, 2013. **19**(8): p. 2208-15.

32. Ascierto, P.A., et al., Biomarkers for immunostimulatory monoclonal antibodies in combination strategies for melanoma and other tumor types. Clin Cancer Res, 2013. **19**(5): p. 1009-20.

33. Seiwert TY, B.B., Weiss J, Gluck I, et al. , A phase Ib study of MK-3475 in patients with human papillomavirus (HPV)-associated and non-HPV–associated head and neck (H/N) cancer., in Proc ASCO. 2014, J Clin Oncol.

34. Wolchok, J.D., et al., Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res, 2009. **15**(23): p. 7412-20.

35. Brahmer, J.R., et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012. **366**(26): p. 2455-65.

36. Ma, B.B., E.P. Hui, and A.T. Chan, Systemic approach to improving treatment outcome in nasopharyngeal carcinoma: current and future directions. Cancer Sci, 2008. **99**(7): p. 1311-8.

37. Foo, K.F., et al., Gemcitabine in metastatic nasopharyngeal carcinoma of the undifferentiated type. Ann Oncol, 2002. **13**(1): p. 150-6.

38. Chua DT, JS Sham, and GK Au, A phase II study of capecitabine in patients with recurrent and metastatic nasopharyngeal carcinoma pretreated with platinum-based chemotherapy. Oral Oncol, 2003. **39**(4): p. 361-6.

39. Lim WT, et al, A Phase II study of pazopanib in Asian patients with recurrent/metastatic nasopharyngeal carcinoma. Clin Cancer Res, 2011. **17**(16): p. 5481-9.

40. Chan A, Hsu MM, Goh BC, et al. Multicenter, Phase II study of cetuximab in combination with carboplatin in patients with recurrent or metastatic nasopharyngeal carcinoma. J Clin Oncol, 2005. **23**(15): p. 3568-76.

41. Quon H, Brizel DM. Predictive and prognostic role of functional imaging of head and neck squamous cell carcinomas. Semin Radiat Oncol. 2012 Jul; 22(3):220-32.

42. Feng W, Zhang J, Hong S, et al. EBV-driven LMP1 and IFN-γ up-regulate PD-L1 in nasopharyngeal carcinoma: Implications for oncotargeted therapy. Published online: Oncotarget,

October 21, 2014

43. Eisenhauer EA, Therasse P, Bogaert J, et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1. 1). Eur J Cancer 45(2): 228-247, 2009.)

44. King AD, Chow KK, Yu KH, et al. Head and neck squamous cell carcinoma: diagnostic performance of diffusion-weighted MR imaging for the prediction of treatment response. Radiology. 2013 Feb; 266(2):531-8.

45. Vandecaveye V, Dirix P, De Keyzer F, et al. Predictive value of diffusion-weighted magnetic resonance imaging during chemoradiotherapy for head and neck squamous cell carcinoma. Eur Radiol. 2010 Jul; 20(7):1703-14

46. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. J Am Stat Assoc 1958; 53: 457-481.

APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: BIOASSAY TEMPLATES

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Immunohistochemical (IHC) Marker Template For Integral Markers in Clinical Trials

This is a template to describe the analytical and clinical performance of an assay that is essential for performance of a trial. It will be used to assess whether assays are ready for use in a trial by Disease Steering Committees and CTEP. The FDA may also use it to evaluate integral assays and diagnostics for their pre-IDE evaluation. Not all parameters may be known a priori. Please enter as much information as you can and N/A for not available or applicable where appropriate.

This template requires detailed information that may be known only by laboratorians, scientists who work in clinical laboratories, and should be collaborating closely with clinical trialists. Please be sure to collect the appropriate responses before filling out this form. The template has the following sections with information needed from trialists and laboratorians:

1. **Assay, Patient and Specimen Information –Trialists and Laboratorians**
2. **Primary Antibody Characteristics – Laboratorians**
3. **Design of Immunohistochemical Assay - Laboratorians**
4. **Assay Performance – Laboratorians**
5. **Laboratory Information – Trialists and Laboratorians**

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Section 1. Assay, Patient and Specimen Information

A. Name of marker (Please use HUGO gene or protein name for molecular marker or the Atlas for Genetics in Hematology and Oncology for cytogenetic or FISH markers)

PD-L1

HUGO Site: http://www.genenames.org/
Atlas Site: http://atlasgeneticsoncology.org/Index.html

B. How will assay and its marker be used in clinical trial?

○ **Integral Marker** ○ **Integrated Marker** ◉ **Research Marker**

- **Integral markers are required for the trial to proceed (e.g., patient eligibility, assignment to treatment, stratification, risk classifier or medical decision-making - often requires performance in a CLIA laboratory).**
- **Integrated markers are performed on all or a statistical subset of patients but are not used for medical decision-making.**
- **Research markers are all other assays and commonly referred to as correlative research.**
- **For other definitions, please see References at end of form.**

B1. Assay Purpose

Other (Specify): Exploratory study on the association between treatment respons

C. Assay type

IHC

D. Will assay be performed in a Central Reference CLIA lab, multiple CLIA-certified labs, or research labs?

○ **Central Reference CLIA Lab** ○ **Multiple CLIA Labs** ◉ **Research Labs**

E. Anatomic source of specimens (organ site)

Oral Cavity
Oropharynx
Other
Pancreas

E1. Type of Specimen

Tumor Tissue

E2. Tissue collection

Mandatory - must be performed on trial

F. Patient conditions or co-morbidities that may affect assay and must be noted:

Only archived, paraffin-embedded nasopharyngeal cancer (NPC) samples will be used.

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

G. Preanalytic Specimen Requirements

G1. Maximum Warm Ischemia time (=time from cutting blood supply to removal from body) allowed in minutes if known:

Not applicable

G2. Maximum Cold Ischemia time (=time until specimen fixed/frozen after removal from body) allowed in minutes if known:

1 hour

G3. Type of stabilization of Specimen: (●) **fixed** ( ) **frozen** ( ) **both**

G3a If fixed, what fixation buffer to be used? Other
Bouin's
10% Neutral Buffered Formalin

G3b. If Other fixative, what is it? (free text)

Not applicable

G3c What is shortest fixation time allowed (Hours or fraction thereof)

(for biopsy) the shortest fixation time allowed is "4 hour

G3d What is longest fixation time allowed (Hours or fraction thereof)

(for biopsy) the longest fixation time allowed is "48 hou

G3e If frozen, how will specimen be frozen:

Embedded in OCT and then frozen

H. How will specimens be stored?

Room Temperature

I. Specimen size to be stored 2 **length** 2 **width** 0.5 **height in cm**

J. Tissue section thickness on slide in microns 4µm

K. Antigen retrieval solution/procedures

IHC on NPC biopsy: PD-L1
All specimens were fixed in 10% buffered formalin and sectioned at a thickness of 4µm. Paraffin-embedded sections were deparaffinized by dissolving the paraffin in xylene and then rehydration with a gradient of ethanol and water. The EnVision™ Detection Systems Perosidase/DAB, Rabbit (Dako) was used according to manufacturer's instructions.
The sections were incubated with anti-PD-L1 antibody (NBP1-03220, Novus) at 1:500 dilution at room temperature for 1 hour. The sections were then treated with 3%H_2O_2 in TBS for 15 minutes to block the endogenous peroxidase activity. After blocking, the sections were washed with TBS and incubate with HRP-conjugated anti-rabbit polymer at room temperature for 1 hour. Bound antibody was visualized with 3,3

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Section 2. Primary Antibody Characteristics

A. Source of primary antibody (purchased from xxx as lot # xxx, or generated in house, etc.)

Anti-PD-L1 antibody (NBP1-03220, Novus) at 1:500 dilution.

B. What was the immunogen (e.g., peptide, oligosaccharide, phosphorylated protein, other)?

○ Protein ◉ Peptide ○ Oligosaccharide ○ Phosphorylated Protein ○ Other

B1. Please describe if Other

C. Species of immunogen (e.g., human or mouse gene product)

Human

D. Are there specific isoform(s) of the immunogen that are recognized (e.g., one or all isoforms or unknown)?

○ One Isoform ◉ All Isoforms ○ Unknown

E. Preparation of immunogen (e.g., purified protein, recombinant, synthetic peptide or oligosaccharide)

○ purified protein ○ recombinant ◉ synthetic peptide ○ oligosaccharide

F. Other attributes of primary antibody (e.g., mono- or polyclonal)

○ Monoclonal ◉ Polyclonal

F1. What species: Rabbit

F1a. If other species, what is it? Include chicken

Not applicable

G. How was the antibody specificity demonstrated?

Western Blot

G1. Please specify if Other

Not applicable

H2. Are there band(s) at the expected mass(es) on Western blot?

(●) Yes ( ) No ( ) Unknown

H2a. If not, please explain

H3. Is immunostaining abolished in knock out/knock-down cells or with epitope-absorbed antibody?

( ) Yes ( ) No (●) Unknown

H4. Is immunostaining abolished when antibody absorbed or blocked with epitope?

( ) Yes ( ) No (●) Unknown

I. What is the targeted organ/tissue/cell (e.g., normal melanocytes? breast ductal carcinoma)?

Only NPC tumor cells are stained.

I1. What non-targeted organ/tissue/cell is also stained?

Non-malignant cells including lymphocytes and other stromal cells in nasopharyngeal carcinoma biopsies are negative for PD-L1 staining.

J. Have any cross-reactive proteins or peptides been identified that may confound interpretation of IHC?

( ) Yes (●) No ( ) Unknown

J1. If yes and known, what are they?

K. Is antigen stable when the period between tissue sectioning and staining is

( ) <7 days (●) 7-30 days ( ) >30 days ( ) Not Known

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Section 3. Design of Immunohistochemical Assay

A. Assay Design (Complete assay details are needed if multiple labs will perform the assay).

A1. Describe the platform of the assay, e.g. instrument (manufacturer, model, UDI number if known)

A1a. Platform The assay is conducted manually.

A1b. Manufacturer Not applicable

A1c. Model Number Not applicable

A1d. UDI Number (Universal Device Number) Not applicable

A1e. Is the platform cleared or approved by the FDA

○ Yes ○ No ◉ Unknown

A2. Is there an SOP?

○ Yes ○ No ◉ Unknown

A2a. Is the SOP attached as an Appendix?

○ Yes ○ No ◉ Unknown

B. Type of Immunoassay

B1. Is the assay qualitative, semiquantitative or quantitative

○ Qualitative ◉ Semiquantitative ○ Quantitative

B1a. If an image analyzer is used, what manufacturer and model was used?

Not applicable

B1b. Is it cleared or approved by the FDA

○ Yes ○ No ◉ Unknown

B2. Nature of reporter signal

Not applicable

B3. Assay method (e.g. direct, indirect, 3-step immunoperoxidase assay)

○ Direct ○ Indirect ◉ 3-step immunoperoxidase ○ Other

If other, please specify

Not applicable

B3a. What secondary reagent(s) is used for the indirect or 3-step assay

See above

C. Are there positive and negative controls for the assay

○ Yes ◉ No ○ Unknown

C1. If there are controls, what are they?

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

D. Specimen size – What is the smallest specimen that can be analyzed by the assay in cm?

0.1 **cm**

D1. Is the minimum specimen size determined by a particular characteristic of the tissue?

(●) **Yes** ( ) **No** ( ) **Unknown**

D1a. If so, is it (●) **Number of cell nuclei** ( ) **Nuclear area** ( ) **Cytoplasmic area** ( ) **Other**

D1b. Please specify if Other

Not applicable

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Section 4. Assay Performance

A. Details regarding how the analyte is measured

A1. What statistical test(s) were used to validate the assay results.

Not applicable

A2. How was a clinically relevant threshold selected?

Literature

A3. Were results obtained on retrospective or prospective data sets?

Retrospective
Prospective

Sample Size 20 (pilot stud

A3a. Training sets or other validation method Other Method, please specify: Not applicable

A4. What is the cut-off?

>/= 1% of NPC tumor cells or >/= 1% Tumor infiltrative lymphocytes (TIL) stained is considered as positive

A5. How well was the cut-off validated before using it in these trials?

Not applicable

A6. Were assay conditions standardized to minimize variance, e.g., automated tissue processors and/or stainers)?

○ Yes ◉ No ○ Unknown

A6a. If yes, what tissue processor/stainer was used?

A7. If calibrators or controls were used, were they stained separately with each batch of slides, included on each slide or internal controls?

A7a. Were calibrators/controls used?

○ Yes ◉ No ○ Unknown

A7b. Were the controls stained as separate slides with slides?

○ Yes ◉ No ○ Unknown

OR **A7c. Were the controls included in each slide and stained as internal controls?**

○ Yes ◉ No ○ Unknown

OR **A7d. Were the controls not stained in each staining run?**

○ Yes ◉ No ○ Unknown

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

B. Reproducibility of assay

B1. Was reproducibility assessed?

○ Yes ◉ No ○ Unknown

B1a. If yes, please describe the specimen type(s) used

B1b. If not, please explain

Research biomarker only

B2. How many replicates were done?

Triplicates

B3. What is the intra-lab reproducibility (%CV)?

Not applicable - research tool only

B4. What is the inter-lab reproducibility (same specimens, different lab, number of different technicians)?

Not applicable - research tool only

B4a. How many on the same specimens?

Not applicable - research tool only

B4b. How many different labs?

Not applicable - research tool only

B4c. How many different technicians?

Not applicable - research tool only

B4d. What types of specimens (e.g., tissue sections, TMA)?

Not applicable - research tool only

B4e. Over how many different days?

Not applicable - research tool only

B4f. How many readers?

Not applicable - research tool only

B5. What is the agreement between readers?

Intepretation by one pathologist

B5a. How are differences resolved?

Different runs of the same assay

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

C. Image Measurement

C1. What strategy was used to select the fields to be analyzed?

Select the tumor area by an experienced pathologist under light microscopic examination

C2. How was a threshold to distinguish positive from negative determined?

>/= 1% of NPC tumor cells or >/= 1% Tumor infiltrative lymphocytes (TIL) stained is considered as positive. Otherwise is considered as negative

C3. How were the cells of interest distinguished from other cells?

The NPC tumor cells, Tumor infiltrative lymphocytes (TIL) are recognized and distinguished from other cells by an experienced pathologist

C4. Was reference material used to generate a standard curve?

○ Yes ◉ No ○ Unknown

C4a. What was the reference material?

C4b. Has it been cleared by the FDA?

○ Yes ○ No ◉ Unknown

D. Assay Discrimination

D1. What is the accuracy of the assay for detecting the analyte?

(Not applicable)

D2. How are staining and tissue artifacts identified and handled (especially if image analysis is used)?

(Not applicable)

LOI/Concept/Protocol #: LOI: 9742 Protocol Investigator: Brigette Ma

Section 5. Laboratory Information

A. Is the lab a research or clinical lab?

(●) **Research** ( ) **Clinical**

B. Does the lab meet GLP standards

(●) **Yes** ( ) **No** ( ) **Unknown**

C. What is the training and experience of the Technicians/Operators?

The immunohistochemistry analysis is performed in the research laboratory of Prof Kwok Wai Lo, (Ph.D. (Chinese university of Hong Kong, CUHK); M.Phil (CUHK); BSc. (CUHK); Associate of Institute of BioMedical Science, U.K. (AIBMS). Hist research interest is in the molecular genetics of nasopharyngeal carcinoma (NPC); Roles of EBV in NPC tumorigenesis; New therapeutic approaches for NPC; Tumor microenvironment and cancer stem-like cells in NPC.

References

Section	Ref #	Citation
1.	20215558	Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, Parchment R, Ratain MJ, Shankar LK, Stadler WM, True LD, Gravell A, Grever MR; Biomarkers Task Force of the NCI Investigational Drug Steering Committee. Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res. 2010 Mar 15;16(6):1745-55.
1.A	18327244	Deutsch EW, Ball CA, Berman JJ, Bova GS, Brazma A, Bumgarner RE, Campbell D, Causton HC, Christiansen JH, Daian F, Dauga D, Davidson DR, Gimenez G, Goo YA, Grimmond S, Henrich T, Herrmann BG, Johnson MH, Korb M, Mills JC, Oudes AJ, Parkinson HE, Pascal LE, Pollet N, Quackenbush J, Ramialison M, Ringwald M, Salgado D, Sansone SA, Sherlock G, Stoeckert CJ Jr, Swedlow J, Taylor RC, Walashek L, Warford A, Wilkinson DG, Zhou Y, Zon LI, Liu AY, True LD. Minimum information specification for in situ hybridization and immunohistochemistry experiments (MISFISHIE). Nat Biotechnol. 2008 Mar;26(3):305-12.
1.B	OBBR	https://brd.nci.nih.gov/BRN/brnHome.seam?conversationId=12427
1.F1	12414521	Dash A, Maine IP, Varambally S, Shen R, Chinnaiyan AM, Rubin MA. Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. Am J Pathol. 2002 Nov;161(5):1743-8.
1.F2	19734848	Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkarni S. Delay to formalin fixation effect on breast biomarkers. Mod Pathol. 2009 Nov;22(11):1457-67.
1.F1-2.	19415952	Middleton LP, Price KM, Puig P, Heydon LJ, Tarco E, Sneige N, Barr K, Deavers MT. Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases. Arch Pathol Lab Med. 2009 May;133(5):775-80.
2.E	20093391	Brevet M, Arcila M, Ladanyi M. Assessment of EGFR mutation status in lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR. J Mol Diagn. 2010 Mar;12(2):169-76.
	20359301	Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques. 2010 Mar;48(3):197-209.
	18796405	Pradidarcheep W, Labruyère WT, Dabhoiwala NF, Lamers WH. Lack of specificity of commercially available antisera: better specifications needed. J Histochem Cytochem. 2008 Dec;56(12):1099-111.

2.E7 NCBI Blast Search http://blast.ncbi.nlm.nih.gov/Blast.cgi

4. 20524870 Fitzgibbons PL, Murphy DA, Hammond ME, Allred DC, Valenstein PN. Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. Arch Pathol Lab Med. 2010 Jun;134(6):930-5.

ISBN978-0-387-72805-6 Discrete Multivariate Analysis: Theory and Practice. Authors: Paul W. Holland (Author)Stephen E. Fienberg (Author)Yvonne M. Bishop (Author)F. Mosteller (Contributor)R.J. Light (Contributor). Springer. 30 July 2007. English. Paperback, 558 pages

18829475 Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. Clin Cancer Res. 2008 Oct 1;14(19):5967-76.

Appendix to CLSI document IL-28a

Appendix to CLSI document IL-28a

4.B5 2189948 Cicchetti DV, Feinstein AR. High agreement but low kappa: II. Resolving the paradoxes. J Clin Epidemiol. 1990;43(6):551-8.

4.B 11459866 Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001 Jul 18;93(14):1054-61.

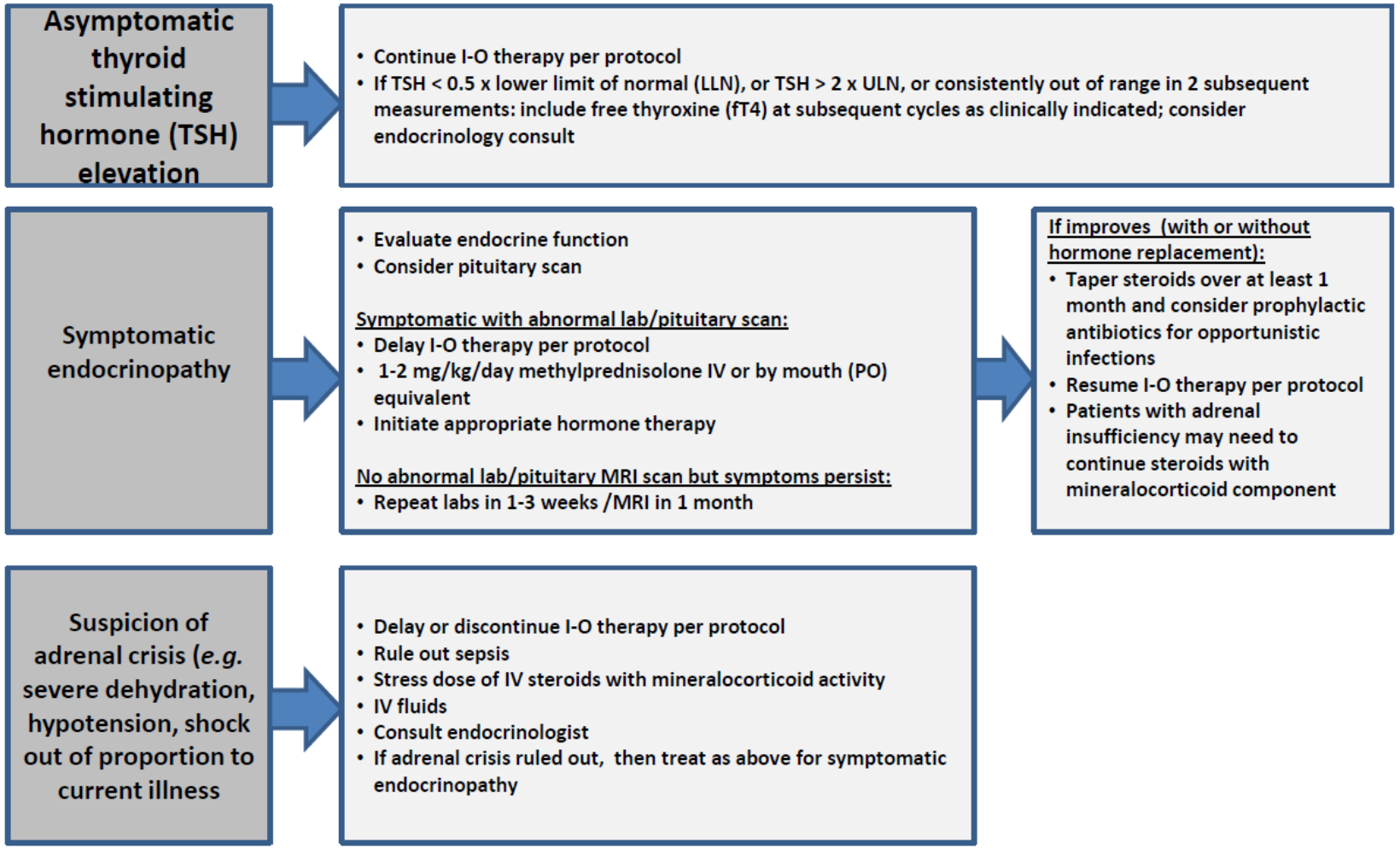
4.B 20586616 Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010 Jul;134(7):e48-72.

4.8 2578140 Gross DS, Rothfeld JM. Quantitative immunocytochemistry of hypothalamic and pituitary hormones: validation of an automated, computerized image analysis system. J Histochem Cytochem. 1985 Jan;33(1):11-20.

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

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

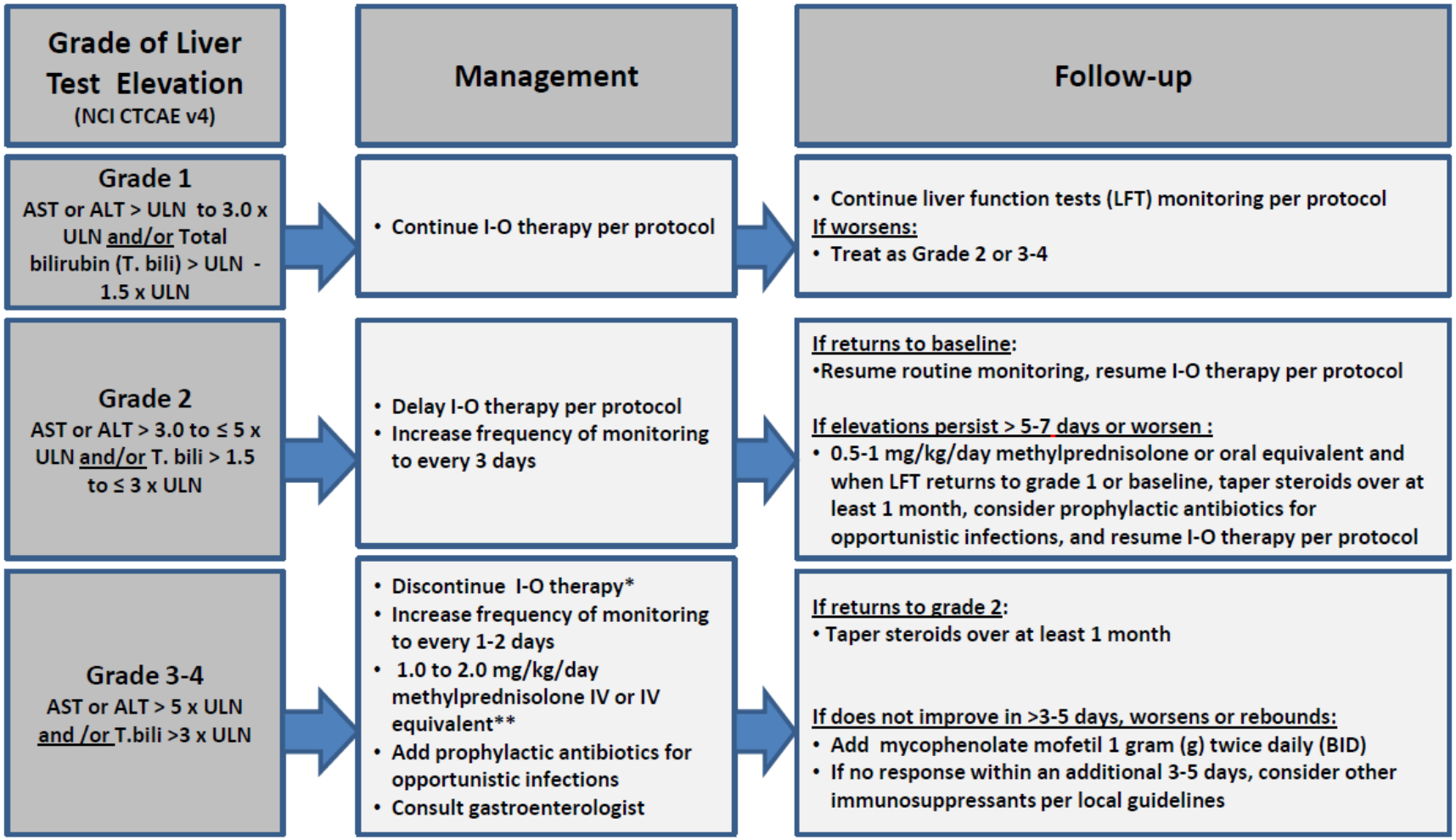
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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.

Pulmonary Adverse Event Management Algorithm

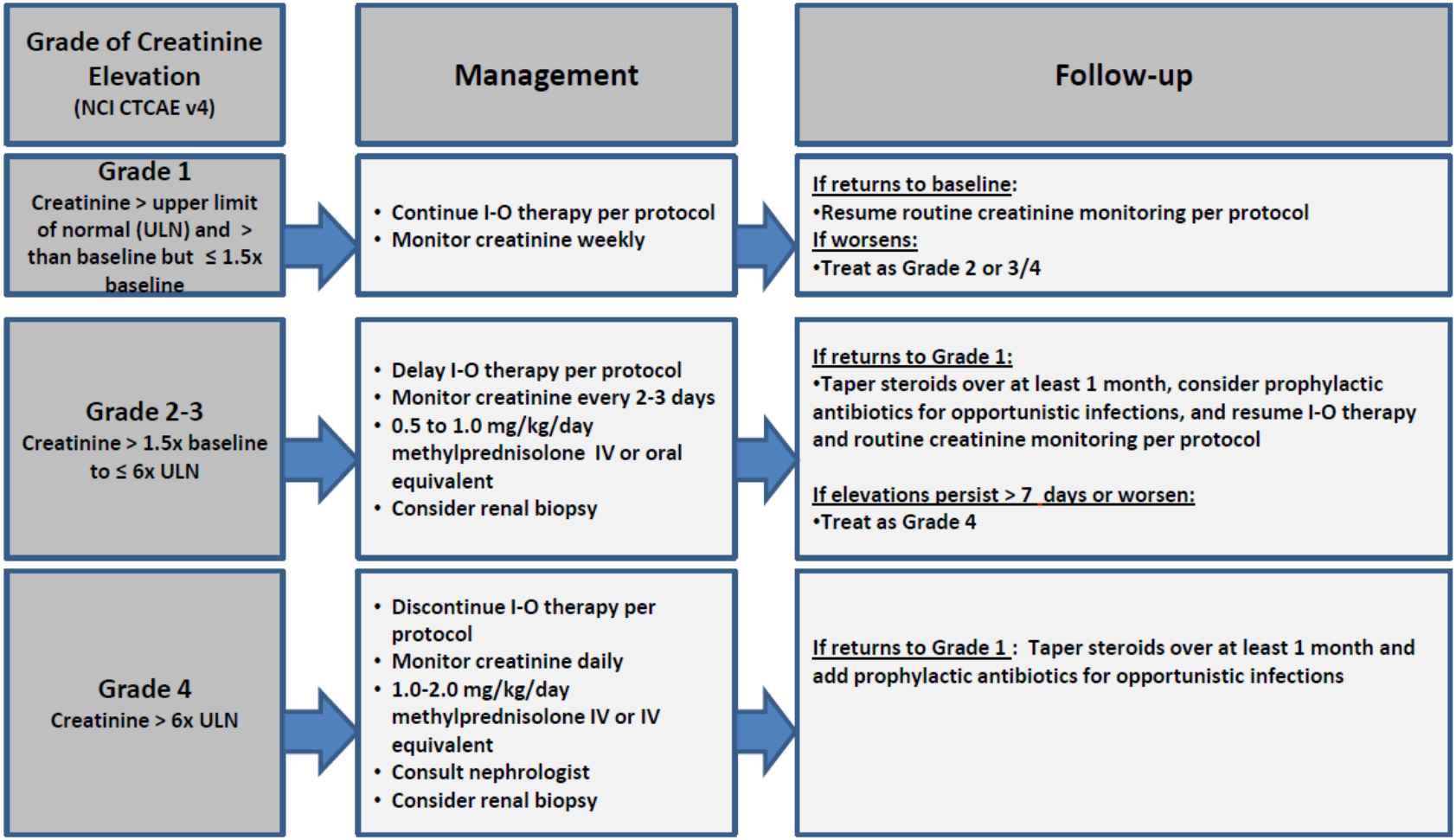
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

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

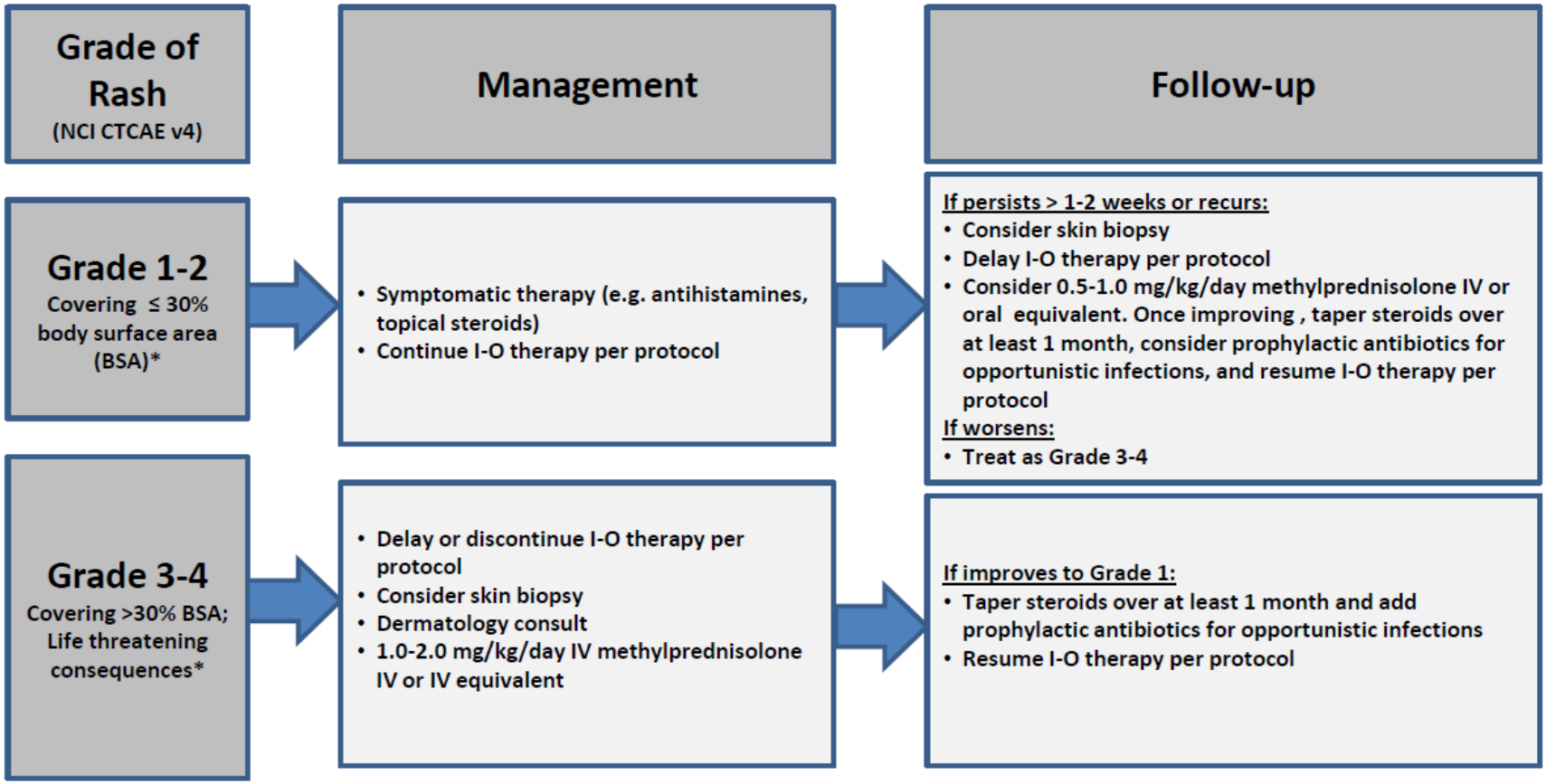
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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.



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 D: CARD FOR STUDY PARTICIPANTS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using nivolumab. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

Although nivolumab is not expected to interact with drugs that are processed by your liver, it is still very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements.
Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**.

These are the things that you and they need to know:

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is: _____ and he or she can be contacted at _____.

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using nivolumab**.** This clinical trial is sponsored by the NCI. Although nivolumab is not expected to interact with drugs that are processed by your liver, you should:

- Tell your doctors if you stop taking regular medicine or if you start taking a new medicine.
- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your study doctor's name is _____

  and can be contacted at _____.