SUMMARY OF CHANGES – Protocol

For Protocol Amendment #5

NCI Protocol #: 9925
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#	Section	Page(s)	Change
1	Global		**Updated protocol version number and date.** *Changed from:* Version 9.0 / September 8, 2017 *Changed to:* Version 10.0 / October 27, 2017
2	Cover page	1	**Added the following Non-Member Collaborator:** IL042 John H Stroger Jr Hospital of Cook County Chicago, IL Paul Rubinstein, MD Telephone: 312-864-7277 Fax: 312-864-9002 prubinstein@cookcountyhhs.org
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NCI Protocol #: 9925

Local Protocol #: 125462

ClinicalTrials.gov Identifier: NCT02631746

TITLE: Phase II Trial of Nivolumab for HTLV-Associated Adult T Cell Leukemia/Lymphoma

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Version 8.0 / May 5, 2017
Version 9.0 / September 8, 2017
Version 10.0 / October 27, 2017

SCHEMA

Nivolumab will be given at 240 mg every 2 weeks for 4 initial doses, followed by up to 42 doses if tolerated. Therapy is continued in each individual until sign of tumor progression or intolerable toxicity, or other endpoint is reached.

Imaging will be performed every 12 weeks for individuals with abnormal imaging at study initiation or during the course of the study.

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1. OBJECTIVES

1.1 Primary Objective

1. To determine the safety and tolerability of nivolumab for patients with HTLV-associated ATLL.

2. To determine the efficacy of nivolumab for patients with HTLV-associated ATLL.

1.2 Secondary Objectives

1. To determine effects of nivolumab on HTLV-1 proviral DNA and RNA loads.

2. To determine the effects of nivolumab on anti-HTLV-1 and anti-ATLL immune responses.

3. To determine effects of nivolumab on HTLV-1 integration site clonality.

2. BACKGROUND

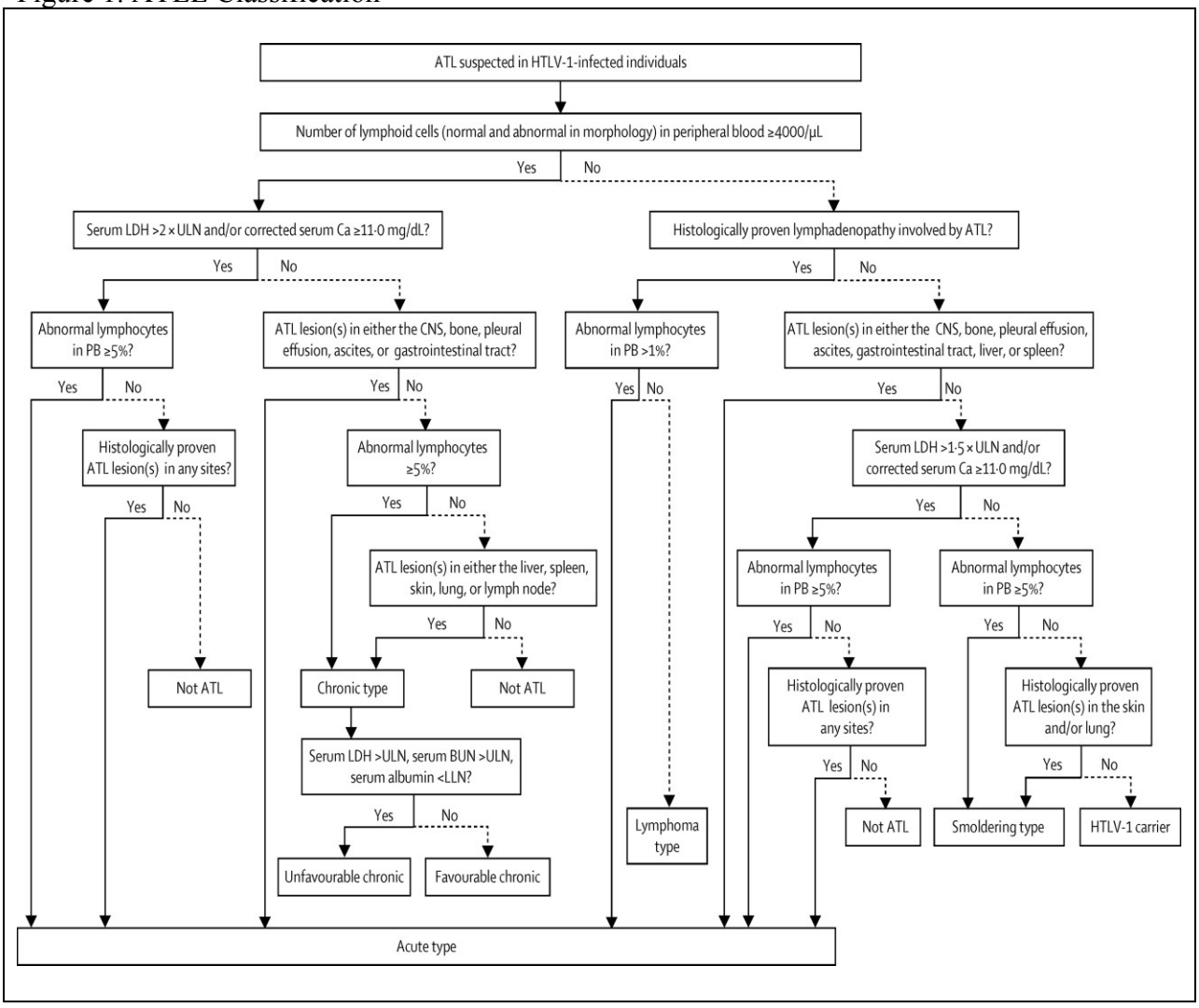
2.1 HTLV-1 Infection

Human T-cell leukemia virus type 1 (HTLV-1) is a delta retrovirus [1]. It is closely related to simian T-cell leukemia virus type 1 (STLV-1) and phylogenetic trees show that STLV-1 and HTLV-1 strains are intermingled [2]. A somewhat more distantly related virus, with 60% nucleotide sequence homology to HTLV-1, is HTLV-2. These infections can be distinguished by differences in antibody reactivity to recombinant proteins or by polymerase chain reaction assays. HTLV-1 infections are prevalent in southern Japan, the Caribbean, and many parts of Central and South America, the Middle East, and Africa, where 10-15% of the population is infected [1, 3]. In the United States, 0.25% of volunteer blood donors are infected with HTLV-1 or HTLV-2. Infections by both viruses are not unusual among intravenous drug abusers. HTLV-2 is endemic in native North, Central, and South American populations. The HTLV-1 genome includes *gag*, *pro, pol,* and *env* genes, encoding the inner capsid of the virion, the viral protease, the viral reverse transcriptase and integrase enzymes, and the glycoprotein on the virion surface necessary for viral entry [4]. Regulatory proteins include Tax, a potent transcriptional trans-activator, and Rex, a mediator of nuclear export of viral RNAs. Accessory genes encode proteins designated, p12, p13, p27, p30, and HBZ whose functions remain to be fully characterized.

There is considerable evidence that the Tax protein is critically important for the leukemogenic activity of the virus [5, 6]. Although most viral genes are not expressed in ATLL samples, Tax expression is usually detectable, although often at very low levels. Tax together with Ras is capable of transforming rodent fibroblasts, and when expressed in a retrovirus of *Herpes samirii* vector is capable of immortalizing human lymphocytes. Constitutive Tax expression in transgenic mice results in several different types of malignancies, whereas expression in the lymphoid compartment of a transgenic mouse results in a lymphoproliferative malignancy. Tax functions at transcriptional and post-transcriptional steps. Tax upregulates several transcriptional pathways to

enhance the expression of the viral promoter as well as several interleukins and their receptors (e.g. IL2, IL2Rα, IL15, IL15Rα), cytokines (e.g. IFNγ, GM-CSF), adhesion molecules (e.g. ICAM-1, VCAM-1, VLA-4), growth promoting factors (e.g. c-myc, c-sis, c-fos, egr-1, cyclin D2, and E2F-1), and apoptosis inhibiting factors (e.g. bcl xL, A20). Moreover, Tax can directly bind and enhance or inhibit the activity of several growth promoting (e.g. cyclins D2 and D3, E2F) or inhibiting factors (e.g. p15, p16, and p53 tumor suppressor proteins and cell cycle checkpoint protein MAD1), respectively. In addition Tax promotes the phosphorylation and inactivation of the p53 tumor suppressor protein.

Figure 1. ATLL Classification



Recent findings implicate HBZ as a maintenance factor in ATLL [7]. HBZ is ubiquitously expressed in ATLL cells and HTLV-1 infected cells *in vivo*. Moreover, HBZ promotes proliferation of HTLV-1 infected cells. Interactions between Tax and HBZ on HTLV-1 infected cells are complex, and in some cases opposite. For example, Tax activates the AP-1, NFAT, and

CREB pathways, whereas HBZ suppresses them. Conversely, Tax inhibits TGFbeta signaling, whereas HBZ activates it. Tax activates both the canonical and non-canonical NFkB pathways, whereas HBZ inhibits only the canonical NFkB pathway. Tax promotes cell proliferation but also induces cellular senescence by induction of p21 and p27, whereas HBZ prevents senescence by inhibiting p65. Lastly, HBZ suppresses the canonical Wnt pathway, but enhances the non-canonical pathway, suggesting that HBZ modulates the intra-cellular environment of peripheral T cells targeted by the virus.

2.1.1 HTLV-1 Associated Adult T-Cell Leukemia Lymphoma

Although HTLV-2 is not clearly associated with a clinical disorder, HTLV-1 is closely associated with two clinical disorders [1]. About 5% of HTLV-1 infected individuals eventually develop a form of myelopathy known as HTLV-1 myelopathy (HAM) or tropical spastic paraparesis (TSP). It is characterized by lower limb spasticity, bowel and bladder disturbances, and slow but steady progression over several years. HTLV-1 has also been associated with other inflammatory clinical conditions, including uveitis, arthropathy, myopathy, pneumopathy, and a Sjogren's disease-like syndrome [8]. Some investigators have suggested that these disorders are related to high virus load, overstimulation of dendritic cells, resulting in chronic production of high levels of type 1 interferons and interferon-stimulated gene expression.

About 5% of HTLV-1 infected individuals develop a T-cell non-Hodgkin's lymphoma designated adult T-cell leukemia lymphoma (ATLL). It occurs almost exclusively in individuals who acquired HTLV-1 as a result of breast feeding, although four or five decades are generally required before development of disease. ATLL is characterized by frequent blood and bone marrow involvement, hypercalcemia, and lytic bone lesions. It is most commonly a CD4+ leukemia/lymphoma, although occasional examples of CD8+ lymphoma have been described. ATLL may fit into a variety of pathological subtypes as classified by the International Working Party Formulation. Clinical classifications of ATLL have included a "smoldering" disorder characterized by rash and minimal blood involvement, as well as a chronic form of ATLL, which have median survivals of two years or more (Fig 1) [9]. In contrast, a "lymphomatous" form of ATLL and an "acute" form of ATLL have median survivals of 3-6 months.

2.1.2 HTLV-1 Proviral DNA and Cell-Associated and Virion RNA Load and Protein Expression

Assays of HTLV-1 proviral DNA load using PCR techniques have been developed and applied to clinical samples. These studies demonstrated levels of about 0.02 copies/PBMC in asymptomatic individuals and 0.08 copies/PBMC in HAM patients [10]. RT-PCR assays of cell-associated viral and virion RNA have been developed and used to demonstrate that the majority of infected cells do not express viral products [11]. However, quantitative data on transcript levels has not been obtained with clinical samples.

2.1.3 HTLV-1 Clonality

In ATLL, there is a clonal expansion of T cells with clonal T-cell receptor gene rearrangements

and clonal sites of provirus integration into chromosomal DNA, as determined by Southern blot hybridization [12]. More recently, a ligation mediated PCR technique has been utilized as a more sensitive way to detect different clonal populations in asymptomatic infected individuals, HAM patients, and ATLL patients [13]. These studies have demonstrated that the expanded clone of malignant ATLL has arisen from an oligoclonal expansion of HTLV-1 infected cells. These studies suggest that HTLV-1 dissemination can occur by viral replication and clonal expansion of infected cells.

A recent study examined the genomic characteristics of the integration sites in 197 ATL cases [14]. In 91% of cases, a single predominant provirus was present; in 11% of the total 2 proviruses were present in a single tumor clone. In 9% of cases, no significant difference in the abundance of two integration sites was seen. A complete provirus was present in 46% of cases, 39% of cases contained a defective provirus, 7% contained a nonsense mutation of the *tax* gene, and 8% contained a hypermethylated promoter in the 5'LTR. The host genomic characteristics of the integration site in the large ATL clones closely resembled those of low-abundance clones present in subjects with nonmalignant HTLV-1 infection. These authors concluded that the major determinant of the risk of ATL is the absolute number of clones, i.e. the larger the number, the greater the chance of malignant transformation. They also suggested that the number of HTLV-1 infected clones present in an individual during chronic infection is determined chiefly by the efficiency of the host's CTL response to the virus.

Recently, whole genome/exome and RNA seq was performed on 50 paired ATL samples [15]. The mutation rate of 89 (44-227) per samples was found, significantly higher than in acute myeloid leukemia (7.3-13) and chronic lymphocytic leukemia (11.5). In addition to previously reported targets of mutation (p53, TCF8, and Fas), and known targets frequently mutated in other lymphoid malignancies (CARD11, GATA3, IRF4, POT1, and RHOA), they identified recurrent mutations in genes involved in T-cell development, activation and migration, VEGF and WNT signaling, immunosurveillance, and transcriptional regulation. Although Tax itself underwent gene silencing in most cases, alternative oncogenic mechanisms result in acquisition of somatic mutations or copy number alterations in Tax-related pathways.

2.1.4 Apoptosis Markers in HTLV-1 Infected Cells

HTLV-1 infected cells are resistant to inducers of apoptosis. This may be a result of Tax inactivation of the p53 gene, or up-regulation of apoptosis inhibitor A20 or bcl-XL [5, 6]. Arsenic trioxide and Interferon Alpha-2a have been used to induce apoptosis in HTLV-1 infected T-cell lines [16].

2.1.5 Treatment of HTLV-1 Associated non-Hodgkin's Lymphoma

Various chemotherapy regimens have been utilized to treat patients with ATLL with complete response rates of only about 30%, which are not durable [17-20]. In a large cooperative study in Japan, 4-year survival of patients with acute or lymphomatous forms of ATLL was only 4%. There is no standard therapy recommendation for smoldering or chronic forms of ATLL, and generally no therapy is recommended. Therapy recommendations are provided in Table 1 of ref 19, provided

below:

Table 1. Recommended Strategy for the Treatment of ATL

Smoldering- or favorable chronic-type ATL
Consider inclusion in prospective clinical trials
Symptomatic patients (skin lesions, opportunistic infections, and so on): consider AZT/IFN-α or watch and wait
Asymptomatic patients: consider watch and wait
Unfavorable chronic- or acute-type ATL
Recommend: inclusion in prospective clinical trials
If outside clinical trials, check prognostic factors (including clinical and molecular factors if possible):
Good prognostic factors: consider chemotherapy (VCAP-AMP-VECP evaluated by a randomized phase III trial against biweekly CHOP) or AZT/IFN-α (evaluated by a retrospective worldwide meta-analysis)
Poor prognostic factors: consider chemotherapy followed by conventional or reduced-intensity allogeneic HSCT (evaluated by retrospective or prospective Japanese analyses, respectively)
Poor response to initial therapy with chemotherapy or AZT/IFN-α: consider conventional or reduced-intensity allogeneic HSCT
Lymphoma-type ATL
Recommend: inclusion in prospective clinical trials
If outside clinical trials, consider chemotherapy (VCAP-AMP-VECP)
Check prognostic factors and response to chemotherapy (including clinical and molecular factors if possible):
Favorable prognostic profiles and good response to initial therapy: consider chemotherapy
Unfavorable prognostic profiles or poor response to initial therapy with chemotherapy: consider conventional or reduced-intensity allogeneic HSCT
Options for clinical trials (first line)
Test the effect of up-front allogeneic HSCT
Test promising targeted therapies such as arsenic trioxide + IFN-α, bortezomib + chemotherapy, or antiangiogenic therapy
Consider a phase II global study testing pegylated IFN and AZT
Options for clinical trials (relapse or progressive disease)
Test the effect of promising targeted therapies such as arsenic trioxide and IFN-α, bortezomib, a purine nucleotide phosphorylase inhibitor, histone deacetylase inhibitors, monoclonal antibodies, antiangiogenic therapy, and survivin, β-catenin, syk, and lyn inhibitors, etc.
Consider conventional or reduced-intensity allogeneic HSCT when possible
Abbreviations: ATL, adult T-cell leukemia-lymphoma; AZT, zidovudine; IFN-α, interferon alfa; VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin, and prednisone; doxorubicin, ranimustine, and prednisone; and vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; HSCT, hematopoietic stem-cell transplantation.

Two reports describe major response rates of 58% and 100%, respectively, in the acute form of ATLL, with the use of a combination of zidovudine and Interferon Alpha-2a [21, 22]. However, other investigators reported lower response rates with this approach [23]. One group reported an 81% response rate with the use of interferon and zidovudine with concurrent chemotherapy in 73 patients with aggressive ATLL [24]. Other approaches have included the use of antibodies to IL2Rα with or without conjugates to radioisotopes, or the use of an IL2-diptheria toxin product [25].

A phase II trial of aurora kinase inhibitor MLN8237 (alisertib) is underway in PTCL, including ATLL. (NCT01466881) Also, a randomized phase 3 study of alisertib in patients with relapsed/refractory peripheral T cell lymphoma (NCT01482962) is underway.

Mogamulizumab, or KW-0761, is a defucosylated humanized monoclonal antibody against CCR4. A phase II study of Mogamulizumab in CCR4-positive relapsed ATLL, given at 1.0 mg/kg/wk x 8, showed a response rate of 50% [26]. The most common adverse reactions were lymphopenia (96%), neutropenia (52%), thrombocytopenia (52%), acute infusion reaction (89%), and skin eruption (63%). A phase I/II trial in relapsed or refractory PTCL showed a response rate of 42%. Global phase II trials of mogamulizumab are underway for relapsed/refractory ATLL and PTCL. A randomized phase II study of mogamulizumab plus VCAP-AMP-VECP (mLSG15) versus mLSG15 alone for newly diagnosed aggressive ATLL was recently presented [27]. Patients received 4 courses of chemotherapy with 8 courses of mogamulizumab. Complete response rates were 52% in patients treated with mogamulizumab plus chemotherapy compared to 33% in those treated with chemotherapy alone. Median overall survival was not reached, although length of follow up was not described. The most common treatment-related toxicities were neutropenia (100% vs 96%), thrombocytopenia (100% vs 90%), leukopenia (100% vs 92%), and febrile neutropenia (90% vs 88%).

Experience with mLSG15 is limited outside of Japan as several components are unavailable. Additionally, the rates of myelosuppression and febrile neutropenia are very high [28]. Moreover, it is not clear that LSG15 is superior to other combination chemotherapy regimens used for lymphoma treatment. Experience with DA-EPOCH infusional chemotherapy in two US multi-investigator trials of ATLL, as well as its use in HIV-associated lymphomas, Burkitt lymphomas, and relapsed diffuse large B cell lymphomas suggests this is an appropriate chemotherapy regimen for use in aggressive ATLL. In a study of 19 patients treated with EPOCH (not dose-adjusted, using 750mg/m^2 cyclophosphamide) followed by zidovudine, lamivudine, and alpha interferon 2a, 4 patients came off study before completion of 2 cycles of therapy due to toxicity, 3 patients had progressive disease, and 12 patients had responses (including 2 CRs; 67% ORR; 11% CR) [29]. In our ongoing trial of aggressive ATLL, treated with DA-EPOCH (375 mg/m2 cyclophosphamide starting dose, all myelosuppressive drugs dose increased or decreased each cycle) combined with bortezomib and raltegravir, two subjects achieved complete remission lasting for >12 mos, 10 subjects had a partial remission, and 3 subjects had stable disease as their best response.

2.1.6 Antiviral Therapy for HTLV-1 Infection

Although the AZT/Interferon Alpha-2a combination was used in ATLL patients, it is unclear whether this combination had antiviral, antitumor, and/or immunomodulatory activity towards HTLV-1. A report of the use of lamivudine in 5 HAM patients suggested antiviral activity, as demonstrated by a 1 log median decrease in virus load [30]. This was associated with clinical improvement in one patient. Use of anti-retroviral agents has recently been shown to decrease HTLV-1 proviral DNA [31]. Our studies have examined various diketone integrase inhibitors for HTLV-1 infection [32]. We found that raltegravir inhibited HTLV-1 replication with IC50 of 35 nM.

In addition to direct anti-viral effects, viral suppression may be necessary in the treatment of cancer. We demonstrated this in our recent phase II clinical trial of infusional chemotherapy with etoposide, doxorubicin, and vincristine, daily prednisone, and bolus cyclophosphamide (EPOCH) given for two to six cycles until maximal clinical response, and followed by antiviral therapy with daily zidovudine, lamivudine, and alpha interferon-2a for up to one year. Seven patients were on study for less than one month due to progressive disease or chemotherapy toxicity. Eleven patients achieved an objective response with median duration of response of thirteen months, and two complete remissions. During chemotherapy induction, viral RNA expression increased (median 190-fold), and virus replication occurred, coincident with development of disease progression. Thus, while EPOCH chemotherapy followed by antiretroviral therapy is an active therapeutic regimen for adult T-cell leukemia-lymphoma, viral reactivation during induction chemotherapy may contribute to treatment failure. Alternative therapies are sorely needed in this disease that simultaneously prevent virus expression, and are cytocidal for malignant cells.

2.1.7 HTLV-1 Immune Responses

Although the HTLV-I-specific antibody titer is high in infected individuals, antibody surveillance is thought to be largely ineffective [33]. Similarly, there is little evidence that natural killer cells play an important role in controlling HTLV-1 infection. CD56+CD3- NK cells have no detectable lytic activity against HTLV-1 infected target cells. This is consistent with the finding that HTLV-1, unlike other viruses, does not down-modulate inhibitory NK ligands or upregulate activating ligands. In addition, MHC class I expression is not down regulated in primary HTLV-1-infected cells. Lastly, ligands for NK cell activating receptors are not expressed by HTLV-1 infected cells.

In contrast, there is compelling evidence that the CD8+ cytotoxic T lymphocyte (CTL) response is an important determinant of the outcome of HTLV-1 infection [33]. There is a strong association between certain human leukocyte antigens alleles and the outcome of infections. HTLV-1 is genetically stable, and while viral variants that can escape CTLs do occur, there is little evidence that these variants have a net selective advantage in patients. It has been possible to rank HTLV-1 proteins by whether binding their peptides was associated with a reduced viral load. Thus, protein targets associated with reduced proviral load include Gag, Pol, and HBZ, whereas, Env, is least associated. The Tax protein is intermediate in this rank order list.

Although, CTLs are frequently chronically activated and have immediate effector function ex vivo, HTLV-1 is rarely, if ever, cleared. Several explanations have been offered for the lack of immunogenicity of infected cells. First, HBZ interacts with cellular factors to suppress Tax-mediated transactivation, thereby inhibiting expression of HTLV-1 genes. In addition, HBZ has been shown to inhibit IFN-gamma production, enhance sensitivity to TGFbeta, and enhance the expression of the regulatory transcription factor Fox P3, critical for Treg cells. Second, the HTLV-1 provirus acquires mutations which prevent expression of viral proteins. Third, ATL cells often have a hypermethylated or deleted 5'LTR, thus preventing transcription of genes on the forward strand.

Histone deacetylase inhibitor, valproate, activates Tax and Gag expression, enhances CTL activity, and results in reduced proviral load [33]. Other recent data suggest that HTLV-1 specific CTLs

can be induced in patients with aggressive types of ATLL, and that these CTLs can contribute to treatment and inhibit relapse [34].

2.1.8 Immune Check Point Blockade

Tumors are able to evade detection and destruction by the immune system, despite the fact that many tumors appear to elicit a strong immune response that is evident in lymphocyte infiltrates of the primary lesion. Tumor immune evasion can be divided into 2 main mechanisms: 1) the induction of immune tolerance and 2) resistance to killing by activated immune effector cells [35]. The concept of ‘‘immunoediting’’ relates to the manner in which tumors manipulate their microenvironment through tumor-derived cytokines, chemokines, and other soluble factors [36]. By the time tumors have become clinically detectable, they have already evolved mechanisms to evade immune response mounted by the host that must be overcome to create effective and durable antitumor immunity.

Antigen-induced activation and proliferation of T cells are regulated by the temporal expression and binding of both co-stimulatory and co-inhibitory receptors. The signaling through these receptors in adaptive cellular immunity modulates the initiation, escalation, and subsequent resolution of host immune responses. In the absence of co-inhibitory signaling, persistent T cell activation can lead to excessive tissue damage in the setting of infection as well as autoimmunity. In the context of cancer immunology, in which immune responses are directed against antigens specifically or selectively expressed by cancer cells, these immune checkpoints can represent major obstacles to overcoming tumor-specific tolerance and generating clinically meaningful tumor control. Therefore, efforts have been made in the clinical arena to investigate blockade of immune checkpoints as novel therapeutic approaches to tumors which may exploit several distinct pathways to actively evade immune destruction, including endogenous “immune checkpoints” that normally terminate immune responses after antigen activation.

These observations have resulted in intensive efforts to develop immunotherapeutic approaches for cancer, including immune-checkpoint-pathway inhibitors such as anti–CTLA-4 antibody (ipilimumab) and anti-PD1 antibody (nivolumab).

2.2 Nivolumab

Nivolumab (BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor (Investigator Brochure, 2014). 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 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) (Investigator Brochure, 2014). 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 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 (Woo *et al*., 2012). 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 survival in many tumors, including melanoma [37], renal [37], esophageal [38], gastric [39] 2006), ovarian [40], pancreatic [41], lung [42], and other cancers (Investigator Brochure, 2014).

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. Recent work suggests that PD1 expression on infected T cells contributes to the impairment or exhaustion of CTL function in HTLV-1 infected individuals [43].

2.2.1 Pre-Clinical Development

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated (Investigator Brochure, 2014). 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.

2.2.2 Clinical Development

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 (Investigator Brochure, 2014). 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.

2.2.3 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab were 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 (Investigator Brochure, 2014). 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.

2.2.4 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

[44]. 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 [45]. In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year (Drake et al., 2013).

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 [46]. 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.

2.2.4.1 **Activity in Lymphomas**

In a phase 1 study of 23 heavily treated patients with relapsed or refractory Hodgkin's lymphoma were treated with 3 mg/kg nivolumab every 2 wks [47]. This resulted in an objective response in 20 subjects, including 4 subjects with a complete response, and progression-free survival of 86% at 24 wks. Copy-number gains in PDL1 and PDL2 gene resulted in enhanced JAK-STAT activity, which may explain the activity seen.

A phase I study of nivolumab in patients with relapsed or refractory lymphoid malignancies included 29 subjects with B-NHL and 23 patients with T-NHL, resulting in overall response rates of 28% and 17%, respectively [48]. This study, as well as two previous studies with pidilizumab in subjects with follicular lymphoma or diffuse large B cell lymphoma confirm the safety of this approach [49, 50].

2.2.5 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined [51]. 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.).

It is recognized that HTLV-1 is associated with inflammatory diseases, whose etiology is unclear. These include forms of myelopathy, uveitis, arthropathy, pneumonitis, and Sjogren's disease-like manifestations. Aberrant immune responses resulting in cross-reaction between virus-induced antigens and cellular antigens may be at least partially responsible. Thus, there is a theoretical risk that immune checkpoint inhibitors may promote these disorders, to which there will be careful attention.

2.2.6 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count (Wolchok et al., 2013). 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. 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. Recent findings suggest a possible correlation to higher mutational load and expression of a subset of mutant tetrapeptide motifs in tumor antigens [52].

2.3 Rationale

The rationale for the use of nivolumab in HTLV-1 associated ATLL is to enhance immune clearance of infected malignant cells.

2.4 Correlative Studies Background

See Section 2.1 for HTLV-1 Proviral DNA and Cell-Associated and Virion RNA Load and Protein Expression (2.1.2), HTLV-1 Clonality (2.1.3) and HTLV-1 Immune Responses (2.1.7).

See Section 2.2 for Pharmacodynamics/Biomarkers (2.2.6).

3. PATIENT SELECTION

3.1 Inclusion Criteria

1. Patients with any stage of pathologically confirmed CD3+ acute, lymphoma, chronic, or smoldering subtypes of ATLL.

2. Documentation of HTLV infection (ELISA) in individual with confirmation of HTLV-1 infection (by immunoblot or PCR) or a consistent clinical picture [including two of the following: 1) CD4+ leukemia or lymphoma, 2) hypercalcemia, and/or 3) Japanese, Caribbean or South American birthplace].

3. Patients with acute or lymphoma forms must have received at least one cycle of combination chemotherapy (with or without mogamulizumab) or interferon (with or without zidovudine and/or arsenic). Individuals with chronic or smoldering ATL are not required to have had prior treatment or could have received any number of previous courses of therapy.

4. ECOG performance status 0-2 (Karnofsky ≥60%, see Appendix A).

5. Age at least 18. Because no dosing or adverse event data are currently available on the

use of nivolumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

6. Life expectancy >12 weeks.

7. Patients must have normal organ and marrow function as defined below:
   a. leukocytes ≥ 3,000/mcL
   b. absolute neutrophil count ≥ 1,500/mcL
   c. platelets ≥ 100,000/mcL
   d. total bilirubin within normal institutional limits
   e. AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional upper limit of normal
   f. creatinine within normal institutional limits
      OR
      creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

8. The effects of nivolumab on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or 2 barrier methods of birth control; or abstinence) prior to study entry and for the duration of study participation. Women should continue birth control for 23 weeks after stopping nivolumab, and men should continue birth control for 31 weeks after stopping nivolumab. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 31 weeks after completion of nivolumab administration.

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

3.2 Exclusion Criteria

1. Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

2. Patients who are receiving any other investigational agents.

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

4. Prior allogeneic transplantation, since such individuals have increased risk of complications from nivolumab.

5. 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.

6. Any condition requiring >10 mg/d prednisone equivalents

7. Current or prior HTLV-1 associated inflammatory diseases, including but not limited to myelopathy, uveitis, arthropathy, pneumonitis, or a Sjögren's disease-like disorder.

8. Prior treatment with anti-PD-1, anti-PD-L1, anti-PD-L2 antibody.

9. Grade 2 or greater toxicity from prior therapy.

10. Grade 2 or greater diarrhea.

11. Autoimmune disease: Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids, should be excluded. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barré 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 (other than HTLV-associated arthropathy), 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.

    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).

12. Patients who have had evidence of active or acute diverticulitis, intra-abdominal abscess, GI obstruction and abdominal carcinomatosis which are known risk factors for bowel perforation should be evaluated for the potential need for additional treatment before coming on study.

13. Patients who have Hepatitis C (both reactive anti-HCV antibody and detectable HCV RNA) and Hepatitis B (HBsAg positive and anti-HBc-Total positive), may be enrolled, provided their total bilirubin: ≤1.5× institutional upper limit of normal (ULN) AST (SGOT) /ALT (SGPT): ≤2.5 X institutional upper limit of normal.

14. Patients with concurrent HIV infection may be enrolled if compliant with 3 or more drug anti-retroviral regimen and virus load less than 50 copies/ml and CD4 count greater than 250 cells/ml, and no concurrent opportunistic infection or other malignancy.

15. Any other prior malignancy from which the patient has been disease free for less than 3 years, with the exception of adequately treated and cured basal or squamous cell skin cancer, superficial bladder cancer, carcinoma in situ of any site or any other cancer.

16. Pregnant women are excluded from this study because nivolumab is an agent with the potential for teratogenic or abortifacient effects. 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.

3.3 Inclusion of Women and Minorities

Women and members of minority groups and their subpopulations will be included in this study.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

4.1.1 CTEP Registration Procedures

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

Registration requires the submission of:

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

Fillable PDF forms and additional information can be found on the CTEP website at http://ctep.cancer.gov/investigatorResources/investigator_registration.htm.

For questions about Investigator Registration, 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 required to access all CTEP applications and, if applicable (*e.g.*, all Network trials), all Cancer Trials Support Unit (CTSU) applications and websites.

Additional information can be found on the CTEP website at http://ctep.cancer.gov/branches/pmb/associate_registration.htm.

For questions about Associate Registration or CTEP-IAM Account Creation, please contact the ***CTEP Associate Registration Help Desk*** by email at 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 Central IRB (CIRB)-Early Phase 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 Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study so that the study approval can be applied to those institutions. Other site registration requirements (*e.g.*, 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 Requirements for 9925 Site Registration

- CTSU IRB Certification (for sites not participating via the NCI CIRB)

- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)
- Local informed consent document (if required by LPO)

4.2.2 Downloading Regulatory Documents

Site registration forms may be downloaded from the NCI #9925 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 1 Grants, followed by LAO- NC010 and protocol #9925.
- 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.)

4.2.3 Submitting Regulatory Documents

Submit required forms and documents 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)

4.2.4 Checking Site Registration Status

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

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

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

4.3 Patient Registration

In cooperation with the Corresponding Organization (Duke Cancer Institute LAO), the Lead Protocol Organization or LPO (Washington University School of Medicine) is utilizing an online 'study portal' for key study communication from participating sites to the Lead Principal Investigator and Study Coordinator. The study portal operates through REDCap, a secure, web-based application, managed by the Duke Cancer Institute LAO. Participating sites will not require REDCap user accounts or passwords to access the study portal for this study.

The **9925 Study Portal** may be accessed through the following link: **http://j.mp/2g4kpUG** .

Prior to registering patients into Oncology Patient Enrollment Network (OPEN), participating sites are instructed to access the 9925 Study Portal for the following:

- **Subject Consent** – Within 24 business hours of subject signing consent for the study, the Site Coordinator accesses the study portal to upload consents and notify the Study Coordinator of subject consent. Upon receipt of consent notification, the Study Coordinator will email the Site Coordinator with a subject screening ID number.

- **Eligibility Confirmation** – After completion of all screening evaluations and if possible, within at least 48 business hours of anticipated study treatment start, the Site Coordinator accesses the study portal to upload completed eligibility checklist and de-identified supporting source documentation including sufficient tumor tissue pathology confirmation documentation. After review of eligibility documents by the Lead Principal Investigator or designee(s), the Study Coordinator will email the Site Coordinator with confirmation or questions/comments regarding subject eligibility. If eligibility is confirmed, the Site Coordinator will be instructed by the Study Coordinator via email to proceed to patient registration in OPEN.

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.

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: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar.
- 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.
- 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.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 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.

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Nivolumab	None	in 100 ml NS	IV over 60 minutes (+/- 10 minutes)	Every 2 weeks	14 days
***Doses as appropriate for assigned dose level.*					

A flat dose of 240 mg will be administered. There will be no dose modifications allowed.

Nivolumab is to be administered as a 60-minute (+/- 10 minutes) IV infusion, using a volumetric pump with a 0.2/1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be

below 1.0 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

5.2.1 Supportive Care

All supportive measures consistent with optimal patient care will be given throughout the study.

See Section 5.1 for supportive care considerations for nivolumab administration.

Patients requiring chemotherapy or radiation therapy during the study will be taken off study treatment. Any exceptions must be discussed with the Principal Investigator.

5.2.2 Other Medications

Recommended prophylaxis for opportunistic infections includes Bactrim DS every other day. Subjects must be instructed to inform the investigators of the current or planned use or all other medications during the study (including prescription medications, over-the-counter medications, vitamins and herbal and nutritional supplements). It is the responsibility of the investigator to ensure that details regarding all medications are documented. Bisphosphonates started prior to screening activities or initiated during the course of the study to control bone pain may be used with caution.

Prophylactic growth factor (G-CSF) administration is not recommended. Based on observed toxicities, protocol-specified dose modification guidelines should be followed for subsequent therapy cycles. Management of anemia will be at the discretion of the treating physician, No concurrent investigational agents are permitted.

5.2.3 Prohibited Therapies

Patients in this study may not use vaccines for the treatment of cancer for up to one month pre and post dosing with nivolumab. Concomitant systemic or local anti-cancer medications including biologics, or radiation therapy treatments are prohibited in this study while receiving nivolumab.

Patients may not use any of the following therapies during the study:

- Any non-study anti-cancer agent (investigational or non-investigational)
- Any other investigational agents
- CTLA4 antagonists, CD137 agonists, PD-1 inhibitors
- Immunosuppressive agents, unless indicated to manage study therapy induced immune-related adverse events (irAEs)
- Chronic systemic corticosteroids, unless indicated to manage study therapy induced irAEs or chronic graft-versus-host disease (GVHD) at a stable dose prior to study entry

- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug, however it is suggested that routine vaccinations, including seasonal influenza, be given at least 2 weeks prior to study treatment).

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for the 4 starting doses plus 42 cycles (approximately 23 months) or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Patient decides to withdraw from the study,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or
- Unacceptable adverse event(s) which include the following (see also Section 6 for dosing delays/dose modifications and Appendix E for specific algorithms):
    - Any toxicity requiring high dose corticosteroids except patients with grade 2 or 3 skin lesions, pruritis, thyroiditis, or n. VII palsy can be restarted after a tapered dose of corticosteroids.
    - Any dosing interruption lasting more than 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 more than 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting more than 6 weeks, the Principal Investigator must be consulted.
    - 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
    - Requiring additional immune suppressive treatment beyond steroids

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

5.4 Duration of Follow Up

Patients will be followed for 1 year after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.6 Criteria to Resume Treatment

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

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

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

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

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol the treatment should resume at the earliest convenient point that is within the 6 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.

For patients treated with corticosteroids:

- Grade 2 events must resolve to ≤ Grade 1 before considering retreatment.
- Grade ≥ 2 events should have nivolumab held until resolution to ≤ Grade 1 for at least 2 weeks following complete removal from steroid treatment except for maintenance replacement doses for adrenal insufficiency (preferably no greater than 10mg prednisone equivalent daily).
- 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 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.

5.7 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, pruritus, arthralgia, hypo- or hypertension, bronchospasm, or other symptoms.

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

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

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

Remain at bedside and monitor subject until recovery from symptoms. Infusion rate may be slowed. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be

increased to 100% of the original infusion rate. Monitor patient closely.

The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations, slowing infusion rate as above.

For Grade 2 symptoms:
(Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; close observation for recurrence and treatment medications may need to be continued for 24-48 hours).

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor patient until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. If symptoms recur, then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the patient until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF).

The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and (acetaminophen) (or paracetamol) 325 to 1000 mg should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 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 ventilatory support indicated.)*

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

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor patient until recovery from symptoms.

In the case of late-occurring hypersensitivity symptoms (*e.g.*, appearance of a localized or generalized pruritus 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.

5.8 Treatment Beyond Progression

Patients are allowed to continue treatment for 4-6 weeks and reassessed. Treatment may continue up to an additional 30% total single diameter increased over baseline, if no change in performance status, no new serious adverse event, and no need for intervention. New measureable lesions are not permitted with this schema.

6. DOSING DELAYS/DOSE MODIFICATIONS

There will be no dose adjustments allowed. Subjects with infusion delays > 49 days (i.e., 2 missed doses + 7 days) should discontinue the study drugs.

Please refer to Appendix E 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.

Generally, we strongly encourage early evaluation, withholding drug, and appropriate treatment as indicated in the management tables and following event specific guidelines.

ALL OTHER EVENTS*	**Management/Next Dose for Nivolumab** ***Not agent related**, or agent related non-immunologically mediated
≤ 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.
Recommended management: As clinically indicated.	

ALL OTHER EVENTS**	**Management/Next Dose for Nivolumab** **Immunologically mediated
≤ Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1 OR baseline. When resolved, resume at same dose level.
Grade 3	Off protocol therapy (exceptions noted in Section 5.3)
Grade 4	Off protocol therapy
Recommended management: As clinically indicated.	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.*
Grade 2	No change in dose.* May continue on treatment at investigator discretion.
Grade 3	Hold* until ≤ Grade 1. 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/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: See Appendix E for Skin AE management algorithm.	

Liver Function AST, ALT, Bilirubin	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose. May continue on treatment at investigator discretion.
Grade 2	Hold until UNL or baseline. Resume at same dose level. Consider steroid treatment > 7 days.
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
Continued treatment of active immune mediated hepatitis may exacerbate ongoing 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. For individuals who are hepatitis B or C positive, viral DNA or RNA load, respectively, should be measured to determine if worsening LFTs are due to immune-mediated hepatitis or infectious hepatitis.	
Recommended management: See Appendix E for Hepatic AE management algorithm.	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold until Grade 0 or baseline. When resolved, resume at same dose level.
Grade 2	Hold until Grade 0 or baseline. Consider steroid treatment > 7 days.
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
Patients with Grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Patients who require steroids should be taken off study treatment. 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 food borne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: See Appendix E for GI AE management algorithm for management of symptomatic colitis.	

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab**
≤ Grade 1	If asymptomatic without evidence of pancreatitis, liver or gallbladder disease, or new onset diabetes, may continue on treatment at investigator discretion.
Grade 2	If asymptomatic without evidence of pancreatitis, liver or gallbladder disease, or new

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab**
	onset diabetes, may continue on treatment at investigator discretion.
Grade 3	If symptomatic, hold until Grade 0. If asymptomatic, without clinical, radiographic, or ultrasound evidence of pancreatitis, and no evidence of associated liver or gall bladder disease or new onset diabetes, may continue on treatment at investigator's discretion. Patients who develop symptomatic pancreatitis or DM should be taken off protocol therapy.
Grade 4	If symptomatic, hold until Grade 0. If asymptomatic, without clinical, radiographic, or ultrasound evidence of pancreatitis, and no evidence of associated liver or gall bladder disease or new onset diabetes, may continue on treatment at investigator's discretion. Patients who develop symptomatic pancreatitis or DM should be taken off protocol therapy.
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated.	
Recommended management: See Appendix E for the Hepatic AE management algorithm for treatment management of symptomatic pancreatitis.	

Pneumonitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and resolution to ≤ Grade 0 or baseline including baseline pO2. Resume no change in dose after pulmonary and/or ID consultation.
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	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation only if lymphocytic pneumonitis is excluded. 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 Appendix E for Pulmonary AE management algorithm.	

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

Other GI N-V	**Management/Next Dose for Nivolumab**
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. When resolved, 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. When resolved, 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), GB syndrome, myasthenia gravis should be off study.	
Recommended management: See Appendix E for Neurologic AE management algorithm.	

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
≤ Grade 1	With asymptomatic TSH elevation may continue on study with appropriate evaluation, and endocrine consultation suggested.*
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 protocol therapy.
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 Appendix E for Endocrine AE management algorithm.	

Fever	**Management/Next Dose for Nivolumab**
≤ Grade 1	Antipyretic therapy if no evidence of infection.
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 protocol therapy.
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	

Renal Acute Toxicity	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold for evaluation and treatment. Resume at same dose level.
Grade 2	Hold for evaluation and treatment until ≤ Grade 1. Resume at same dose level if an inflammatory response is excluded.
Grade 3	Hold for evaluation and treatment until ≤ Grade 1. Resume at same dose level if an inflammatory response is excluded.
Grade 4	Off protocol therapy. Evaluation and treatment.

Infusion Reaction	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
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	
Recommended management: See Section 5.8 for treatment of infusion reactions.	

Cardiac *	**Management/Next Dose for BMS-936558 (Nivolumab) 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.

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

Patients requiring > 2 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.

For subjects who are HIV co-infected and compliant with antiviral medications, nivolumab will be held if viral load increases to >500 copies/ml or CD4 count falls to <100 cells/mL. CD4 and virus load will be repeat 2-4 weeks later, and if the above parameters are still found, then nivolumab will be permanently discontinued.

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)

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)***
	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	

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%)**	
		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)	
		Myositis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy	
		Facial nerve disorder[5]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)	

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%)**	
		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 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in Section 7.3.4.

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

7.3 Expedited Adverse Event Reporting

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.

7.3.1 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 or Lead 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.

7.3.2 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. 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 (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **<u>MUST</u>** immediately report to the sponsor (NCI) **<u>ANY</u>** 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 **<u>ANY</u>** of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- 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).

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

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes

<table>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td>Not required</td><td>10 Calendar Days</td></tr>
<tr><td colspan="4">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

<u>Expedited AE reporting timelines are defined as:</u>
o “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.
o “10 Calendar Days” - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.</td></tr>
<tr><td colspan="4">[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:
• All Grade 4, and Grade 5 AEs
Expedited 10 calendar day reports for:
a) Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
a) Grade 3 adverse events

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

Effective Date: May 5, 2011</td></tr>
</table>

7.4 Routine Adverse Event Reporting

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

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

7.5 Secondary Malignancy

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

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

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

- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

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

7.7 Adverse Event Monitoring

Safety and tolerability will be monitored by AEs determined every 2 weeks while on treatment. It is expect that about 10% of subjects will have intolerable toxicity, requiring discontinuation of therapy. If the number of subjects discontinuing therapy is greater than 10% ($p<0.05$), this therapy will be considered intolerable in this population.

Participating sites will enter data every two weeks at a minimum. Participating sites will also notify the coordinating center of Grade 3 and higher adverse events and serious adverse events within 24 hours of occurrence or notification. The study chair (PI) will be notified of such events by the coordinating center team at Washington University, the Lead Protocol Organization (LPO) and Duke University, the Lead Academic Organization (LAO). The coordinating center team and LAO and LPO investigators will have a weekly conference call to review subject enrollment, study progress, and available safety data including those generated from the study database. In addition, there will be monthly conference calls with the coordinating center, study chair, and participating sites (study nurses/ coordinators/ investigators) to review study progress and safety data.

8. PHARMACEUTICAL INFORMATION

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

8.1 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 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with butyl rubber stoppers and aluminum seals.

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 1 mg/mL.

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

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

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

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

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

Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, 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 12.2).

8.2 Agent Ordering and Agent Accountability

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.

8.2.1 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 drugs received using the NCI Drug Accountability Record Form (DARF) (available on the CTEP home page (*http://ctep.cancer.gov*) or by calling the Pharmaceutical Management Branch at 301-496-5725). The DARFs document the drug delivery date to the site, inventory at the site, use by each study participant, and disposal of the drug (if applicable). A site-specific accountability record, either manual or electronic, may be used if it includes all the information required on the NCI Investigational Drug Accountability Record and if the paper printout is identical to the NCI accountability record. A separate DARF is required for each protocol using the same agent. The investigator will ensure that the drugs are used only in accordance with this protocol.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Integral Laboratory or Imaging Studies

N/A

9.2 Investigational Device Information

N/A

9.3 Integrated Correlative Studies

N/A

9.4 Exploratory/Ancillary Correlative Studies

9.4.1 HTLV-1 Studies - Biological Markers of Anti-Tumor Response

A. HTLV-1 DNA Proviral Load

Hypothesis: Nivolumab will enhance anti-ATLL immune responses and result in a decrease in HTLV-1 proviral load.

Rationale: An effective immune response against HTLV-1 should decrease the number of HTLV-1 infected cells.

Preclinical and Clinical Results: See Section 2.1 for HTLV-1 Proviral DNA and Cell-Associated and Virion RNA Load and Protein Expression (2.1.2).

Assay Methodology: Genomic DNA will be extracted from PBMCs isolated from whole blood obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, every 12 weeks thereafter, and at the end of study time point, and tumor tissue at baseline and end of study time point (if available). The HTLV-1 DNA assay is performed with PBMCs, prepared in a BSL3 facility, measuring the number of copies of integrated or unintegrated viral genome with a Biorad digital drop PCR assay [53]. PCR is performed with *tax* primers that amplify a 154 bp region, and a FAM/MGB probe. Additionally, in a duplex PCR, a cellular housekeeping gene, ribonuclease P protein subunit P30 is amplified and detected with VIC/MGB probe. DNA will be digested with BamHI, mixed with the primers and probes and Bio-Rad 2× Supermix, and then emulsified using a QX-200 droplet generator, and PCR performed in a 96 well plate, and analyzed on the QX200 droplet reader. QuantaSoftware version 1.3.2.0 is used to quantify the copies/µl. Thresholds are determined manually for each experiment, according to the negative controls, which include a no template control and DNA from a healthy volunteer. Droplet positivity is determined by fluorescence intensity; only droplets above a minimum amplitude threshold are counted as positive. The PVL is calculated as the percentage of infected cells Data normalization is accomplished by applying the log (base 2) transformation, calculating the mean and standard deviation (SD), and defining the lower (mean-2SD) and upper (mean+2SD) values of the expected range for each assay type. These values are used to convert the log transformed values to a percentage of the expected range for each assay type, by subtracting the lower range value for the assay first then dividing by the difference of the upper and lower values of the expected range for the assay. Linear relationship of the post-

normalized values is assessed using Pearson correlation.

Investigator's Experience and Competence with the Assay: Dr. Lee Ratner, the Principal Investigator, used this assay in previous publications [29, 54, 55], and has funding for these studies PO1 100730, R01 CA63417, and P50 CA094056

Justification for Number of Patients and Specimens: Blood samples will be obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and every 12 weeks thereafter, and at the end of study treatment, in order to examine the correlation between proviral load, clinical response, and other correlative studies. Tumor tissue sample is obtained at baseline and off-study time point (if available) to determine proviral load in the tumor.

B. HTLV-1 RNA Levels

Hypothesis: Nivolumab will enhance anti-ATLL immune responses and result in a decrease in HTLV-1 RNA levels in PBMCs and tumors.

Rationale: Nivolumab will enhance anti-ATLL immune responses and result in a more dramatic decrease in HTLV-1 RNA levels than HTLV-1 DNA levels, since not all infected cells will be expressing viral antigens.

Preclinical and Clinical Results: See Section 2.1 for HTLV-1 Proviral DNA and Cell-Associated and Virion RNA Load and Protein Expression (2.1.2).

Assay Methodology: RNA will be extracted from PBMCs isolated from whole blood obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, every 12 weeks thereafter, and at the end of study, and tumor tissue at baseline and end of study time point (if available). The HTLV-1 RNA assay is performed with PBMCs. RNA extraction, complementary DNA (cDNA) synthesis, and then digital drop PCR is performed. The extracted RNA is converted to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The conversion is performed using a GeneAmp 9700 thermocycler (Applied Biosystems, Grand Island, NY) with the following parameters: 10 min at 25°C, 120 min at 37°C, 5 min at 85°C, and a hold at 4°C. HTLV-1 tax and HBZ primers and FAM/MGB probes were designed for ddPCR using NCBI Primer Blast and Primer3Plus. Additionally, we amplify a housekeeping gene, Hypoxanthine-guanine phosphoribosyltransferase (HPRT), using a commercially available mix (Life Technologies, Frederick, MD). The final concentrations in the ddPCR reaction are 900 nM of each primer and 250 nM of each probe.

Digital droplet PCR is performed as described in assay methodology noted above for HTLV-1 DNA proviral load. Briefly, the cDNA is mixed with the HTLV-1 tax (or HBZ) and HPRT1 primers and probes and Bio-Rad 2x Supermix, which is then emulsified with droplet generator oil (Bio-Rad, Hercules, CA) using a QX-200 droplet generator according to the manufactuter's instructions. The droplets are transferred to a 96-well reaction plate (Eppendorf, Hauppage, NY) and heat-sealed with pierceable sealing foil sheets (Thermo

Fisher Scientific, West Palm Beach, FL.). PCR amplification is performed using a GeneAmp 9700 thermocycler (Applied Biosystems, Grand Island, NY) with the following cycling parameters: 10 min at 95°C, 40 cycles consisting of a 30-s denaturation at 94°C and a 60-s extension at 59°C, followed by 10 min at 98°C and a hold at 12°C. Following PCR amplification, the 96-well plate was transferred to a QX100 droplet reader (Bio-Rad, Hercules, CA). Each well was queried for fluorescence to determine the quantity of positive events.

QuantaSoft software version # 1.7.4.0917 (Bio-Rad, Hercules, CA) is used to quantify the copies/µl of each queried target per well. Droplet positivity was determined by fluorescence intensity. Thresholds are determined manually for each experiment, according to negative controls, which included a no template control and cDNA from a HTLV-I seronegative healthy volunteer. All samples are run in duplicate and the gene expression is the average of the two measurements. The gene expression was normalized to the housekeeping gene expression using the following formula: Gene expression = ((quantity of HTLV-1 tax or HBZ) / (quantity of housekeeping gene))*100.

Primers and Probe Sequences:

Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe Sequence (5'-3')
Tax cDNA	ATCCCGTGGAGACTCCTCAA	CCAAACACGTAGACTGGGTATCC	6FAM CCCCGCCGATCCCAAA MGBNFQ
HBZ cDNA	AGAACGCGACTCAACCGG	TGACACAGGCAAGCATCGA	6FAM ATGGCGGCCTCAGGGCT MGBNFQ

Investigator's Experience and Competence with the Assay: Dr. Lee Ratner, the Principal Investigator, used this assay in previous publications [29, 54, 55], and has funding for these studies PO1 100730, R01 CA63417, and P50 CA094056.

Justification for Number of Patients and Specimens: Blood samples will be obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and every 12 weeks thereafter, and at the end of study treatment, in order to examine the correlation between viral RNA levels, clinical response, and other correlative studies. Tumor tissue sample is obtained at baseline and off-study time point (if available) to determine viral RNA levels in the tumor.

C. HTLV-1 Clonality Analysis

Hypothesis: Nivolumab will enhance anti-ATLL immune responses and result in a decrease in the clonality index of integrated HTLV-1 proviruses.

Rationale: Nivolumab will enhance anti-ATLL immune responses and result in a decrease in the clonally expanded population of infected cells, as quantified by integration site analyses.

Preclinical and Clinical Results: See Section 2.1 for HTLV-1 Clonality (2.1.3).

Assay Methodology: Genomic DNA will be extracted from PBMCs isolated from whole blood obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, every 12 weeks thereafter, and at the end of study time point and with tumor tissue at baseline and end of study time point (if available). Clonality is determined from deep sequencing of integration sites [56]. Ten micrograms of DNA from uncultured PBMCs or tumor cells is sheared by sonication. DNA ends are then end-repaired using T4 DNA polymerase, DNA polymerase I Klenow fragment, T4 polynucleotide kinase. Addition of an adenosine at the 3' ends of the DNA is performed with Klenow Fragment 3' to 5' exo. One hundred pmol of a partially double stranded DNA linker is ligated to the DNA ends. Twenty six different linkers are constructed, each one with a specific 6bp tag to allow multiplexing of DNA samples during the sequencing. The ligated product is then split into 3 aliquots and each aliquot used in a separate PCR reaction. For each PCR reaction, ligated product is 50 pmol of B3 primer (binds HTLV-1 LTR), 10 pmol of B4 primer (which anneals to the strand of the linker generated by the amplification from Bio3), Phusion DNA polymerase. The 3 PCR1 products, derived from the same sample are then pooled, and PCR2 performed with PCR1 product using 25pmol of P5B5 primer (binds HTLV-1 LTR), 25 pmol of P7 primer (binds the linker), Phusion DNA polymerase. A library is constructed by pooling the different PCR2 products (each one possessing a specific tag). Quantification of the libraries is made by QPCR using primers P5 and P7 libraries are diluted to 0.5 pM and clustered on the Illumina flow cell. Paired-end reads (read1 and read2 each 50bp) plus a 6 bp tag read (read 3) are acquired on a GA II. The library construction pipeline is split into four steps: 1. DNA isolation and shearing; 2. Pre-PCR manipulations (ends repair and ligation); 3. PCR1 and PCR2 and library QPCR; 4. Library sequencing. Each step is carried out in a specific room and the sample flow is unidirectional, to minimize the risk of PCR contamination.

Read 1 and read 2 are mapped against the HTLV-1 and the human genome. First, overlapping clusters are exclude and then the following quality control filters: i) the single-read alignment scores of read 1 and read 2 must be higher than 10, ii) the strand orientation of the 2 reads must be opposite; iii) the length of the amplicons must be smaller than 1kb. Because the PCR is not specific to the 3'LTR, we discard the amplicons generated from the 5'LTR that contained only HTLV-1 sequences. We identify read 1 sequences that start with ACACA (the five bases at the 3' terminus of the HTLV-1 LTR). Read 1 and read 2 sequences are used to map the insertion site and the shear site. Read 3 is used to allocate the insertion site to a particular sample. Together with the mapping of a large number of UISs, the goal of our approach is to quantify the abundance of each unique integration site (UIS). Because PCR preferentially amplifies short products, the number of amplicons cannot be used to quantify the abundance of a given UIS. Random DNA fragmentation by sonication is a key feature to allow the quantification of the UIS abundance because, it is not biased to particular nucleotide sequences. For each UIS, we count the number of amplicons of different length. Additionally, misreading of the tag index during the sequencing could lead to the attribution of a particular insertion site to different samples. We solve this issue by taking into account not only the 6bp tag information but also the total number of distinct shear sites and the total number of reads

for a given insertion site to attribute the insertion site to the correct sample. Finally, control DNA (from a human T-cell line (Jurkat) uninfected by HTLV-1) is run on every lane of each flow cell to assess the effectiveness of our quality control procedures. Clonality is then determined from the oligoclonality index.

Investigator's Experience and Competence with the Assay: Dr. Lee Ratner, the Principal Investigator, used this assay in a previous report [55], and has funding for these studies PO1 100730, R01 CA63417, and P50 CA094056.

Justification for Number of Patients and Specimens: Blood samples will be obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and every 12 weeks thereafter, and at the end of study treatment, in order to examine the correlation between clonality, clinical response, and other correlative studies. Tumor tissue samples are obtained at baseline and off-study time point (if available) to determine clonality in the tumor.

D. HTLV-1 Specific CTL Analysis

Hypothesis: Nivolumab will enhance anti-ATLL immune responses, including Tax-specific CTL numbers.

Rationale: Nivolumab will enhance anti-ATLL immune responses, as measured by HTLV-1 specific CTL numbers.

Preclinical and Clinical Results: See Section 2.1 for HTLV-1 Immune Responses (2.1.7).

Assay Methodology: Tax-specific CTLs are determined with PBMCs obtained at baseline, Day 1 of Cycle 3, and end of study endpoint. To stimulate IFN-γ^+ HTLV-1 Tax-specific $CD8^+$ T cells, we use two pools of overlapping 20 mer peptides (offset by 6 amino acids) spanning the full length of the Tax protein. One hundred thousand CD4-depleted PBMCs are incubated at 37°C for 6 h in the presence a range of concentrations (0, 0.01, 0.1, 1, 5 and 10 μM) of the Tax peptide pools in duplicate wells. IFN-γ production by Tax-specific CTLs is quantified by ELISpot. Spot-forming cells (SFCs) were counted using an automated ELISpot reader. For each peptide pool concentration, the frequency of IFN-γ^+Tax-specific CTLs is calculated as follows: $(SFC^{poolA} + SFC^{poolB} - 2 \cdot SFC^{no\ peptide})/(\text{total number of } CD8^+ \text{ T cells})$. Log_{10}[peptide] is then plotted against the number of IFN-γ^+ Tax-specific CTLs. Using Graphpad Prism software, the following equation was fitted to the data: $y = (\max)/(1 + 10^{((\text{LogEC50-}x) \cdot \text{Hillslope})})$; where $x = \log_{10}$ (peptide concentration); y = % $CD8^+$ cells producing IFN-γ at a given peptide concentration; Hillslope = gradient of fitted curve; and max = predicted maximum SFC (i.e., where peptide concentration is not a limiting factor in IFN-γ production by Ag specific cells). The effective concentration of peptide that induced IFN-γ production by half the maximum number of Tax-specific cells (EC_{50}) is estimated using this equation, and $CD8^+$ T cell avidity was defined as the reciprocal of this value ($1/EC_{50}$). Avidity is expressed in units of $10^6\ M^{-1}$.

Investigator's Experience and Competence with the Assay: Dr. Lee Ratner, the Principal Investigator, has unpublished experience in this assay.

Justification for Number of Patients and Specimens: Blood samples will be obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and every 12 weeks thereafter, and at the end of study treatment, in order to examine the correlation between viral RNA levels, clinical response, and other correlative studies. Tumor tissue sample is obtained at baseline and off-study time point (if available) to determine viral RNA levels in the tumor.

9.4.1.1 Collection of Specimens

The risks of obtaining the blood samples are that of venipuncture and are minimal. No additional tumor tissue will be obtained beyond that initially obtained for clinical purposes, but no longer needed for those purposes.

Blood for PBMC

For each subject:

- Collect five 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog no. 366480) OR five 8.5ml yellow-top (acid citrate dextrose or ACD) tubes (BD Vacutainer, Catalog no. 364606) of peripheral blood for the following time point:
    a) Baseline
- Collect three 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog no. 366480) OR three 8.5ml yellow-top (ACD) tubes (BD Vacutainer, Catalog no. 364606) for the following time points:
    a) Day 1 of Cycle 3
    b) Day 1 of Cycle 5
    c) Every 12 weeks (after Day 1 of Cycle 5)
    d) End of study treatment

Tumor Tissue

For each subject:

- Obtain tumor tissue sample (block or core) for the following time points:
    a) Baseline
    b) End of study treatment (if available)

9.4.1.2 Handling of Specimens

Blood for PBMC

- If collected Monday through Thursday, ship tubes on day of collection at room temperature.
- If not shipped on day of collection, isolate and store PBMCs using the PBMC Isolation Procedure (see Table 9.4.1.2 below).
- Label each sample (tube or cryovial) with:

- Study Number NCI9925
- Sample Type **PBMC**
- Subject ID Number
- Collection Date
- Collection Time
- WBC Volume in mL (for cryovials only)

Table 9.4.1.2 PBMC Isolation Procedure

A. Collection

1. Collect blood in specified tube(s).
2. Prepare an ice bucket with dry ice. Chill one 2mL cryovial for each tube to collect the white blood cells isolated in the procedure.

B. Blood Separation

1. Fractionate the whole blood by centrifuging at 1500-2000 *X* g for 10-15 min at room temperature. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the white blood cells (WBCs) / buffy coat. Fractionate the blood as soon as possible after collection.
   NOTE: In a typical clinical centrifuge 1500-2000 *X* g is ~3000-3400 rpm. Check the appropriate settings for your centrifuge using the nomogram in your user's manual.
2. Use a disposable, plastic transfer pipet or Pasteur pipet to slowly and carefully aspirate off the plasma (upper layer) down to about 1 mm above the buffy coat. Do not disturb the buffy coat. Discard the plasma into a 250 mL flask containing bleach.
3. Gently recover the buffy coat (WBCs) with a fresh disposable pipet, Pasteur pipet, or 1000 ul micropipettor with a sterile tip. Try not to uptake the RBC layer below the buffy coat.
4. Place the recovered buffy coat into the WBC labeled cryovial cooled on ice from step 2.
5. Screw on the cryovial cap tightly to prevent isopentane from seeping into the vial.
6. Visually estimate the volume of WBCs recovered using the volume lines on the cryovial and write the information on the cryovial label. Buffy coat volume is greater in samples with high WBC counts. Usually you can expect $\leq$ 0.5 mL total.
7. Proceed to section C, "Freezing Collected Cells".

C. Freezing Collected Cells

1. Set Up Freezing Station
   - Do not perform snap freezing with bare hands. Wear gloves at all times and heavy duty gloves when working with liquid nitrogen or cooled isopentane.
   - Use extreme caution when dispensing liquid nitrogen.
   - Fill a small 100 ml metal beaker about 1/4 full with isopentane
   - Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.

2. Freezing Blood Cells in Cryovial
   1. Lower the 100 ml metal beaker containing isopentane half-way into the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when the isopentane is reaching its freezing point the tone of the boiling will increase for 2-3 seconds.

2. Lift the beaker out of the liquid nitrogen once more than you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
3. Use long forceps to hold the cryovial down into the cooled isopentane. Hold for at least 1 minute.
4. Use the long forceps to take out the cryovial(s).
5. Store vial(s) in -80ºC freezer.

Tumor Tissue

- Formalin fixed tumor biopsy - prepare as 3x3mm core.
- Fresh tumor tissue (flash frozen in liquid nitrogen within 2 hours of collection) - prepare excised tissue or cores measuring at least 10x2x2mm in aggregate.
- Site may opt to send tumor tissue block. Blocks will be handled by the Ratner Laboratory per study specifications (not to exhaust the tissue sample) and the remaining tissue will be returned to the site.
- Label each tissue sample with:
  - Study Number NCI9925
  - Subject ID Number
  - Sample Type **Block or Core**
  - Date of Specimen
  - Corresponding Accession Number

9.4.1.3 Shipping of Specimens

Blood for PBMC

- On Monday through Thursday, overnight ship tubes on day of collection at room temperature OR batch ship cryovials at the end of the study on dry ice to the Ratner Laboratory at the following address:

  Dr. Lee Ratner / John Harding
  Washington University
  McDonnell Science Research Building Room 562
  4523 Clayton Avenue
  St Louis, MO 63110
  Telephone: 314-362-8836

- Please notify the Ratner Laboratory (LRATNER@DOM.WUSTL.EDU) of any incoming blood specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the PBMC Shipment Log (see Appendix F). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

Tumor Tissue

- On Monday through Thursday, batch ship tissue samples at the end of study at room temperature to the Ratner Laboratory at the following address:

  Dr. Lee Ratner / John Harding
  Washington University
  McDonnell Science Research Building Room 562
  4523 Clayton Avenue St Louis, MO 63110
  Telephone: 314-362-8836

- Please notify the Ratner Laboratory (LRATNER@DOM.WUSTL.EDU) of any incoming tissue specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the Tissue Shipment Log (see Appendix G). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

9.4.1.4 Site Performing Correlative Study

Studies will be performed at the Ratner Laboratory at Washington University.

9.4.2 Immune Cell Numbers - Biomarker of Anti-Tumor Activity

Hypothesis: Nivolumab will enhance anti-ATLL immune responses and will decrease the number of CD3+CD4+CD25+ cells carrying HTLV-1 provirus, while increasing the number of CD3+CD8+ cells without affecting CD56+ or CD19+ cell numbers

Rationale: Nivolumab will enhance anti-ATLL immune responses and decrease infected immune responses and increase anti-ATLL immune effectors, which are likely primarily CD8+ cells

Preclinical and Clinical Results: See Section 2.1 for HTLV-1 Immune Responses (2.1.7).

Assay Methodology: Fluorescence Activated Cell Sorting (FACS) assay to be run at each institution.

Investigator's Experience and Competence with the Assay: N/A

Justification for Number of Patients and Specimens: Blood samples will be obtained at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and every 12 weeks thereafter, and at the end of study treatment, in order to examine the correlation between immune cell numbers, clinical response, and other correlative studies. Tumor tissue sample is obtained at baseline and off-study time point (if available) to determine immune cell numbers in the tumor.

9.4.2.1 Collection of Specimens

The risks of obtaining the blood samples are that of venipuncture and are minimal. No additional tumor tissue will be obtained beyond that initially obtained for clinical purposes, but no longer needed for those purposes.

Blood for FACS

For each subject:

- Collect peripheral blood per standard institutional procedure at the following time points:
    a) Baseline
    b) Day 1 of Cycle 3
    c) Day 1 of Cycle 5
    d) Every 12 weeks (after Day 1 of Cycle 5)
    e) End of study treatment

Tumor Tissue

For each subject:

- Obtain tumor tissue sample per standard institutional procedure at the following time points:
    a) Baseline (not required if fresh tissue is not available)
    b) End of study treatment (if available)

9.4.2.2 Handling of Specimens

FACS assay of blood and tumor tissue will be completed per standard institutional procedure. Assay results should include the following: CD3, CD4, CD8, CD19, CD56, and CD25.

9.4.2.3 Shipping of Specimens

N/A

9.4.2.4 Site Performing Correlative Study

FACS assay will be performed at the institution where the patient was enrolled.

9.4.3 PD-1, PD-L1, PD-L2 Expression - Biomarker of Anti-Tumor Activity

Hypothesis: Nivolumab responses will be greater in tumors will high levels of PD-L1 or PD-L2 as compared to tumors with low levels of expression of these checkpoint proteins.

Rationale: Nivolumab responses for solid tumors have been shown to correlate with levels of PD-L1 or PD-L2 expression.

Preclinical and Clinical Results: See Section 2.2 on Pharmacodynamics/Biomarkers (2.2.6).

Assay Methodology: IHC assay.

Investigator's Experience and Competence with the Assay: Dr Ratner, PI of the study, has significant experience with IHC assays.

9.4.3.1 Collection of Specimens

No additional tumor tissue will be obtained beyond that initially obtained for clinical purposes, but no longer needed for those purposes.

Tumor Tissue

For each subject:

- Obtain 10 tumor tissue sample (slides) for the following time points:
    a) Baseline
    b) End of study treatment (if available)

9.4.3.2 Handling of Specimens

Tumor Tissue

- Sections should be freshly cut for IHC staining.
- Use positively charged microscope slides, such as Superfrost Plus Fisher Catalog #22-034-979 or #12-550-15.
- Cut 10 unstained slides with section thickness of 5 microns.
- Slide drying should be performed at 37°C overnight.
- Storage of cut slides at room temperature should ideally not exceed more than 7 days.
- Longer-term storage should be at 4°C, with slide boxes carefully parafilmed to prevent moisture entering the slide box.
- Mark each slide with:
    - Study Number NCI9925
    - Subject ID Number
    - Sample Type **PDL**
    - Date of Specimen
    - Corresponding Accession Number
    - Serial Number of Slide (1, 2, 3…)

9.4.3.3 Shipping of Specimens

Tumor Tissue

- On Monday through Thursday, batch ship tissue samples at the end of study at room temperature to the Ratner Laboratory at the following address:

Dr. Lee Ratner / John Harding
Washington University
McDonnell Science Research Building Room 562
4566 Scott Ave4523 Clayton Avenue
St Louis, MO 63110
Telephone: 314-362-8836

- Please notify the Ratner Laboratory (LRATNER@DOM.WUSTL.EDU) of any incoming tissue specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the Tissue Shipment Log (see Appendix G). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

9.4.3.3 Site Performing Correlative Study

IHC study will be performed in the Ratner Laboratory at Washington University.

9.4.4 Tumor Antigen Analysis - Biomarker for Correlation with Responses

Hypothesis: Nivolumab will enhance anti-ATLL immune responses directed against tumor antigens.

Rationale: Responses to nivolumab in solid tumors correlate with the level of mutant tumor antigens.

Preclinical and Clinical Results: See Section 2.2 for Pharmacodynamics/Biomarkers (2.2.6).

Assay Methodology: Primary tumor or leukemic blood samples and matched normal specimens (skin or CD19+ peripheral blood B cells) are obtained. Only specimens with high tumor cellularity (>75%) will be used. Tumor and skin specimens are snap frozen in liquid nitrogen after surgical resection or biopsy and stored at −80 °C. Sections of tumor are stained with hematoxylin and eosin. DNA is extracted and exon capture is performed using a human All Exon 50MB kit (Agilent) with the addition of capture probes corresponding to the entire HTLV-1 genome. Enriched exome libraries will be sequenced on the HiSeq 2500 platform (llumina) to >100X coverage. Alignment, base-quality score recalibration and duplicate-read removal are performed, germline variants excluded, mutations annotated and indels evaluated. Samples with tumor coverage ≤10X were excluded. Medium-confidence reads (11-34X) are manually reviewed using the Integrated Genomics Viewer for sequencing of candidate mutations. Mutations between clinical groups were compared using the Mann-Whitney test.

HLA typing is performed by either low to intermediate resolution polymerase chain reaction-sequence-specific primer (PCR-SSP) method or by high–resolution SeCore HLA sequence-based typing method (HLA-SBT) (Invitrogen). ATHLATES (http://www.broadinstitute.org/scientificcommunity/science/projects/viral-genomics/athlates)3 is also used for HLA typing and confirmation.

The bioinformatic tool NAseek is used which performs two functions: translation of stretches surrounding each mutation, and comparison between the resulting peptides for homology. First, NASeek translates all mutations in exomes so strings of 17 amino acids are generated for the predicted wild type and mutant, with the amino acid resulting from the mutation is situated centrally. Next, to evaluate MHC Class I binding, wild type and mutant nonamers containing the tetrapeptides common to the complete responders are input into NetMHC v3.4 (http://www.cbs.dtu.dk/services/NetMHC/) or RANKPEP (http://imed.med.ucm.es/Tools/rankpep.html) for patient-specific HLA types, using a sliding window method. These programs generate a predicted MHC Class I binding strength. Finally, NAseek assesses for similarity between nonamers that are predicted to be presented by patient-specific MHC Class I. The logo plot of the amino acid frequencies is executed using Weblogo (http://weblogo.berkeley.edu/logo.cgi) with default parameters. In order to further narrow down the predicted nonamers for testing *in vitro*, nonamers were also evaluated for putative binding to the T cell receptor using the IEDB immunogenicity predictor with patient-specific HLA types (http://tools.immuneepitope.org/immunogenicity/) and CTLPred (http://www.imtech.res.in/raghava/ctlpred/). To evaluate homology to known pathogens' antigens, conserved tetrapeptides are analyzed using Immune Epitope Database (www.iedb.org) and assessed as substrings of immunogens in the database for a positive T cell response in *Homo sapiens* host. The "neoantigen signature" is generated from the nonamers containing the peptides common to patients with long-term benefit. Standard methods for signature derivation using unsupervised hierarchical clustering followed by logistic regression are used to determine predictive models based solely on the discovery set data. We will perform simulation/permutation testing to demonstrate that the neoantigen signature is highly unlikely to result from chance. To assess the null hypothesis that the signature is due to chance, 5 distinct simulation models are evaluated, three with new datasets and two using permutations of our dataset. The simulations are executed using (a) nonamers drawn from the SwissProt database (b) mutations from the TCGA T-cell lymphoma dataset (c) randomly generated nonamers (d) redistribution of the mutations found in our data and (e) reordering of the 9 amino acids within each nonamer predicted to be presented in our dataset. In each simulation, the nonamers are distributed randomly, and in proportion to our data (for example, if an actual sample harbored 150 nonamers predicted to bind MHC Class I, then the "virtual" sample is assigned 150 nonamers). Simulation testing is then conducted by applying the same iterative model used on the actual data applied to this virtual dataset, and repeating this process 1,000 times, recording the frequency of signatures greater than the actual signature to determine the p value. P value was calculated as the proportion of iterations with a signature greater that correctly classified segregation of the clinical cohorts, divided by the 1,000 iterations.

Mann-Whitney test is used to compare non-synonymous exonic mutational burden between clinical groups. Log-Rank test was used to compare the Kaplan-Meier curves for overall survival in the discovery and validation sets. As described above, simulation testing was used with the null hypothesis that all tetrapeptides contribute equally to clinical benefit to determine if a signature of the size we found happened by chance.

Investigator's Experience and Competence with the Assay: Dr. Lee Ratner, the Principal Investigator, has used this assay in a previous report [55], and has funding for these studies PO1 100730, R01 CA63417, and P50 CA094056

Justification for Number of Patients and Specimens: Tumor samples will be obtained at baseline and end of study time point. A skin biopsy is obtained at baseline, at the same time as the bone marrow biopsy to provide germline DNA not contaminated with PBMCs.

9.4.4.1 Collection of Specimens

No additional tumor tissue will be obtained beyond that initially obtained for clinical purposes, but no longer needed for those purposes.

Tumor Tissue

- The same tumor tissue samples obtained for HTLV-1 studies will be used for this analysis.

Skin

For each subject:

- Obtain a 3 or 4 mm punch biopsy of skin per standard institutional procedure at the following time point:
    a) Baseline (at the same time as bone marrow biopsy)

9.4.4.2 Handling of Specimens

Tumor Tissue

- The same tumor tissue samples obtained for HTLV-1 studies will be used for this analysis.

Skin

- Skin punch biopsy should be flash frozen in liquid nitrogen within 2 hours of collection.
- Label each skin sample with:
    - Study Number NCI9925
    - Subject ID Number
    - Sample Type **Skin**
    - Collection Date
    - Collection Time

9.4.4.3 Shipping of Specimens

Tumor Tissue

- The same tumor tissue samples obtained for HTLV-1 studies will be used for this analysis.

Skin

- On Monday - Thursday, batch ship tissue samples at the end of study at room temperature to the Ratner Laboratory at the following address:

  Dr. Lee Ratner/ John Harding
  Washington University
  McDonnell Science Research Building Room 562
  4566 Scott Ave4523 Clayton Avenue
  St Louis, MO 63110
  Telephone: 314-362-8836

- Please notify the Ratner Laboratory (LRATNER@DOM.WUSTL.EDU) of any incoming tissue specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the Tissue Shipment Log (see Appendix G). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

9.4.4.4 Site Performing Correlative Study

Studies will be performed at the Ratner Laboratory at Washington University.

9.4.5 Protein Markers Associated with Sensitivity or Resistance to Nivolumab

Multiplex ELISA assays have been developed to analyze over 25 angiogenic, inflammatory and immune-related markers in less than 0.5 ml of plasma. Coefficients of variation for most analytes are <10%. This platform has been successfully applied to several in-house phase I and II studies, as well as several phase III, Alliance-conducted studies with bevacizumab in colorectal and other cancers at Duke University. Markers of inflammation will be analyzed in the Duke Phase I Biomarker Lab, which serves as a core lab for these analyses for the US Cooperative Group, the Alliance.

Analyses will be performed on pre-treatment and on-treatment plasma samples. Analyte levels, and changes in analyte levels, will be correlated with clinical outcome (PFS, OS). Plasma will also be evaluated for protein markers that may be associated with sensitivity or resistance to ibrutinib. These may include CRP and other markers of inflammation, including but not limited to IFNγ, IL1β, IL6, sILR6R, sGP130, IL4, IL7, IL10, IL12, IL17A, IL17E, and IL23. Additional markers of proteins regulated by the PD-1/PD-L1 interaction and the IL6/JAK-STAT axis will also be assessed, including but not limited to VEGF, HER and TGFβ family members.

9.4.5.1 Collection of Specimens

Plasma Collection and Processing:

- Draw one 10ml lavender top (K_2EDTA) tube (BD Vacutainer, Catalog no. 366643) at the

following time points:

a) Baseline;
b) Every restaging;
c) End of study treatment.

- Invert tubes 10 times to mix blood.
- Centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions).
- Remove plasma from each tube and transfer equally into two separate clean 15ml polypropylene tubes (or institutional equivalent).
- Repeat centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions).
- Aliquot approximately 1.0ml of plasma from each tube evenly into four 2.0ml cryovials.
- Label each cryovial with:
  - Study Number NCI9925
  - Subject ID Number
  - Sample Type **Plasma**
  - Collection Date
  - Collection Time
  - Frozen Time

9.4.5.1 Handling of Specimens

Plasma samples will be stored at -70°C until further use.

9.4.5.2 Shipping of Specimens

In the end of trial accrual, all protein marker (plasma) and pharmacogenomics (whole blood) specimens will be batched (approximately 2 batches per institution) and shipped on dry ice by courier guaranteeing overnight delivery (Monday-Thursday) to the following address:

Phase I Biomarker Laboratory
ATTN: Andrew Nixon, PhD
Duke University Medical Center
395 MSRB, Research Drive
Durham, NC 27710

- Please notify the Phase I Biomarker Laboratory (andrew.nixon@duke.edu) of any incoming blood specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the Biomarker Shipment Log (see Appendix H). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

9.4.5.4 Site Performing Correlative Studies

Protein marker analysis will be performed at the Duke Phase I Biomarker Laboratory.

9.4.6 Pharmacogenomics Assessment

A one-time, blood sample will be drawn prior to initiation of therapy for assessment of variants in genes anticipated to be involved in the pharmacokinetics or pharmacodynamics of nivolumab.

9.4.6.1 Collection of Specimens

Whole Blood Collection and Processing:

- Draw one 6ml pink top (K2EDTA) tube (BD Vacutainer, Catalog no. 367899) at the following time point:
  a) pre-study (i.e., baseline) only.
- Invert tube 10 times to mix blood.
- Label the tube with:
  - Study Number NCI9925
  - Sample Type **Whole Blood**
  - Subject ID Number
  - Collection Date
  - Collection Time
  - Frozen Time

9.4.6.2 Handling of Specimens

Whole blood samples will be stored at -70°C until further use.

9.4.6.3 Shipping of Specimens

In the end of trial accrual, all protein marker (plasma) and pharmacogenomics (whole blood) specimens will be batched (approximately 2 batches per institution) and shipped on dry ice by courier guaranteeing overnight delivery (Monday-Thursday) to the following address:

Phase I Biomarker Laboratory
ATTN: Andrew Nixon, PhD
Duke University Medical Center
395 MSRB, Research Drive
Durham, NC 27710

- Please notify the Phase I Biomarker Laboratory (andrew.nixon@duke.edu) of any incoming blood specimen shipments by providing notification two days prior to shipment via the 9925 Study Portal. The shipment notification will include upload of the Biomarker Shipment Log (see Appendix H). The 9925 Study Portal may be accessed through the following link: **http://j.mp/2g4kpUG**

9.4.6.4 Site Performing Correlative Studies

Pharmacogenomic analysis will be performed at the Duke Phase I Biomarker Laboratory.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done <4 weeks prior to the start of therapy.

PROCEDURE/TEST*		Week*								Cycle*	
	Pre-study	1	2	3	4	5	6	7	8	5 - 46	Off-study[5]
Nivolumab		A		A		A		A		A	
Informed Consent	X										
Demographics	X										
Medical History	X										
Concurrent Medications	X	X----------								----------X	
Physical Exam	X	X		X		X		X		X	X
Neurological Exam [1]	X	X		X		X		X		X	X
Vital Signs	X	X		X		X		X		X	X
Height	X										
Weight	X	X		X		X		X		X	X
Performance Status	X	X		X		X		X		X	X
HTLV Antibody Assay	X										
CBC w/diff and Platelets	X	X	X	X	X	X	X	X	X	X	X
Serum Chemistry [2]	X	X	X	X	X	X	X	X	X	X	X
Hormone Levels [3]	X										
B-HCG [4]	X										
EKG	X										
Echocardiogram[14]	X[14]										
Cardiac Evaluations[15]		X----------								----------X	X
Adverse Event Evaluation		X----------								----------X	X
Radiologic Evaluation	X	Radiologic evaluations should be performed every 12 weeks.									X
Tumor Measurements	X	Tumor measurements are repeated every 12 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.									X
Bone Marrow and Skin Biopsy	X	Bone marrow biopsy is repeated if positive at baseline and complete response (CR) is obtained by all other criteria.									
CORRELATIVE STUDIES											
HTLV-1 Studies [6] - PBMC	X[6]					X[6]				X[6]	X[6]
HTLV-1 Studies [7] - Tumor Tissue (block/core)	X[7]										X[7]
Immune Cell Numbers [8] - Blood	X[8]					X[8]				X[8]	X[8]
Immune Cell Numbers [9] - Tumor Tissue	X[9]										X[9]
PD-1,-L1,-L2 Expression [10] - Tumor tissue (slides)	X[10]										X[10]
Tumor Antigen Analysis [11] - Skin	X[11]										

PROCEDURE/TEST*		Week*								Cycle*	
	Pre-study	1	2	3	4	5	6	7	8	5 - 46	Off-study[5]
Protein Markers [12] - Plasma	X[12]									X[12]	X[12]
Pharmacogenomic [13] - Whole Blood	X[13]										

*: +/- 1 day permissible for procedures, labs, and study drug administration
A: Nivolumab, 240 mg every 2 weeks
1: See Appendix H
2: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
3: TSH and am (i.e., before midday) cortisol are drawn at baseline.
4: Serum pregnancy test for women of childbearing potential only.
5: Off-study evaluation. Follow up assessments will be every 3 months (+/- 2 weeks) if patient is < 1 year from study completion. However, patients with ongoing toxicities should be seen more often as clinically indicated. Patients who develop recurrent disease will be followed for survival and for information on salvage patterns. The schedule of clinical follow up for these patients will be at the discretion of the treating physician and according to established Standard of Care. Adverse events assessment on the study will continue for all patients until 90 days after the last study drug administration.
6: Blood for PBMCs will be obtained at the following time points (+/- 2 days) for HTLV-1 DNA proviral load, RNA levels, clonality analysis, and specific CTL analysis: at baseline (50mL), Day 1 of Cycle 3 (30mL), Day 1 of Cycle 5 (30mL), and then every 12 weeks thereafter (30mL) and at the end of study treatment (30mL). Refer to Section 9.4.1 for sample collection, handling, and shipping.
7: Tumor tissue will be obtained at the following time points (+/- 2 days) for HTLV-1 DNA proviral load, RNA levels, clonality analysis, and specific CTL analysis: at baseline and end of study treatment (if available). Refer to Section 9.4.1 for sample collection, handling, and shipping.
8: Blood will be obtained at the following time points for immune cell numbers: at baseline, Day 1 of Cycle 3, Day 1 of Cycle 5, and then every 12 weeks thereafter and at the end of study treatment. FACS to assay to be performed per institutional standard procedure.
9: Tumor tissue will be obtained at the following time points for immune cell numbers: at baseline and at the end of study treatment (if available). FACS to assay to be performed per institutional standard procedure. This is not required at baseline if not done prior to study and fresh tissue is not available.
10: Slides from tumor tissue will be obtained at the following time points for PD-1, PD-L1, PD-L2 Expression: at baseline and at the end of study treatment (if available). Refer to Section 9.4.3 for sample collection, handling, and shipping.
11: Skin from punch biopsy collected at the same time as baseline bone marrow biopsy will be obtained for tumor antigen analysis. Refer to Section 9.4.4 for collection, handling, and shipping.
12: Blood for plasma will be obtained at the following time points for protein markers at baseline (10mL), at every restaging (10mL), and at the end of study treatment (10mL). Refer to Section 9.4.5 for sample collection, handling, and shipping.
13: Whole blood will be obtained at baseline (6mL) for pharmacogenomics. Refer to Section 9.4.6 for sample collection, handling, and shipping.
14: Only for patients with a history of congestive heart failure or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated.
15: As clinically indicated for patients with evidence of congestive heart failure, myocardial infarction, cardiomyopathy or myositis. To include EKG, CPK, troponin and echocardiogram as indicated.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect

For the purposes of this study, patients should be re-evaluated for response every 12 weeks. In

addition to a baseline scan, confirmatory scans should also be obtained 12 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using criteria suggested by Tsukasaki et al [19].

11.1.1 Definitions

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

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

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

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

11.1.3 Methods for Evaluation of Measurable Disease

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

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

Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

All subjects with skin disease and/or erythema using the SWAT and will have photographs taken using a digital camera. Special care should be taken to shield the patient's identity

Photos must be provided labeled with patient's protocol number and initials and date.

The body is divided into 12 regions with preassigned percentage total body surface area (%TBSA) based on methodology used to assess burns: head is 7% BSA, neck 2%, ant trunk 13%, post trunk 13%, buttocks 5%, genitals 1%, upper arms 8%, forearms 6%, hands 5%, thighs 19%, lower leg 14%, feet 7%.

Biopsies at baselines and subsequent times can be performed to confirm a CR at the discretion of the investigator.

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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

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

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

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not

advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

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

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

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

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

11.1.4 Response Criteria

Response Criteria for Adult T-Cell Leukemia-Lymphoma							
Response	Definition	Lymph Nodes	Extranodal Masses	Spleen, Liver	Skin	Peripheral Blood	Bone Marrow
Complete Remission[1]	Disappearance of all disease	Normal	Normal	Normal	Normal	Normal[2]	Normal
Uncertified Complete Remission[1]	Stable residual mass in bulky lesion	≥75% decrease[3]	≥75% decrease[3]	No increase	≥50% decrease	≥ 50% decrease	Normal
Partial Remission[1]	Regression of disease	≥50% decrease[3]	≥50% decrease[3]	No increase	≥50% decrease	≥50% decrease	Irrelevant
Stable Disease[1]	Failure to attain complete/partial remission and no progressive disease	No change in size	No change in size	No change in size	No chance in size	No change	No change
Relapse Disease or Progressive Disease	New or increased lesions	New or ≥50% increase [4]	New or ≥50% increase [4]	New or ≥50% increase	New or ≥50% increase	New or ≥50% increase [5]	Re-appearance
Not Assessable							

[1] Require each criterion to be present for a period of at least 4 weeks
[2] Provided that <5% of flower cells remained, complete remission was judged to have been attained if the absolute lymphocyte count, including flower cells, was $<4x10^9/L$
[3] Calculate by the sum of the products of the greatest diameters of measurable disease
[4] Defined by ≥50% increase from nadir in the sum of the products of measurable disease
[5] Defined by ≥50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of $>4x10^9/L$

11.1.4.1 Evaluation of Best Overall Response

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

11.1.5 Duration of Response

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

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

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

11.1.6 Progression-Free Survival

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

11.1.7 Response Review

Simultaneous review of the patients' files and radiological images will be reviewed by an independent expert.

11.2 Other Response Parameters

Skin involvement will be assessed by the severity-weighted assessment tool (SWAT) scores obtained for patients at each tumor assessment visit. This score represents the product of the percentage total surface area (%TBSA) involvement of each lesions type (patch, plaque, and tumor or ulceration), multipled by a weight factor: SWAT = (patch%TBSA x1) + (plaque %TBSA x 2) + (tumor or ulcer %TBSA x 3). In addition, the standard measurements of TBSA involvement and physician global assessments are recorded for comparison.

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 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

The Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators to review accrual, progress, and pharmacovigilance.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.2 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.

12.2.1 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. 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.

12.2.2 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 website:
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.

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

12.3 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 Objectives and Endpoints

The primary objective of this study is to determine safety, tolerability, and efficacy of nivolumab for patients with HTLV-associated ATLL. The safety and tolerability will be measured by the frequency of adverse events (AEs) and the number of patients who discontinue therapy due to intolerable toxicity, while the efficacy will be measured as overall response (Complete Response/CR+Partial Response/PR) rate.

The secondary objectives include

- To determine effects of nivolumab on HTLV-1 proviral DNA and RNA loads;
- To determine the effects of nivolumab on anti-HTLV-1 and anti-ATLL immune responses;
- To determine effects of nivolumab on HTLV-1 integration site clonality.

13.2 Study Design

At least 20 evaluable patients will be enrolled for this study. Evaluable patients will be those patients who received study medication. The sample size of 20 patients is based on Simon's Optimal 2-stage design, with the assumption that an overall response rate of 40% is the minimum response rate that would be considered for further analysis of this approach, compared to a rate of 10% under null hypothesis. Thus, 20 subjects are required to with a one-side type 1 error rate of 5% and a power of 90%. If 1 or fewer responses are seen among the first 9 subjects enrolled, the trial is stopped early for futility. In contrast, if 2 or more responders are observed out of the 9 patients in the first stage, additional 11 patients will be enrolled in the 2nd stage. In a multicenter study such as this, however, it is difficult for a trial to reach the planned sample exactly. The investigators would not turn away patients who have already been approached for participation just because the number of patients needed has been met. Therefore, we adopted a flexible Phase II design (Chen and Ng 1998) to allow the actual number at final stage to deviate slightly from

what is planned. Due to the expected relatively low accrual rate, we expect that the interim analysis could be performed at the designed sample size. Only the decision boundaries at the 2nd-stage are determined following Chen and Ng's flexible design and programed by the study statistician [57]. That is, if 5 or more responders are observed out of 20-23 patients, or 6 or more responders are observed out of 24-25 patients, we would conclude that preliminary evidence for efficacy exists and that further investigation of the treatment is warranted.

The interim analysis will be based upon independent review of response, with a minimum time frame of 2 months for the interim analysis. Accrual will not be halted during the interim analysis.

13.3 Data Analysis

Data analysis of the study will be descriptive in nature. Demographic and clinical characteristics of the sample, toxicity by grade, as well as tumor response, duration of response, and length of follow-up will be summarized using descriptive statistics. Binomial proportions and their 90% confidence intervals will be used to estimate the response rates of therapy. Binomial proportions and their 90% confidence intervals will also be calculated for the efficacy based on immune response criteria (Appendix D). The Kaplan-Meier method will be used to evaluate the response duration. Analysis of variance methods will be used to evaluate the effects of treatment and time on the viral load measurements, as well as measurements of viral transcripts. The incidence of toxicities will be estimated using the binomial proportion and its 90% confidence interval. A proportional hazards analysis with viral load measures as time dependent covariates will be used to evaluate the effects of these measures on duration of response. Detailed analysis of correlative studies and biomarkers are listed in Section 9.

The safety and tolerability will be monitored every 2 weeks. All patients who receive treatment with nivolumab will be eligible for safety and tolerability analysis. A Bayesian sequential monitoring method [58] will be used for safety analysis and the stopping rule is defined as $Pr(\theta>0.10 \mid data)>0.90$, where θ denotes the proportion of patients who discontinue therapy due to intolerable toxicity. That is, enrollment will be stopped early whenever, given the accumulated observed data, there is >90% probability that the discontinuation of therapy is greater than 10%. Specifically, enrollment will be stopped early if 3 patients discontinue therapy in the first 12 patients, or 4 patients discontinue therapy in the first 19 patients, or 5 patients discontinue therapy before the last patient enrolled. These boundaries were obtained using the free-download software Multc99 (version 2.1) from M.D. Anderson Cancer Center. We assume that the parameter θ follows a prior distribution of beta (α, β), with α and β representing the number of patients discontinuing and continuing therapy respectively.

PLANNED ENROLLMENT REPORT

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	**0**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
Asian	1	1	0	0	**2**
Native Hawaiian or Other Pacific Islander	1	1	0	0	**2**
Black or African American	6	6	0	0	**12**
White	1	1	1	1	**4**
More Than One Race	0	0	0	0	**0**
Total	**9**	**9**	**1**	**1**	**20**

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13.4 Reporting and Exclusions

13.4.1 Evaluation of Toxicity

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

13.4.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 8) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 8 usually designates the "unknown" status of any type of data in a clinical database.]

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

All conclusions should be based on all eligible patients. Subgroup analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subgroup analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B LIST OF PROHIBITED MEDICATIONS

The following medications may not be taken while participating on this study through 6 weeks after the last dose of protocol treatment:

- Any non-study anti-cancer agent (investigational or non-investigational)
- Any other investigational agents
- Any other CTLA-4 inhibitors or agonists
- CD137 agonists, PD-1 inhibitors
- Immunosuppressive agents, unless indicated to manage study therapy induced irAEs
- Chronic systemic corticosteroids, unless indicated to manage study therapy induced irAEs or chronic GVHD at a stable dose prior to study entry
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug, however it is suggested that routine vaccinations, including seasonal influenza, be given at least 2 weeks prior to study treatment)

APPENDIX C IMAGING METHODOLOGIES

Contrast enhanced CT or enhanced MRI are the preferred imaging modalities to be used. Chest x-rays performed during the screening phase (and repeated at anytime during the study if clinically indicated) may be used as supportive data, as an accessory to the CT chest scans.

The same imaging techniques used at screening MUST be used at all subsequent time points to permit accurate, comparable measurement of lesions. All imaging data are to be collected on film or in digital format.

CT/MRI of the Chest/Abdomen/Pelvis

CT/MRI imaging of the chest, abdomen and pelvis is required at Screening and at each tumor assessment visit as indicated in Table 1, Table 2, and Table 3, regardless of the location of known metastases. In addition, CT/MRI scans must be obtained of anatomic regions not covered by the chest, abdomen and pelvic scans, in subjects where there is clinical suspicion of deep soft tissue metastases (eg, lesions in the thigh). Such additional CT/MRIs will be required at Screening only when deep soft tissue disease is known/suspected and must be consistently repeated at all subsequent tumor assessment visits.

Non-radiographic Assessments/Digital Photography

These should be made at the sites and recorded in the source documents. Visible skin lesions should be measured clinically and documented digitally using standardized photographic images, including a ruler for scale as part of the image.

Image Acquisition Guidelines

Similar methods of tumor assessment and similar techniques must be used to characterize each identified and reported lesion at Screening and during the Induction and Maintenance Phases. All measurable and non-measurable lesions should be assessed at Screening and at the defined tumor assessment time points (see Table 1, Table 2, and Table 3). Additional assessments may be performed, as clinically indicated, if there is a suspicion of progression or for confirmation of response. The Investigator will base response to treatment on the irRC (see Appendix D). Imaging-based evaluation is preferred to physical examination. Helical (spiral) CT scans of chest, abdomen and pelvis are preferred. If not available, conventional (non-helical, non-spiral CT) should be used.

IV contrast should be used for all CT scans. If i.v. contrast is contraindicated, CT may be performed without contrast. Alternatively, MRI can be used to assess lesions in the subject. Subjects who develop contrast allergy after study enrollment may be followed by MRI for subsequent tumor measurements. Sections should be contiguous, similarly sized and consistent from visit-to-visit. Section thickness must be based on institutional standards (eg, from 5 to 8 mm; 10 mm cuts are not recommended). Chest x-ray, ultrasound, and PET scans are not acceptable methods to measure disease for the purposes of this study.

APPENDIX D IMMUNE-RELATED RESPONSE CRITERIA

Immune-related Response Criteria (irRC) are derived from modified World Health Organization (mWHO) conventions. Assessments of lymph nodes are derived from current RECIST guidelines.[1]

1.1.1.1.1 Definitions of Measurable/non-Measurable Lesions

All measurable and non-measurable lesions should be assessed at Screening and at the defined tumor assessment time points (see Table 1). Additional assessments may be performed, as clinically indicated for suspicion of progression. The Investigator will base response to treatment using the irRC.

1.1.1.1.2 Measurable Lesions

Measurable lesions are lesions that can be accurately measured in 2 perpendicular diameters, with at least 1 diameter ≥ 20 mm and the other dimension ≥ 10 mm (10 mm x 10 mm for spiral CT with cuts of 5 mm). The area will be defined as the product of the largest diameter with its perpendicular.

Lymph nodes may also be considered measurable. To be considered pathologically enlarged and measurable, a lymph node must be at least 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

1.1.1.1.3 Non-Measurable Lesions

Non-measurable (evaluable) lesions are all other lesions, including unidimensional measurable disease and small lesions (lesions without at least 1 diameter ≥ 20 mm, or pathological lymph nodes with short axis ≤ 15 mm), and any of the following: lesions occurring in a previously irradiated area (unless they are documented as new lesions since the completion of radiation therapy), bone lesions, leptomeningeal disease, ascites, pleural or pericardial effusion, lymphangitis cutis/pulmonis, abdominal masses that are not pathologically/cytologically confirmed and followed by imaging techniques and cystic lesions. Lymph nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

1.1.1.1.4 Definitions of Index/non-Index Lesions

Measurable lesions, up to a maximum of 5 lesions per organ and ten lesions in total, must be identified as index lesions to be measured at Screening. The index lesions should be representative of all involved organs. In addition, index lesions must be selected based on their size (eg, lesions with the longest diameters), their suitability for accurate repeat assessment by imaging techniques, and how representative they are of the subject's tumor burden. At Screening, a Sum of the Product Diameters (SPD) for all index lesions will be calculated and considered the baseline SPD. The baseline sum will be used as the reference point to determine

[1] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Can. 2009;45: 228 247.

the objective tumor response of the index lesions at tumor assessment.

1.1.1.1.5 Non Index Lesions

Measurable lesions, other than index lesions, and all sites of non-measurable disease, will be identified as non-index lesions. Non-index lesions will be evaluated at the same assessment time points as the index lesions. In subsequent assessments, changes in non-index lesions will contribute only in the assessment of complete response.

1.1.1.1.6 Calculation of Sum of Product of Diameters (SPD)

Sum of Product of Diameters is an estimate of tumor burden. The 2 greatest perpendicular diameters are used to estimate the size of each tumor lesion. The SPD is calculated as the sum of the product of the diameters for index tumor lesions. Several variations of the SPD are identified for the purpose of classification of tumor responses.

SPD at Baseline: The sum of the product of the diameters for all index lesions identified at baseline prior to treatment on Day 1.

SPD at tumor assessment: For every on-study tumor assessment collected per protocol or as clinically indicated, the SPD at tumor assessment will be calculated using tumor imaging scans. All index lesions and all new measurable lesions that have emerged after baseline will contribute to the SPD at tumor assessment (irSPD).

SPD at NADIR: For tumors that are assessed more than 1 time after baseline, the lowest value of the SPD (SPD Baseline or SPD at tumor assessment) is used to classify subsequent tumor assessments for each subject. The SPD at tumor assessment using the irRC for progressive disease incorporates the contribution of new measurable lesions. Each net percentage change in tumor burden per assessment using irRC accounts for the size and growth kinetics of both old and new lesions as they appear. In this study the irRC as defined by the Investigator will serve as the basis of key endpoints for efficacy analyses and guide clinical care.

1.1.1.1.7 Definition of Index Lesion Response

Immune-related Complete Response (irCR), which is defined as complete disappearance of all index lesions. Lymph nodes that shrink to < 10 mm short axis are considered normal.

Immune-related Partial Response (irPR), which is defined as a decrease, relative to baseline, of 50% or greater in the sum of the products of the 2 largest perpendicular diameters of all index and all new measurable lesions (ie, Percentage Change in Tumor Burden), in the absence of irCR.

Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥ 25% when compared to SPD at nadir

Immune-related Stable Disease (irSD), which is defined as not meeting the criteria for irCR or

irPR, in the absence of immune-related progressive disease (irPD)

Immune-related Progressive Disease (irPD), which is defined as at least a 25% increase in Tumor Burden (ie, taking sum of the products of all index lesions and any new measurable lesions) when compared to SPD at nadir.

1.1.1.1.8 Definition of Non-Index Lesion Response

Immune-related Complete Response (irCR), which is defined as complete disappearance of all non-index lesions. Lymph nodes that shrink to < 10 mm short axis are considered normal.

Immune-related Partial Response (irPR), non-index lesion(s) are not considered in the definition of PR, this term does not apply.

Immune-related Stable Disease (irSD), non-index lesion(s) are not considered in the definition of SD, this term does not apply.

Immune-related Progressive Disease (irPD), increases in number or size of non-index lesion(s) does not constitute progressive disease unless/until Tumor Burden increases by 25% (ie, the SPD at nadir of index lesions and any new measurable lesions increases by the required amount).

1.1.1.1.9 Impact of New Lesions on irRC

New lesions alone do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

1.1.1.1.10 Definition of Overall Response Using irRC Will Be Based on the Following Criteria:

- **Immune-related Complete Response (irCR):** Complete disappearance of all tumor lesions (index and non-index), together with no new measurable or unmeasurable lesions, for at least 4 weeks from the date of documentation of irCR. All lymph nodes short axes must be < 10 mm.
- **Immune-related Partial Response (irPR):** The sum of the products of the 2 largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the 2 largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of the Product of the Diameters (irSPD). A decrease, relative to baseline of the irSPD of 50% or greater is considered an irPR, in the absence of irCR. Must be confirmed no less than 4 weeks from the first irPR.
- **Immune-related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- **Immune-related Progressive Disease (irPD):** It is recommended in difficult cases (eg, increase in SPD or irSPD accompanied with significant individual lesion regression, “mixed response”, or in presence of stable or improving performance status/clinical condition) to confirm PD at the following tumor assessment. Any of the following will

constitute progressive disease:

- At least 25% increase in the SPD of all index lesions over nadir SPD calculated for these lesions.
- At least a 25% increase in the SPD of all index lesions and new measurable lesions (irSPD) over the nadir SPD calculated for the index lesions.

irRC Definitions

Index Lesion Definition	Non Index Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	% Change in irSPD Tumor Burdon (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	-100%	irCR
Partial Response	Any	Any	Any	≤-50% >-50% to <+25% ≥ +25%	irPR irSD irPD
Stable Disease	Any	Any	Any	>-50% to <+25% ≥ +25%	irSD irPD
Progressive Disease	Any	Any	Any	≥ +25%	irPD

Best Overall Response and Date of Progression Using irRC (irBOR)

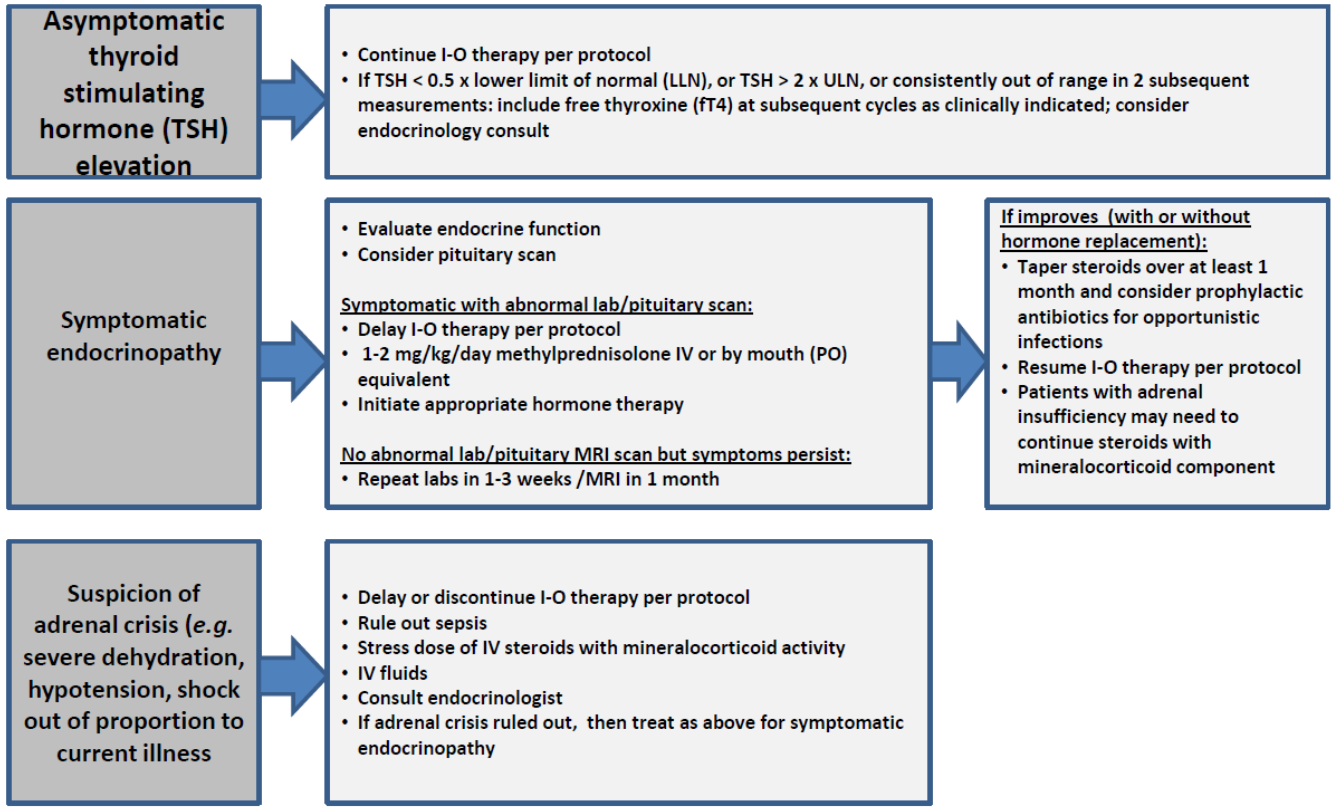
The Investigator will be asked to provide all responses on study and date(s) of progression, if applicable, and the best overall response will be calculated by the Sponsor or designee based on the time point responses and tumor measurements provided by the Investigators.

APPENDIX E ADVERSE EVENT MANAGEMENT ALGORITHMS

Management algorithms for the above-mentioned adverse events are provided on the following pages.

Endocrinopathy Management Algorithm

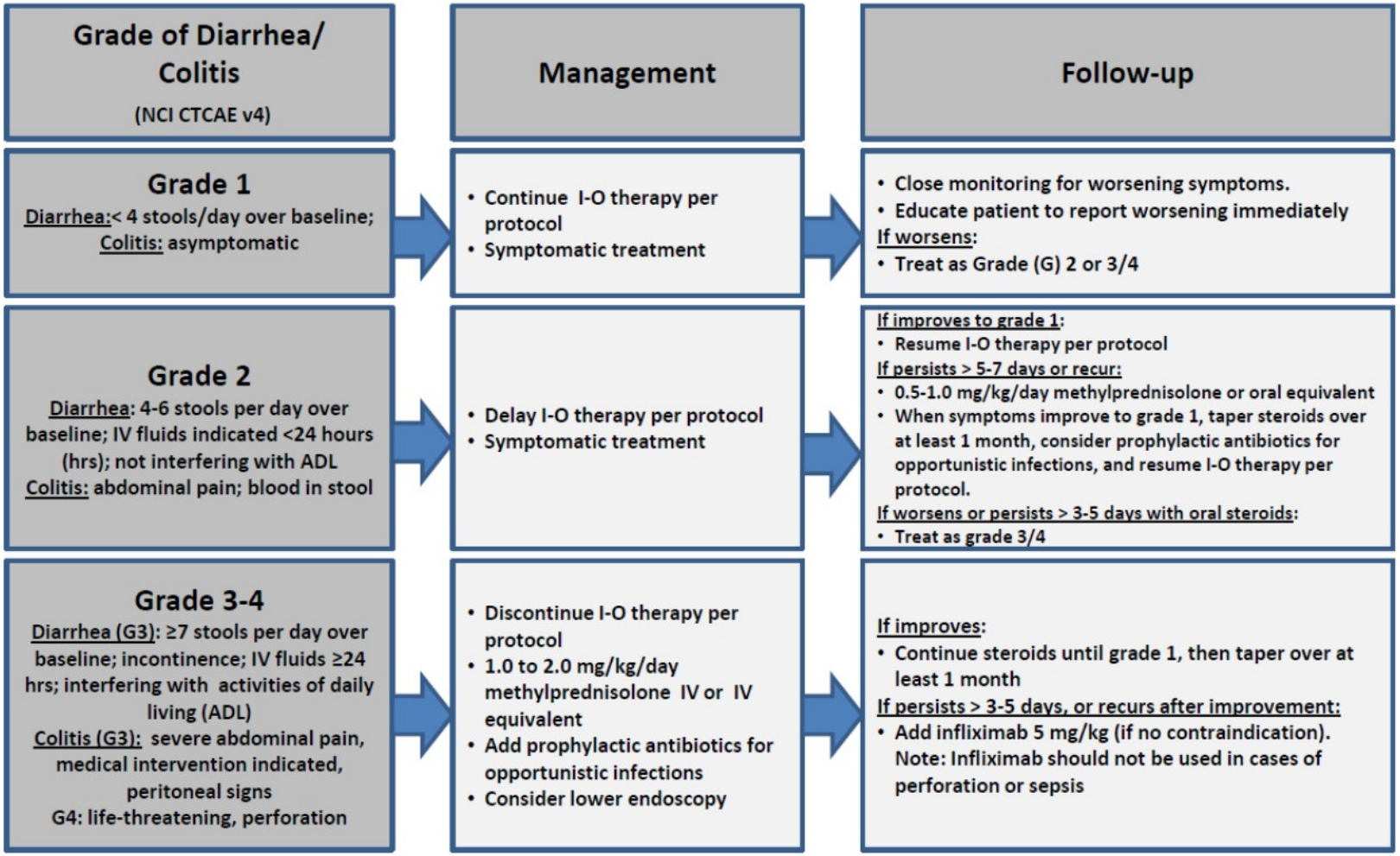
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy.
Consider visual field testing, endocrinology consultation, and imaging.



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

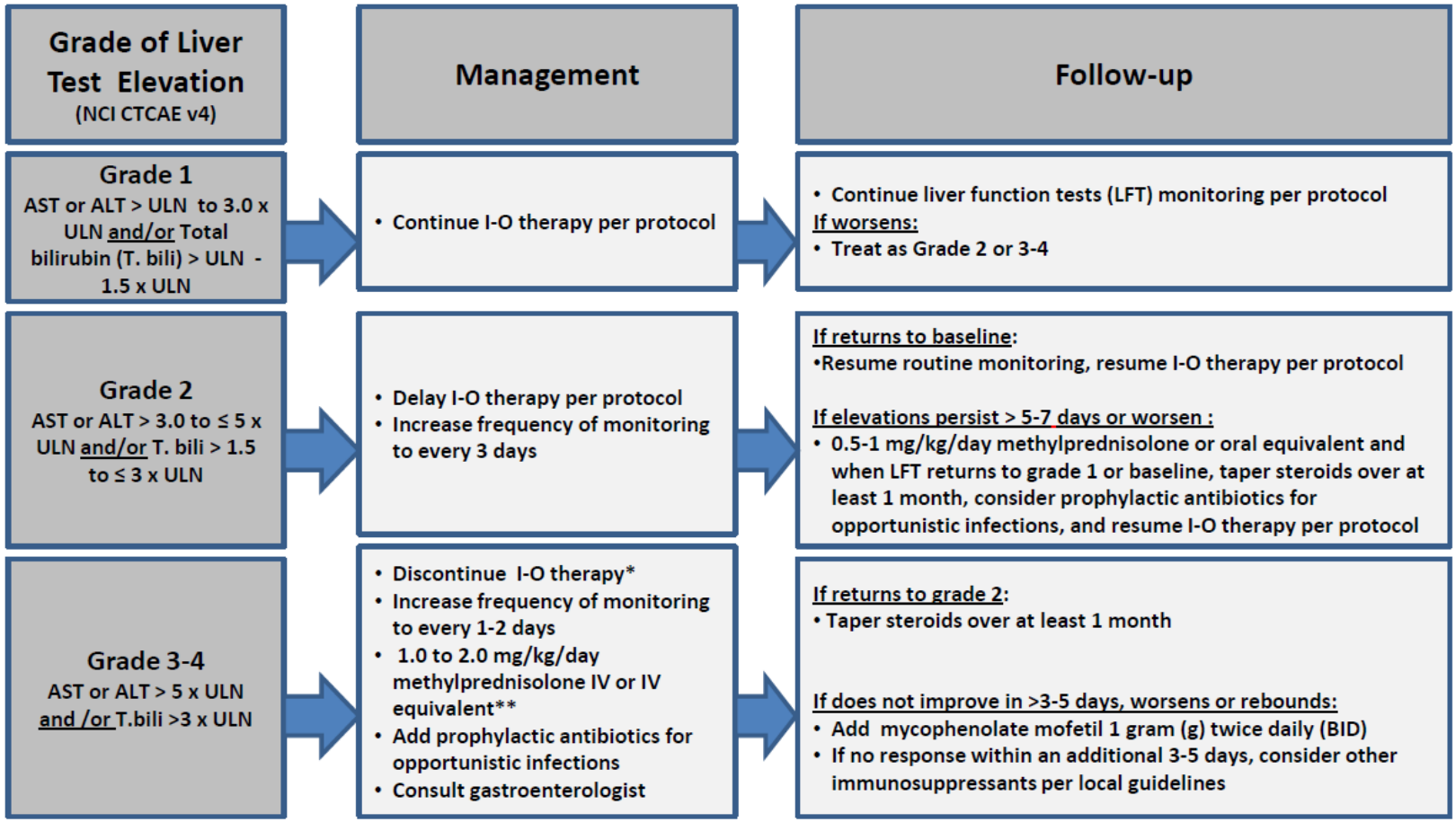
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

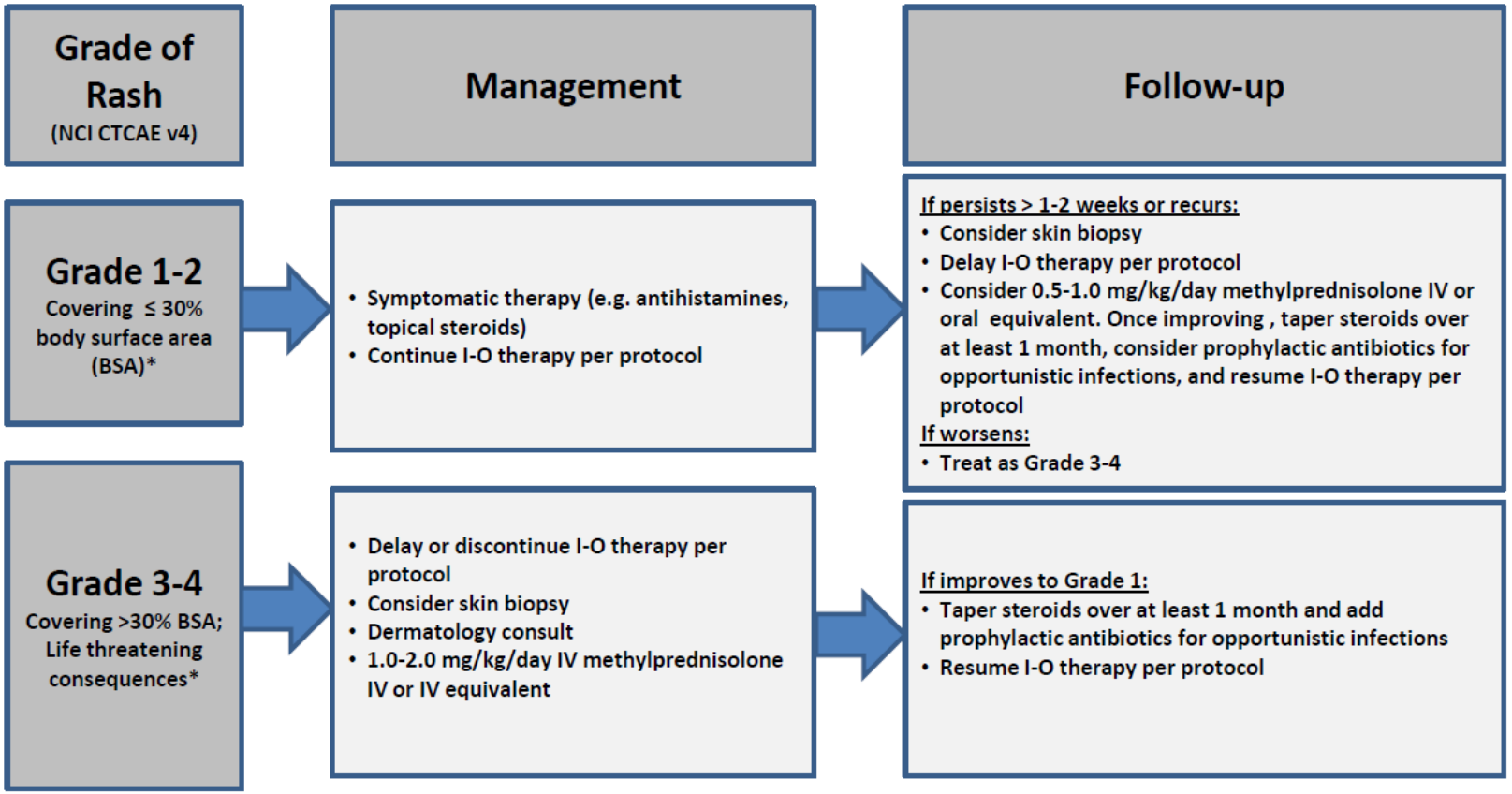
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	<u>If returns to baseline</u>: •Resume routine creatinine monitoring per protocol <u>If worsens:</u> •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy	<u>If returns to Grade 1:</u> •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol <u>If elevations persist > 7 days or worsen:</u> •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	<u>If returns to Grade 1</u> : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

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

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 F PBMC SHIPMENT LOG

Duke-UNC-Washington University (DUNCWU)
An Early Therapeutics Clinical Trials Network (ETCTN) Partnership

NCI9925 PBMC Shipment Log

Site Name: _____

Date of Shipment: _____ FedEx ☐ UPS ☐

Shipment Tracking Number: _____

Insert copy of this log with shipment and email to lratner@dom.wustl.edu.

Subject ID#	**Collection Date** (mm/dd/yyyy)	**Study Visit** (Baseline, Cycle# Day#, or End of Treatment)	**Tube Type** (sodium heparin or ACD)	**Number of Tubes/Cryovials**

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APPENDIX G TISSUE SHIPMENT LOG

Duke-UNC-Washington University (DUNCWU)
An Early Therapeutics Clinical Trials Network (ETCTN) Partnership

NCI9925 Tissue Shipment Log

Site Name: _____

Date of Shipment: _____ FedEx ☐ UPS ☐

Shipment Tracking Number: _____

Insert copy of this log with shipment and email to lratner@dom.wustl.edu.

Subject ID#	**Tissue Specimen Date** (mm/dd/yyyy)	**Study Visit** (Baseline or End of Treatment)	**Sample Type** (block, core, or slides)	**Corresponding Accession Number**

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APPENDIX H BIOMARKER SHIPMENT LOG

Duke-UNC-Washington University (DUNCWU)
An Early Therapeutics Clinical Trials Network (ETCTN) Partnership

NCI9925 Biomarker Shipment Log

Site Name: _____

Date of Shipment: _____ FedEx ☐ UPS ☐

Shipment Tracking Number: _____

Insert copy of this log with shipment and email to andrew.nixon@duke.edu.

Subject ID#	**Collection Date** (mm/dd/yyyy)	**Study Visit** (Baseline, Cycle# Day#, or End of Treatment)	**Sample Type** (plasma or whole blood)	**Number of Tube/Cryovials**

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APPENDIX I – NEUROLOGICAL EXAMINATION

Are there mental status defects? (yes or no)
If yes, elaborate

Are there cranial nerve defects? (yes or no)
If yes, elaborate

Sensory Exam (elaborate for pos answers)
Are there symptoms of tingling, pins and needles, or burning in the extremities? (yes or no)
Is examination of vibratory sense impaired? (yes or no)
Is examination of proprioception impaired? (yes or no)

Motor Exam
Is there a complaint on of lower extremity weakness? (yes or no)
Rate strength on exam as follows (for left and right side):

- 0 = no contraction
- 1 = visible muscle twitch but no movement of the joint
- 2 = weak contraction insufficient to overcome gravity
- 3 = weak contraction able to overcome gravity but no additional resistance
- 4 = weak contraction able to overcome some resistance but not full resistance
- 5 = normal; able to overcome full resistance

Strength of foot flexors:
Strength of foot extensors:
Strength of lower leg flexors:
Strength of lower leg extensors:
Strength of upper leg flexors:
Strength of upper leg extensors:

Deep tendon reflexes (for left and right side), graded as follows:

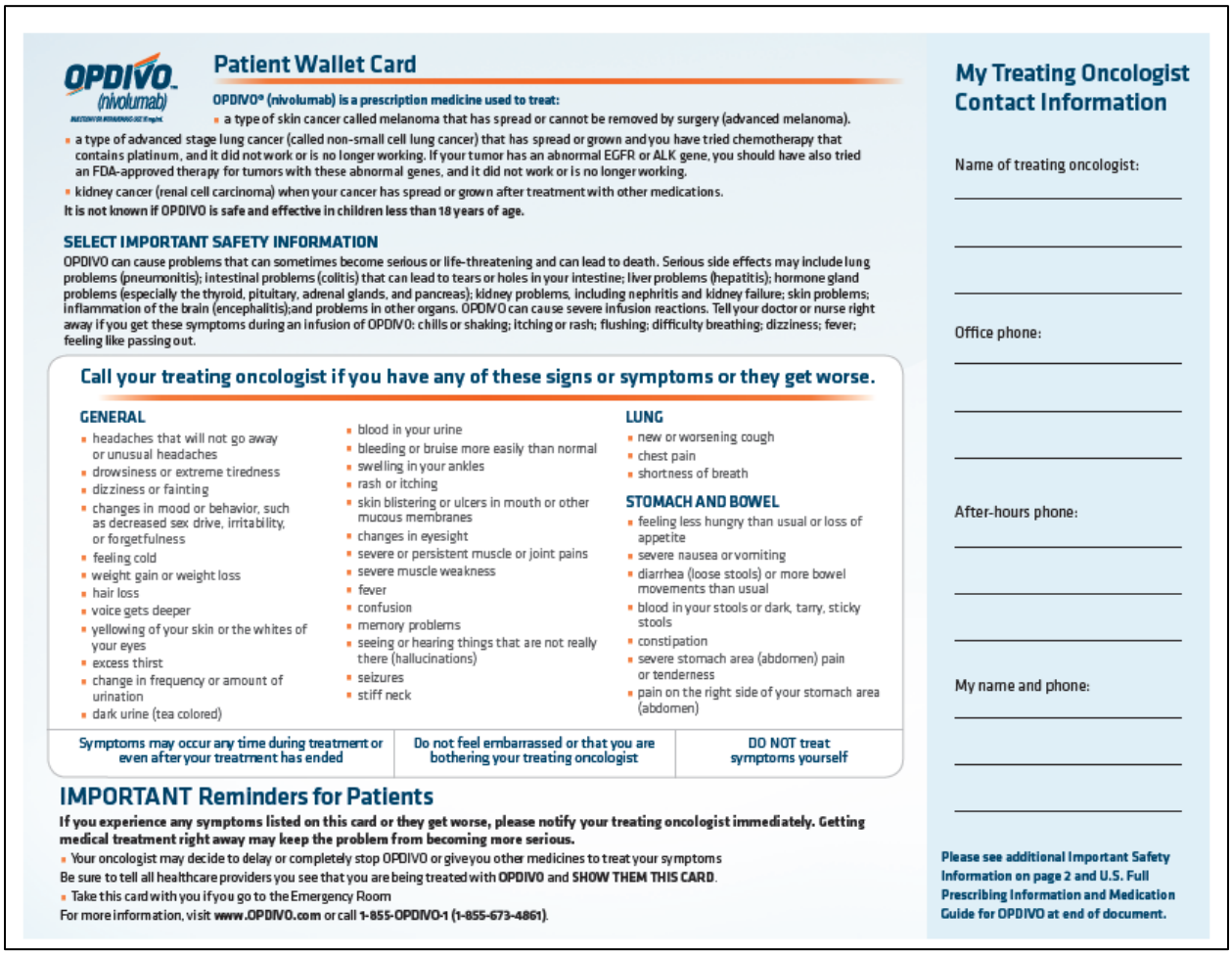
- 0 = absent
- 1 = reduced (hypoactive)
- 2 = normal
- 3 = increased (hyperactive)
- 4 = clonus

Ankle jerk:
Knee jerk:
Biceps:
Triceps:

Is there a disturbance of bladder function? (yes or no)

APPENDIX J – PARTICIPANT INFORMATION AND ALERT CARD

Refer to separate protocol attachment titled '9925 Appendix J - Participant Information and Alert Card' for full size card.



OPDIVO (nivolumab)

Patient Wallet Card

OPDIVO® (nivolumab) is a prescription medicine used to treat:

- a type of skin cancer called melanoma that has spread or cannot be removed by surgery (advanced melanoma).
- a type of advanced stage lung cancer (called non-small cell lung cancer) that has spread or grown and you have tried chemotherapy that contains platinum, and it did not work or is no longer working. If your tumor has an abnormal EGFR or ALK gene, you should have also tried an FDA-approved therapy for tumors with these abnormal genes, and it did not work or is no longer working.
- kidney cancer (renal cell carcinoma) when your cancer has spread or grown after treatment with other medications.

It is not known if OPDIVO is safe and effective in children less than 18 years of age.

SELECT IMPORTANT SAFETY INFORMATION

OPDIVO can cause problems that can sometimes become serious or life-threatening and can lead to death. Serious side effects may include lung problems (pneumonitis); intestinal problems (colitis) that can lead to tears or holes in your intestine; liver problems (hepatitis); hormone gland problems (especially the thyroid, pituitary, adrenal glands, and pancreas); kidney problems, including nephritis and kidney failure; skin problems; inflammation of the brain (encephalitis);and problems in other organs. OPDIVO can cause severe infusion reactions. Tell your doctor or nurse right away if you get these symptoms during an infusion of OPDIVO: chills or shaking; itching or rash; flushing; difficulty breathing; dizziness; fever; feeling like passing out.

Call your treating oncologist if you have any of these signs or symptoms or they get worse.

GENERAL

- headaches that will not go away or unusual headaches
- drowsiness or extreme tiredness
- dizziness or fainting
- changes in mood or behavior, such as decreased sex drive, irritability, or forgetfulness
- feeling cold
- weight gain or weight loss
- hair loss
- voice gets deeper
- yellowing of your skin or the whites of your eyes
- excess thirst
- change in frequency or amount of urination
- dark urine (tea colored)
- blood in your urine
- bleeding or bruise more easily than normal
- swelling in your ankles
- rash or itching
- skin blistering or ulcers in mouth or other mucous membranes
- changes in eyesight
- severe or persistent muscle or joint pains
- severe muscle weakness
- fever
- confusion
- memory problems
- seeing or hearing things that are not really there (hallucinations)
- seizures
- stiff neck

LUNG

- new or worsening cough
- chest pain
- shortness of breath

STOMACH AND BOWEL

- feeling less hungry than usual or loss of appetite
- severe nausea or vomiting
- diarrhea (loose stools) or more bowel movements than usual
- blood in your stools or dark, tarry, sticky stools
- constipation
- severe stomach area (abdomen) pain or tenderness
- pain on the right side of your stomach area (abdomen)

Symptoms may occur any time during treatment or even after your treatment has ended | Do not feel embarrassed or that you are bothering your treating oncologist | DO NOT treat symptoms yourself

IMPORTANT Reminders for Patients

If you experience any symptoms listed on this card or they get worse, please notify your treating oncologist immediately. Getting medical treatment right away may keep the problem from becoming more serious.

- Your oncologist may decide to delay or completely stop OPDIVO or give you other medicines to treat your symptoms

Be sure to tell all healthcare providers you see that you are being treated with **OPDIVO** and **SHOW THEM THIS CARD**.

- Take this card with you if you go to the Emergency Room

For more information, visit **www.OPDIVO.com** or call **1-855-OPDIVO-1 (1-855-673-4861)**.

My Treating Oncologist Contact Information

Name of treating oncologist: _____

_____

_____

Office phone: _____

_____

_____

After-hours phone: _____

_____

_____

My name and phone: _____

_____

_____

Please see additional Important Safety Information on page 2 and U.S. Full Prescribing Information and Medication Guide for OPDIVO at end of document.