SUMMARY OF CHANGES

A Phase I Study of Ipilimumab and Nivolumab in Advanced HIV-Associated Solid Tumors, with Expansion Cohorts in HIV-Associated Solid Tumors and a Cohort of HIV-Associated Classical Hodgkin Lymphoma

Version 15.0

NCI Protocol #: AMC-095
Local Protocol #: AMC-095

NCI Version Date: 24AUG2023
Protocol Date: 24AUG2023

I. Changes Required by Request for Rapid Amendment for Nivolumab from Dr. Howard Streicher (streicherh@ctep.nci.nih.gov), dated 03AUG2023:

#	Section	Description of Change

1.	6.1	The nivolumab CAEPR was updated to version 2.5 dated June 10, 2023. Changes include:
		• Added New Risk:
		  ◦ Rare but Serious: Blood and lymphatic system disorders – Other (lymphatic dysfunction)
		  ◦ Rare but Serious: Immune system disorders – Other (Sarcoid-granuloma)
		• Added a footnote for eye disorder – other (Vogt-Koyanagi-Harada)
		• Replaced immune-mediated hepatitis with immune-related hepatitis
		• Deleted footnote for Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)
		• Replaced immune-mediated nephritis with immune-related nephritis
		• Added an abbreviation for bronchiolitis obliterans with organizing pneumonia (BOOP)
		• Replaced skin and subcutaneous disorder with skin and subcutaneous tissue disorders
		• Removed references to BMS-936558, MDX-1106 to align with version 2.5 of the CAEPR.

2.	ICF - Risks of Nivolumab	The condensed risk profile has been modified • Added New Risk: ◦ Rare: Swelling of arms and legs which may cause a feeling of heaviness and tightness • Occasional Risk:

#	Section	Description of Change
		• Urination was replaced with urine Additionally, references to BMS-936558 and MDX-1106 were removed to align with the language provided in the Nivolumab risk profile.

II. Administrative and Editorial Changes:

#	Section	Description of Change
3.	Global	The protocol version/version date was updated to version 15.0 dated 24AUG2023.

AIDS MALIGNANCY CONSORTIUM

AMC PROTOCOL #095:
A Phase I Study of Ipilimumab and Nivolumab in Advanced HIV-Associated Solid Tumors with Expansion Cohorts in HIV-Associated Solid Tumors and a Cohort of HIV-Associated Classical Hodgkin Lymphoma

A Trial of the AIDS Malignancy Consortium (AMC)

Sponsored by:	National Cancer Institute Office of HIV and AIDS Malignancy (OHAM)
NCT Registration Number:	NCT02408861
Pharmaceutical Support Provided by:	Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC #748726) Ipilimumab (MDX-010, Yervoy™) (NSC # 732442)
	IND #: [redacted] IND Sponsor: DCTD, NCI
Protocol Chair:	Lakshmi Rajdev, MD, MS
Protocol Co-Chair:	Mark Einstein, MD, MS Dirk Dittmer, PhD Paul Rubinstein, MD
Participating Sites:	AMC Domestic Sites

Version 15.0 24 August 2023
NCI Version Date: 24 August, 2023

AMC PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site ____, agree to conduct and follow this protocol: **AMC Protocol #095 – A Phase I Study of Ipilimumab and Nivolumab in Advanced HIV-Associated Solid Tumors with Expansion Cohorts in HIV-Associated Solid Tumors and a Cohort of HIV-Associated Classical Hodgkin Lymphoma (Version 15.0, 24AUG2023),** as written according to AMC, NCI, and FDA guidelines. I understand that no deviations from the protocol eligibility criteria or waivers for protocol deviations will be permitted.

_____ _____

Signature Date (mm/dd/yyyy)

TABLE OF CONTENTS

LIST OF TABLES

PROTOCOL ROSTER

AMC Protocol #095
A Phase I Study of Ipilimumab and Nivolumab in Advanced HIV-Associated Solid Tumors with Expansion Cohorts in HIV-Associated Solid Tumors and a Cohort of HIV-Associated Classical Hodgkin Lymphoma

Protocol Chair:
Lakshmi Rajdev, MD, MS
Northwell Health
130 East 77th Street,
New York, NY 10021
Tel: 212-434-4736
Fax: 212-434-2246
Email: lrajdev2@gmail.com

Protocol Co-Chair (Hematologic Malignancies):
Paul Rubinstein, MD
Stroger Hospital of Cook County
Department of Medicine
Section of Hematology/Oncology
Administration Building
1900 W. Polk Street, Suite 755
Chicago, IL 60612
Tel: 312-864-7277
Fax: 312-864-9002
Email: prubinstein@cookcountyhhs.org

Protocol Co-Chairs:
Mark H. Einstein, MD, MS
Department of Obstetrics, Gynecology and Women's Health
Clinical Research Unit
Medical Science Building, Room E506
Rutgers, The State University of New Jersey
185 South Orange Avenue
Newark, NJ 07103
Tel: 973-972-5266
Fax: 973-972-4574
Email: Mark.Einstein@Rutgers.edu

Dirk P. Dittmer, PhD
Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill
450 West Dr. Rm. #12-038, CB #7295
Chapel Hill, NC 27599-7290
Tel: 919-966-7960
Fax: 919-962-8103
Email: ddittmer@med.unc.edu

Protocol Statisticians:
Himanshu Joshi, PhD, MSc, MPH, MBBS
Icahn School of Medicine at Mount Sinai
1425 Madison Avenue,
New York, NY 10029
Tel: 212-659-9635
Fax: 212-423-2998
Email: himanshu.joshi@mountsinai.org

Mayuri Jain, MPH
Icahn School of Medicine at Mount Sinai
1425 Madison Avenue
New York, NY 10029
Email: mayuri.jain@mountsinai.org

Data Management/Operations:
AMC Operations and Data Management Center
The Emmes Corporation
401 N. Washington Street, Suite 700
Rockville, MD 20850
Tel: 301-251-1161
Fax: 240-238-2842
Email: amc-095@emmes.com

Protocol Research Nurse:
Yoko Eng, NP
Montefiore Medical Center
#100 Hofheimer Building
Bronx, NY 10461
Tel: 718-920-2090
Fax: 718-405-7412
Email: yeng@montefiore.org

AMC Biorepository Director:
Jeffrey M. Bethony, PhD
Chair, AMC Laboratory Resource Committee
Department of Microbiology, Immunology & Tropical Medicine
The George Washington University
2300 I Street NW, Room 523
Washington, DC 20052
Tel: 202-994-2663
Cell: 202-590-8342
Email: jbethony@gwu.edu

AMC NADC Working Group Chair:
Elizabeth Chiao, MD MPH
Baylor College of Medicine
Internal Medicine/Infectious Diseases
One Baylor Plaza
Houston, TX 77030
Tel: 713-440-4485
Fax: 713-748-7359
Email: echiao@bcm.edu

AMC Lymphoma Working Group Chair
Ariela Noy, MD
Memorial Sloan Kettering Cancer Center
Lymphoma Service
1275 York Avenue
New York, NY 10065
Tel: 212-639-7423
Fax: 646-422-2284
Email: noya@mskcc.org

ABBREVIATIONS LIST

ACSR	AIDS and Cancer Specimen Resource
AE	Adverse event
AERS	Adverse Event Reporting System
AIN	Anal intraepithelial neoplasia
AMC	AIDS Malignancy Consortium
AML	Acute myelocytic leukemia
ANA	Anti-nuclear antibody
ANC	Absolute neutrophil count
ART	antiretroviral therapy
BMS	Bristol-Myers-Squibb
CAEPR	Comprehensive Adverse Events and Potential Risks List
cART	Combined antiretroviral therapy
CHF	Congestive heart failure
cHL	Classical Hodgkin lymphoma
CLIA	Clinical laboratory improvement amendments
CMV	Cytomegalovirus infection
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DARF	Drug Accountability Record Form
DCTD	Division of Cancer Treatment and Diagnosis
DHHS	Department of Health and Human Services
DL	Dose level
DLBCL	Diffuse large B cell lymphoma
DLT	Dose-limiting toxicity
EBV	Epstein-Barr virus
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
FDA	Food and Drug Administration
FDF	Financial Disclosure Form
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HIV-cHL	HIV-associated classical Hodgkin lymphoma
HPV	Human papillomavirus
hRS	Hodgkin and Reid Sternberg cell
IAM	Identity and Access Management
IBD	Inflammatory bowel disease
IDF	Investigator Data Form
IRB	Institutional review board

IRC	Immune response criteria
irAE	Immune-related adverse event
IV	Intravenous
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma-associated herpesvirus
LFT	Liver function tests
MDS	Myelodysplastic syndrome
MRI	Magnetic resonance imaging
MSS	Microsatellite stable [colorectal cancer]
MTD	Maximum tolerated dose
mWHO	Modified World Health Organization conventions
NADC	Non-AIDS-defining cancer
NCI	National Cancer Institute
NHL	Non-Hodgkin lymphoma
OAOP	Online Agent Order Processing
ODMC	Operations and data management center
PMB	Pharmaceutical Management Branch
PCR	Polymerase chain reaction
PVC	Polyvinyl chloride
RECIL	International Working Group consensus response evaluation criteria in lymphoma
RR	Response rate
SAE	Serious adverse event
SPD	Sum of Product of Diameters
SPEER	Specific Protocol Exceptions to Expedited Reporting
TEN	Toxic epidermal necrolysis
ULN	Institutional upper limit of normal
WHO	World Health Organization
WOCBP	Women of childbearing potential

PROTOCOL SYNOPSIS

Title:	A Phase I Study of Ipilimumab and Nivolumab in Advanced HIV-Associated Tumors with Expansion Cohorts in HIV-Associated Solid Tumors and a Cohort of HIV-Associated Classical Hodgkin Lymphoma
Phase of Study:	Phase I
Participating Institutions:	This protocol will be open to all AMC domestic member sites.
Accrual Target:	Minimum of four participants with a maximum of 96 participants.
Population:	Participants with histologically confirmed solid malignancy and HIV infection. Solid malignancy must be metastatic or unresectable and standard curative or palliative measures are nonexistent or no longer effective. Uncontrolled Kaposi sarcoma is permitted. Participants with relapsed refractory HIV-associated classical Hodgkin lymphoma (HIV-cHL) as a separate cohort.
Regimen:	In the dose de-escalation cohort, participants will be enrolled in separate strata: Stratum 1 will enroll participants with lymphocyte T CD4+ count above 200/mm^3. Stratum 2 will enroll participants with lymphocyte T CD4+ count between 100-200/mm^3. Enrollment will occur sequentially, initially in Stratum 1 and then in Stratum 2 after Stratum 1 has completed. Stratum 1 dosing will start with a full dose of nivolumab 3 mg/kg (dose level 1), and one dose de-escalation is allowed; after evaluating dosing for single agent nivolumab then participants will be treated with 240mg of nivolumab and 1mg/kg of ipilimumab will be added to evaluate combination therapy (dose level 2) with one dose de-escalation allowed. No intra-participant dose escalations will be allowed. The safety evaluation period is 6 weeks at a given dose level. Stratum 2 dosing will begin at the single agent therapy maximum tolerated dose (MTD) for Stratum 1 (dose level 1 or -1). Stratum 2 will not be allowed to escalate beyond the MTD for Stratum 1. Only 1 dose de-escalation will be allowed. The study treatment consists of up to 46 intravenous (IV) infusions of nivolumab on Day 1 of each 2-week cycle for the dose level -1 and 1 cohorts. For dose level 2, IV infusions of nivolumab are given on Day 1 of each 2-week cycle and ipilimumab on Day 1 every 6 weeks, i.e., every third cycle (cycles 1, 4, 7, etc.). For dose level -2, IV infusions of nivolumab are given on day 1 of each 2-week cycle, and ipilimumab

is given on day 1 every 12 weeks, i.e., every sixth cycle (cycles 1, 7, 13, etc.). Participants with an overall response of stable disease (SD), partial response (PR), complete response (CR), or unconfirmed progressive disease (PD) at Week 8 will continue treatment (Refer to Section 9.1 for Response Evaluation Criteria in Solid Tumors (RECIST) Criteria for up to 84 weeks, (42 IV infusions of nivolumab every 2 weeks, see the table below for the ipilimumab schedule if on dose level 2 or -2).

Dose Level	**Nivolumab* q2 wk (46 doses)**	**Ipilimumab****
-1	1mg/kg	-
1	3mg/kg	-
2	240mg	1mg/kg q 6 wk(16 doses)(day 1 of nivolumab cycles 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46)
-2	1mg/kg	1mg/kg q 12 wk (8 doses)(day 1 of nivolumab cycles 1, 7, 13, 19, 25, 31, 37, and 43)

* Treatment will continue until documented disease progression, discontinuation due to toxicity, withdrawal of consent or end of study, up to 46 doses of nivolumab.

** After evaluating single agent nivolumab dosing, the next dose level will be 2 if dose level 1 is the MTD for single agent nivolumab and dose level -2 if dose level -1 is the single agent MTD. It is also possible to de-escalate from dose level 2 to -2.

The combination therapy MTD will then be studied in a dose expansion cohort (12 participants) limited to only participants with Kaposi sarcoma, lung cancer, and anal cancer; CD4+ count eligibility criteria in the expansion cohort will be determined after reviewing results from Strata 1 and 2. If the MTDs differ between the two strata, expansion cohorts of six participants each will be enrolled**.** The study treatment consists of 12 IV infusions of nivolumab, 240 mg, on Day 1 of each 2-week cycle and ipilimumab on Day 1 every 6 weeks with four total ipilimumab doses, i.e., every third cycle (cycles 1, 4, 7, 10). After completion of the 12th cycle of nivolumab, the nivolumab dose will change to a flat dose of 480mg, on Day 1 of each 4-week cycle without ipilimumab. Participants with an overall response of SD, PR, CR, or unconfirmed PD at Week 8 will continue treatment (Refer to Section 9.1 for RECIST Criteria) for up to 84 weeks.

A single agent expansion cohort of 24 participants with solid tumors, excluding those histologies not known to respond to single agent nivolumab (i.e., pancreas, prostate, and microsatellite stable [MSS] colorectal cancer) and with CD4 count above 200 will receive single agent nivolumab at 240mg administered every 2 weeks for up to 46

doses, or until disease progression, toxicity, study termination, or study withdrawal.

Another single agent cohort of 12 participants with relapsed refractory HIV-associated cHL and with CD4 counts $\geq$ 100/mm^3, will also be treated with single agent nivolumab at a flat dose of 240mg administered every 2 weeks for 46 doses or until disease progression, toxicity, study termination, or study withdrawal (See Section 9.3 for the 2017 response evaluation criteria in lymphoma (RECIL) Criteria for evaluation of measurable disease in HIV-cHL and Section 4.7 and 4.9 for study duration and criteria for removal from study). Participants with an overall response of SD, PR, CR, or unconfirmed PD at Week 8 will continue treatment. Of note, this dose showed no dose-limiting toxicity (DLT) in this trial and is the current FDA approved dosing regimen for non-HIV classical Hodgkin lymphoma.

Duration: Maximum of 92 weeks of treatment

Primary Objective: Dose De-Escalation and Expansion Cohorts:

To demonstrate safety and feasibility of ipilimumab and nivolumab at the standard doses of drug in solid tumor and relapsed refractory HIV-cHL participants with HIV infection given the possibility of increased toxicity based on immune activation, co-morbidity, or interference with Highly active antiretroviral therapy (HAART) . The purpose for this would be to provide appropriate experience and guidelines, if necessary, to allow participants with HIV infections to participate in ongoing trials.

Secondary Objectives: Dose De-Escalation Cohort: To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on immune function (HIV viral load in plasma using conventional HIV assay, CD4+/CD8+ cells).

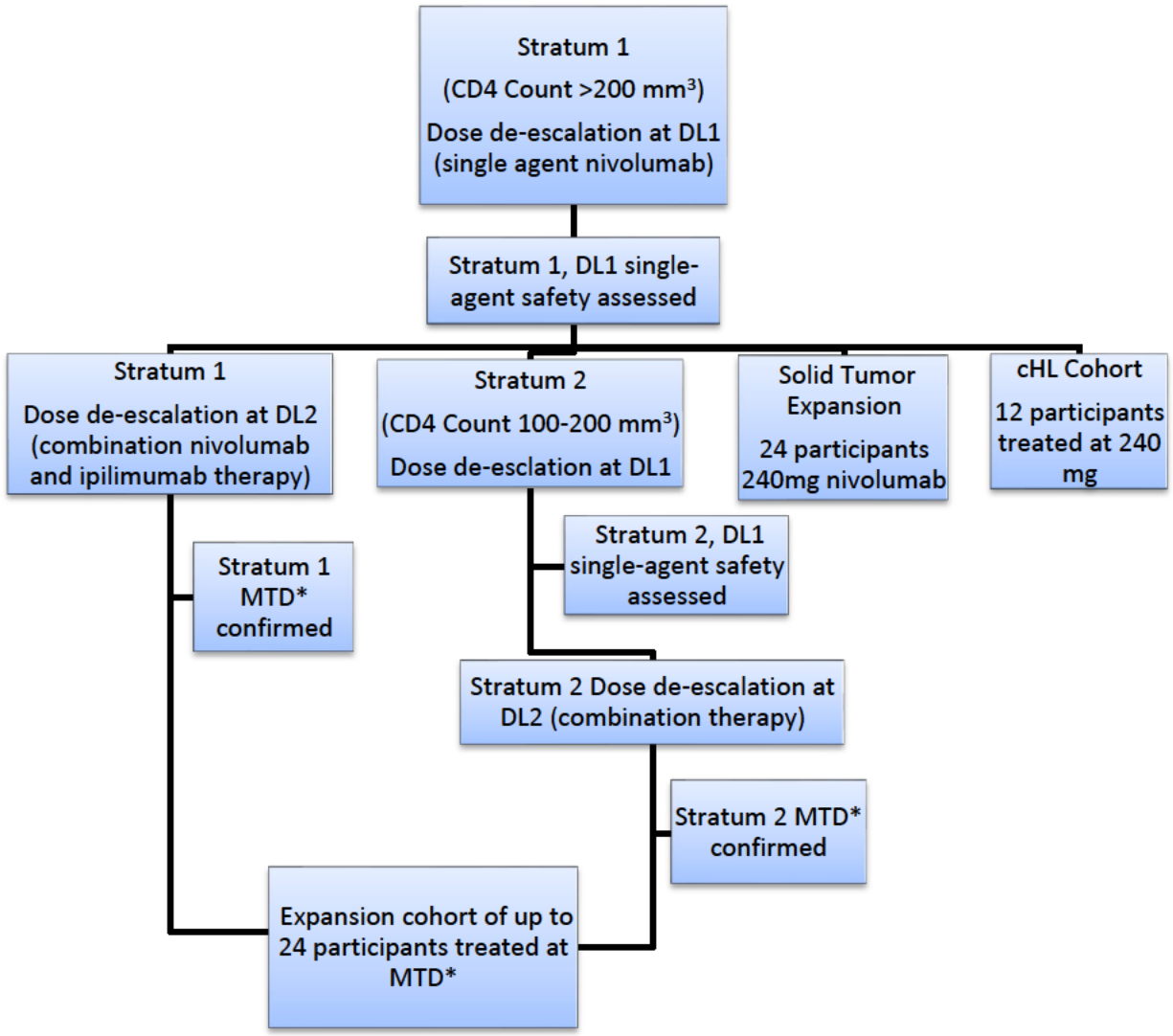
Solid Tumor Dose Expansion and cHL Cohorts: To preliminarily assess objective response rates associated with treatment for commonly represented solid tumors and relapsed refractory HIV-cHL.

Solid Tumor Dose Expansion and cHL Cohorts: To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on immune function (HIV viral load in plasma using conventional HIV assay, CD4+/CD8+ cells). Only single agent nivolumab will be administered in the cHL cohort.

Exploratory Objectives: Dose De-Escalation and Solid Tumor Expansion and cHL Cohorts:

1. Understand the immune response to agent in the context of antiretroviral therapy (ART), of altered immune function, and repertoire due to prior HIV infection.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on intratumor immune cells by IHC for PD1, PDL-1, and others.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on circulating cytokine markers by multiplex assay.
2. To understand the response of human tumor viruses (Human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV)) to agent.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus loads (EBV, KSHV, Cytomegalovirus infection (CMV) in plasma.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent herpesvirus (EBV, KSHV, CMV) in peripheral blood mononuclear cells (PBMC).
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus specific CD8 and CD4 T cells in PBMC.
    - <u>In cases of Kaposi sarcoma</u>, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on viral transcription in tumor biopsies, when feasible.
    - <u>In cases of anal cancer</u>, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HPV types in anal swabs, when feasible.
3. Understand the response of HIV to agent.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent HIV loads in PBMC using outgrowth assay.
    - To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HIV reactive T cells.

PROTOCOL SCHEMA



Abbreviations: DL= dose level; MTD = Maximum Tolerated Dose.

All participants will receive up to 92 weeks of protocol treatment if the participant has stable disease (SD), partial response (PR), complete response (CR), or unconfirmed progressive disease (PD). Dose levels and de-escalation schedules are provided on the following page.

* If combination therapy MTD differs in Stratum 1 and Stratum 2, dose expansion will occur at Stratum 1 Dose in participants with CD4 count > 200/mm^3.

** If the MTDs differ between the two strata, expansion cohorts of 18 and 6 participants respectively will be enrolled.

Dose De-Escalation Schedule for Stratum 1 and Stratum 2		
Dose Level	**Nivolumab* q2 wk x 46 doses**	**Ipilimumab****
Level -1	1mg/kg	-
Level 1	3mg/kg	-
Level 2	240mg	1mg/kg q 6 wk x 16 doses (day 1 of nivolumab cycles 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46)
Level -2	1mg/kg	1mg/kg q 12 wk x 8 doses (day 1 of nivolumab cycles 1, 7, 13, 19, 25, 31, 37, and 43)

*Doses are stated as exact dose in units (e.g., mg/m^2, mcg/kg, etc.) rather than as a percentage.

**When the two drugs are administered, nivolumab will be administered first. Doses of intravenous nivolumab will be administered every 2 weeks up to 46 doses. Ipilimumab will be administered every 6 weeks on dose level 2 or every 12 weeks on dose level -2.
***Treatment will continue until documented disease progression, discontinuation due to toxicity, withdrawal of consent, or the study ends, up to 46 doses of nivolumab (with ipilimumab, if receiving).

NOTE: After evaluating single agent nivolumab dosing, the next dose level will be 2 if dose level 1 is the MTD for single agent nivolumab, and dose level -2 if dose level -1 is the single agent MTD. It is also possible to de-escalate from dose level 2 to -2. Stratum 2 participants will not be allowed to escalate beyond the MTD for Stratum 1; one dose level de-escalation of the nivolumab and ipilimumab combination or to single agent nivolumab will be allowed based on the doses in the schema in the treatment plan.

NOTE: Participants treated on the cHL cohort and participants on the solid tumor expansion cohort of 24 participants will be treated at 240mg q 2 wk.

1.0 OBJECTIVES

1.1 Primary Objective

Dose De-Escalation and Dose Expansion Cohorts: To demonstrate safety and feasibility of ipilimumab and nivolumab at the standard doses of drug in solid tumor and relapsed refractory HIV-cHL participants with human immunodeficiency virus (HIV) infection given the possibility of increased toxicity based on immune activation, co-morbidity, or interference with highly active antiretroviral therapy (HAART). The purpose for this would be to provide appropriate experience and guidelines, if necessary, to allow participants with HIV infections to participate in ongoing trials.

1.2 Secondary Objectives

1.2.1 Dose De-Escalation Cohort: To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on immune function (HIV viral load in plasma using conventional assay, CD4+, and CD8+ cells).

1.2.2 Solid Tumor Dose Expansion and cHL Cohorts: To preliminarily assess objective response rates associated with treatment for commonly represented solid tumors (Kaposi Sarcoma, anal cancer, and lung cancer) and relapsed refractory HIV-cHL.

1.2.3 Solid Tumor Dose Expansion and cHL Cohorts: To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on immune function (HIV viral load in plasma using conventional HIV assay, CD4+, and CD8+ cells). Only single agent nivolumab will be utilized in the cHL cohort.

1.3 Exploratory Objectives

The exploratory objectives are part of both the dose de-escalation phase and the expansion phase. Some of the correlative biomarkers are only present in a subset of tumor types, e.g. HPV in anal cancer. All exploratory objectives will be addressed in batch at the completion of enrollment/study as applicable.

1.3.1 Understand the immune response to agent in the context of antiretroviral therapy (ART), of altered immune function, and repertoire due to prior HIV infection

1.3.1.1 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on intratumor immune cells by IHC such as PD1, PDL-1, and others.

1.3.1.2 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on circulating cytokine markers by multiplex assay, such as: IL-2, IL-4, IL-6, IL-10, IL-8, IP10, CXCL13, IFN-γ, TNF-α, sIL2R-α, sCD27, sTNFR1, and sTNFR2.

1.3.2 To understand the response of human tumor viruses (Human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV)) to agent

1.3.2.1 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus loads (EBV, KSHV, Cytomegalovirus infection (CMV)) in plasma.

1.3.2.2 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent herpesvirus (EBV, KSHV, CMV) in peripheral blood mononuclear cells (PBMC).

1.3.2.3 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus specific CD8 and CD4 T cells in PBMC.

1.3.2.4 In cases of Kaposi sarcoma, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on viral transcription in tumor biopsies.

1.3.2.5 In cases of anal cancer, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HPV types in anal swabs, when feasible.

1.3.3 Understand the response of HIV to agent

1.3.3.1 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent HIV loads in PBMC using outgrowth assay.

1.3.3.2 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HIV reactive T cells.

2.0 BACKGROUND

2.1 Study Disease

HIV infected individuals have an increased propensity to develop malignancy [1, 2]. Early in the AIDS epidemic, a dramatic increase in Kaposi sarcoma (KS), non-Hodgkin lymphoma (NHL), and cervical cancer was noted, and these tumors ultimately were classified as AIDS-defining cancers. With the widespread use of potent combination antiretroviral therapy (cART), there was a decrease in the incidence of KS and NHL and a significant increase in the incidence of several other malignancies (non-AIDS-defining cancers). The risk of dying from non-AIDS-defining cancers (NADC) has been increasing [3]. At present NHL, KS, and NADC each account for approximately 1/3 of cancers in HIV patients on cART.

Malignancies in patients infected with HIV are often characterized by earlier age at onset, atypical pathology (higher tumor grade), more aggressive clinical behavior, and/or more advanced stage at presentation [4, 5].

Multiple factors may contribute to the increased incidence of malignancy in patients infected with HIV. These include immunosuppression, perhaps direct effects of the HIV virus itself, coinfection with other oncogenic viruses, environmental factors, and possibly the use of antiretroviral drugs.

The immunosuppression seen in the HIV infected patients may accelerate the development of neoplasia. The cancers are similar to that observed in solid organ transplant recipients who receive chronic immunosuppressive agents, as well as in patients with profound inherited immune deficiencies. Even after years of successful ART and complete "virologic" response, the T cell repertoire in HIV+ patients remain compromised. It is unclear if and to what degree T cell function remains compromised as well.

2.1.1 HIV-associated neoplasia: the HIV virus and other oncogenic viruses

***Role of the HIV virus*.** A direct molecular role for HIV in cancer remains a matter of discussion. HIV infection may have a direct effect on a variety of cellular processes that contribute to the development of cancer [5]. Postulated mechanisms include the activation of proto-oncogene's, alterations in cell cycle regulation, inhibition of tumor suppressor genes, and possibly other genetic alterations that lead to oncogenesis though the most likely contribution of HIV to tumorigenesis is through immune suppression/ systemic immune activation and modulation of the tumor microenvironment.

Coinfection with oncogenic viruses. Patients infected with HIV are at increased risk of coinfection with other viruses that are known to cause cancer [5]. Furthermore, the natural history of these viral infections may be accelerated in patients with HIV.

HHV-8 infection. KS is caused by a human herpes virus 8 (HHV-8) or KSHV. KSHV has also been associated with the multicentric form of Castleman's disease and primary effusion lymphoma. In the U.S., both of these entities are found primarily in patients with HIV infection.

HPV infection. In addition to its relationship to cervical neoplasia, HPV infection

is associated with cancers of the head and neck (especially the oropharynx and base of tongue) and anogenital (anus, penis, vagina, vulva) regions. Anal cancers have increased in incidence in HIV+ persons since the introduction of cART.

EBV infection. Hodgkin lymphoma (HL) is not an AIDS-defining malignancy, EBV-associated NHL such as CNS lymphoma are AIDS-defining. EBV infection has an established etiologic role in some cases of lymphoma, and EBV is more frequently implicated in HL in HIV infected individuals than in non-HIV infected people.

HBV and HCV infection. Infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are a major cause of hepatocellular carcinoma. Coinfection with HIV and either HBV or HCV can result in more rapid progression of liver disease, resulting in cirrhosis and hepatocellular carcinoma.

Merkel cell polyomavirus. Merkel cell polyomavirus has a causative role in Merkel cell carcinoma, which is much more frequent in HIV infected people.

2.1.2 The immune system and oncogenic viruses: pathogenesis of HIV-associated neoplasia

Oncogenic viruses such as HPV and KSHV are capable of causing cancer not just in the HIV infected individuals but also in recipients of organ and bone marrow transplants who are immunosuppressed as well as in elderly and patients with no prior history of dramatic immune suppression. It is not exactly known what the trigger is that initiates neoplasia development. On the one hand, the immune system continuously controls chronic and persistent virus infection through the production of virus-antigen-specific B and T cells. For instance, approximately 10% of all circulating CD8 T cells are directed against human cytomegalovirus encoded epitopes, fewer against EBV, KSHV, HPV, and other persistent viruses. On the other hand, continuous antigenic stimulation that occurs during chronic virus infections causes severe immune dysfunction [6-8], and a concomitant induction of immuno-regulatory processes, which all result in the loss of tumor immune-surveillance and may lead to cancer establishment and/or progression.

In the HIV infected individual, there is a clear relationship between progressively lower CD4+ T cell levels and increased detection of HPV types in the anus. One of the most consistent risk factors for anogenital IN is a lower CD4+ level, suggesting that HIV-related immune suppression plays an important role in disease pathogenesis [9,10]. Loss of the appropriate immune response to HPV may play its most important role in permitting the development of high-grade IN, which has the potential to progress to cancer. However, progression from high-grade IN to cancer likely depends on additional factors as well. Increasing evidence suggests that one of these factors may be changes in host gene expression secondary to HPV-induced chromosomal instability and copy number abnormalities.

It is speculated that if there is a low number of circulating HPV-specific memory cells, then HPV-specific immunity may be particularly vulnerable to the effects of HIV. Moreover, HPV-specific immunity may not recover fully after immune response is restored, which may explain the relatively limited beneficial effect of

HAART on HPV-associated lesions. HAART does not appear to either prevent the development of AIN, nor the progression of AIN towards SCCA [11].

HIV-associated attenuation of HPV-specific immune responses may allow for persistence of oncogenic HPV, of high-grade IN and for sufficient time for accumulation to accumulate genetic changes that are important in progression to cancer.

KS skin lesions, analogous to melanoma, at times regress spontaneously. The clinical experience in transplant KS shows rapid regression upon tapering of immune suppression [12]. In the HIV infected individual, there is a relationship between progressively lower CD4+ T cell levels and increased KS lesion development, though Maurer et al. as well as a review of AMC studies showed that KS now occurs in patients with undetectable KSHV viral load and near-normal CD4 counts [Even though the exact mechanism has not been elucidated, both immune presenting (Langerhans, NK, macrophage) and CD8+ T lymphocytes have been implicated. KSHV viral epitopes are recognized by human T cells and CD8 T cells attenuation has been observed in progressive KS, including in the setting of HIV [13-15]. In sum, like anal cancer KS is caused by a virus and KS tumor cells express viral T cell epitopes; like melanoma there is evidence of tumor-specific antigens and evidence of tumor regression in response to immune modulation.

The pathogenesis of HIV-associated cHL and NHL is multifactorial, and in part depends on the tumor microenvironment, the presence of oncogenic viruses, and the CD4+ T cell count. The microenvironment of classical HL has been closely linked to the microenvironment. Data by Skinnider et al. has shown that CD4+ T cells initiate trophic signals promoting the survival of the malignant cell associated with HIV-cHL, the Reid Sternberg cell (hRS) [16]. This could also explain the increase in incidence of HIV-cHL from the pre-HAART to the HAART era [1,16,17].

Oncogenic viruses associated with HIV/AIDS-related lymphomas are mainly KSHV and EBV. Approximately 100% in primary effusion lymphoma, 90% of classical HL, 100% of primary CNS lymphoma in the HIV-population, and 80-100% of immunoblastic diffuse large B cell lymphoma (DLBCL) are associated with EBV. All primary effusion lymphoma cells are co-infected with both EBV and KSHV viruses. These viruses can alter mechanisms of apoptosis and cell cycle regulation, activate oncogenes, and inhibit tumor suppressor genes [1].

Chronic antigenic stimulation of B cells and macrophages induced by EBV, HIV, and HHV-8 have also been reported to elicit cytokine and growth factor release that promotes B cell proliferation and the outgrowth of a monoclonal B cell population [1].

PD-1 is an inhibitory receptor expressed by an activated CD4+ and CD8+ T cells. In chronic infection, the PD-1 pathway mediates pathogen-specific and cancer specific CD8+ T cell dysfunction as demonstrated in HIV [24-26], HCV [27,20], HBV [29] infections. In chronic HCV infection, increased PD-1 expression on HCV-specific CD8+ T cells is associated with impaired proliferation and cytokine production [27]. Apart from inhibition of T cell function, PD-1 expression can also

led to spontaneous or FAS-mediated apoptosis of virus-specific T cells [30].

CTLA-4 is another inhibitory receptor expressed by activated CD4+ and CD8+ T cells. It has a higher affinity for the B7 ligands (CD80 and CD86) allowing it to out-compete CD28, hence it is a powerful negative regulator of CD28-dependent T cell responses. It is significantly up-regulated on CD4+ T cells during HIV-1 and HCV infections where it correlates positively with disease progression and negatively with antigen-specific IL-2 production [31]. CTLA-4 is abundantly expressed on Treg as it is required for optimum suppressive function.

For these reasons, anti-PD1 and anti-CTLA-4 may have therapeutic potential in virally mediated neoplasia.

2.1.3 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 two main mechanisms: 1) the induction of immune tolerance and 2) resistance to killing by activated immune effector cells [32]. The concept of ‘‘immunoediting’’ relates to the manner in which tumors manipulate their microenvironment through tumor-derived cytokines, chemokines, and other soluble factors [33]. 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 cancer tumors 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 Study Agents

2.2.1 Nivolumab

Nivolumab (BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor [34]. PD-1 is a negative regulatory molecule that is expressed transiently following T cell activation and on chronically stimulated T cells characterized by an "exhausted" phenotype. Nivolumab binds to cynomolgus monkey PD-1 but not mouse, rat, or rabbit molecules. Clinical activity of nivolumab has been observed in patients with melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC). The combination of nivolumab and ipilimumab (anti-cytotoxic T lymphocyte associated antigen-4 [anti-CTLA-4]) in a phase 1/2 trial showed markedly enhanced clinical activity with an acceptable safety profile in melanoma patients [35].

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) [34]. PD-1 is transiently but highly expressed on activated T cells functioning to limit immune effectors at the site of activation. Chronic stimulation may prevent the re-methylation of the PD-1 gene leading to continuous expression and characterizes a state of "exhausted" T cells that lose function and proliferative capacity while enhancing a suppressive tumor microenvironment. PD-1 may act together with other T cell modulating molecules, including CTLA-4, TIM-3, lymphocyte activation gene 3 (LAG-3) as well as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta (TGF-beta).

Two ligands specific for PD-1 have been identified: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs],

and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). The interaction of PD-1 with PD-L1 and PD-L2 results in negative regulatory stimuli that down-modulate the activated T cell immune response through SHP-1 phosphastase.

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 [36]. Despite minimal immunopathologic sequelae in PD-1 and LAG-3 single knockout mice, dual knockout mice abrogated self-tolerance with resultant autoimmune infiltrates in multiple organs, leading to eventual lethality.

PD-L1 expression is found on a number of tumors and is associated with poor prognoses based on OS in many tumors, including melanoma [37], renal [38-40], esophageal [41], gastric [42], ovarian [43], pancreatic [44], lung [45], and other cancers [34].

PD-L1 is also found on hematological malignancies, in particular cHL. The 9p24.1 locus is found in many copies in cHL, the location of the PD-L1 locus. Increased copy number upregulates PD-L1 expression on the hRS cell. Also located at the 9p24 locus is the Janus kinase 2, and the Janus kinase 2/signal transducer can further induce PD-L1 ligand transcription [18]. Lastly, as stated above, HIV-associated cHL has a 90% association with EBV coinfection of the hRS, another mechanism of PD-L1 overexpression, possibly a viral survival tactic to evade the immune system [1,18]. The high expression of PD-L1 makes this disease, HL, particularly susceptible to anti-PD-1 therapy. (See efficacy in hematological malignancies below.)

The PD-1/PD-L1 axis plays a role in human infections, particularly in HCV and 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.

Non-clinical development of nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated [34]. Combination studies have highlighted the potential for toxicity when combined with ipilimumab, MDX-1408, and BMS-986016. Nivolumab bound specifically to PD-1 (and not to related members of the CD28 family such as CD28, ICOS, CTLA-4, and BTLA) with a K_d = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

Clinical development of nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC, hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications [34]. 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.

In addition, nivolumab, either as a single agent of in combination with ipilimumab or in combination with other immunotherapies or cytotoxic chemotherapies, has been studied across all hematological malignancies, in particular Hodgkin and NHL [18].

Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (C_{max}) and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), with low to moderate inter-participant variability observed at each dose level [34]. 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.

Efficacy in solid malignancies

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 participants, objective responses (ORs) (CR or PR) were seen in NSCLC, melanoma, and RCC. ORs were observed at all doses [46]. 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 [47]. In the RCC cohort, median duration of response was 12.9 months for both doses with five of the ten responses lasting ≥1 year [48].

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered by IV every 3 weeks for four doses followed by nivolumab alone every 3 weeks for four doses (concurrent-regimen) [35]. The combined treatment was subsequently administered every 12 weeks for up to eight doses. In a sequenced regimen, participants previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent-regimen (53 participants), 53% of participants had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with

tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced regimen (33 participants), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC participants, 43 participants were treated with nivolumab + PT-doublet [49]. 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.

Toxicology

A MTD of nivolumab was not defined [50]. Serious adverse events (SAEs) occurred in 32 of 296 participants (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in three deaths. Renal failure, symptomatic pancreatic and diabetes mellitus (DM), neurologic events, and vasculitis have also been reported.). In combination with ipilimumab in the concurrent-regimen group [35], grade 3 or 4 treatment-related events were noted in 53% of participants. Skin rash represents the majority of these events.

Pharmacodynamics/biomarkers

Tumor cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral blood absolute lymphocyte count [35]. With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 participants (38%) were PD-L1–positive. Among participants treated with the concurrent-regimen of nivolumab and ipilimumab, ORs were observed in participants with either PD-L1–positive tumor samples (6 of 13 participants) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among participants with PD-L1–positive tumor samples (4 of 8 participants) than among participants with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in participants treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell $CD8^+$ are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

Efficacy in Hodgkin lymphoma

Based on the efficacy of PD1 antibody blockade in solid tumors, two phase 1 studies were conducted in hematological malignancies. The first was a study analyzing the safety of single agent nivolumab in relapsed refractory NHL, HL, and multiple myeloma [18-20] Doses were escalated from 1 mg/kg to 3 mg/kg. No dose limiting toxicities were identified, and the dose used in the expansion cohort of the

phase I trial, as well as in the phase II study described below, was 3 mg/kg given every 2 weeks until toxicity, progression, or study termination/removal. A second study analyzed pembrolizumab, a similar anti- PD-1 antibody [18-21]. As stated above, the tumor cell in cHL, hRS, through various mechanisms (e.g., EBV status and elevations in 9p24) upregulates PD-L1 expression [18]. The overall response rate of relapsed/refractory cHL with single agent nivolumab was 87%, with a CR rate of 17% in patients pretreated with multiple lines of therapy, including brentuximab vedotin and autologous transplantation [21]. This is higher than any solid tumor assessed to date with nivolumab. Eighty-six percent of these patients had a PFS of 24 weeks. In the study of nivolumab by Ansell *et al*., only 22% of the patients developed a Grade 3 Adverse event (AE) [21].

Recently, a phase II study of single agent nivolumab in patients who failed both brentuximab vedotin and autologous bone marrow transplantation was reported. Patients received single agent nivolumab at 3 mg/kg every 2 weeks until disease progression, death, or toxicity [21]. Of 80 patients treated, 66% achieved an objective response, with on average four prior therapies, and nine patients achieved a CR. Fifty-eight percent (58%) of the patients achieved a partial remission. At 6 months, the PFS was 76.9% with an OS of 98% [21]. The median number of nivolumab doses received was 17. Of the 80 patients, 29 had to discontinue therapy, all due to disease progression and not toxicity. In a recent trial, the combination of nivolumab and ipilimumab achieved a similar ORR of 74% in 31 patients with relapsed refractory cHL, similar to single agent nivolumab; however, both single and combined showed robust activity [22].

Toxicity of nivolumab in patients with relapsed refractory Hodgkin lymphoma.

In the phase I study of nivolumab, 22% of the patients had drug-related grade 3 AEs. There were no grade 4 or 5 drug-related AEs. In the phase II study, when treated with 3 mg/kg, 33% of participants experienced grade 3 AEs and only 8% experienced grade 4 AEs. The dose utilized in the phase II study was based on data by Ansell et al. The most common drug-related grade 3 or 4 AEs were neutropenia, experienced by 5% of participants, and increased serum lipase concentrations in 5% of participants. The most common SAEs was pyrexia (4%). Three patients died during the study though these were not treatment-related [18-21].

Pharmacodynamics/biomarkers

In the phase II trial of patients treated for relapsed refractory HL with single agent nivolumab, those analyzed showed that 56% had gain of copies of PD-L1 and PD-L2 and amplification of 27% of the tumors assessed. In a post- hoc analysis, none of the patients with progressive disease had amplification of PD-L1 or PD-L2. In addition, correlation between PD-L1 expression was correlated with a greater response. All patients who achieved a CR had the highest level of PD-L1 expression, and progressive disease was only identified in those with the lowest staining by immune histochemistry [21].

2.2.2 Ipilimumab

Ipilimumab (BMS-734016, MDX010, MDX-CTLA-4, Yervoy™) is a fully human

monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells [51]. CTLA-4 is a negative regulator of T cell activation. Ipilimumab binds to CTLA-4 and inhibits its interaction with ligands on antigen-presenting cells (APCs). The proposed mechanism of action for ipilimumab's effects in subjects with melanoma is indirect, possibly through T cell potentiation and mediation of antitumor immune responses.

Ipilimumab has been approved for the treatment of unresectable metastatic melanoma in over 40 countries including the United States (U.S., March 2011), the European Union (July 2011), and Australia (July 2011).

BMS and Medarex (acquired by BMS in Sep-2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing >13,800 subjects in several cancer types in completed and ongoing studies, including a compassionate use program [51]. The focus of the clinical program is on melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies.

CTEP's clinical development of ipilimumab focuses on cervical, gastrointestinal, ovarian, prostate cancer, chronic lymphocytic leukemia, head and neck squamous cell carcinoma, solid tumors, Hodgkin and non-Hodgkin lymphomas, melanoma, and myelodysplastic syndrome.

While the toxicity and clinical responses overlap, mechanisms of immune activation and range of responses appear to be different for each of the single agents.

Preclinical data support the combinations of nivolumab and ipilimumab [52].

The combination of ipilimumab with nivolumab has been reported to result in improved responses in advanced melanoma marked by time to response, number of responses, depth, and duration of responses, PFS, and OS compared to single agent ipilimumab [35].

For RCC results have been reported [53].

The combination is being evaluated in other disease settings typically with 3 mg/kg nivolumab and 1mg/kg ipilimumab q 3 weeks x 4 induction doses.

2.3 Study Design and Rationale

2.3.1 Preclinical data: combining ipilimumab and nivolumab

CTLA-4 and PD-1 appear to play complementary roles in regulating adaptive immunity. Anti-CTLA-4 can lead to enhanced priming and activation of antigen-specific T cell and potentially clearance of regulatory T cells from the tumor microenvironment. The blocking of PD-1 or its ligand PD-L1 can remove the inhibition of cancer cell killing by T cells. In preclinical models, combined blockade of PD-1 and CTLA-4 achieved more pronounced antitumor activity than blockade of either pathway alone [79]. Combining a CTLA-4 targeted therapy

(ipilimumab) with a PD-1-targeted inhibitor (nivolumab) appears to enhance the immune activity in patients over either therapy alone. This might be enhanced with pretreatment receptor profiling of the tumor.

2.3.2 Clinical data: combining ipilimumab and nivolumab

In an early phase study, rapid and deep responses (by modified WHO criteria) were observed in participants with melanoma (ORR: 40% [21/72]; CR: 10% [5/72]). Immune-related toxicities were also enhanced in their magnitude, frequency, and onset (53% Grade 3-4 treatment-related toxicities). Although many of these were serious and required treatment, therapy discontinuation, or hospitalization, the durable partial and CRs in melanoma may warrant this approach in some patients. KS is a likely candidate because of its many similarities to melanoma and because it, similarly, as observed in melanoma, tends to regress in response to immune modulation. Combination therapy appeared to most dramatically benefit patients who were less likely to benefit from PD-L1 or PD-1 inhibition alone, because their tumors were PD-L1-negative (6/13 PD-L1-positive and 9/22 PD-L1-negative participants responded to combination therapy).

Successive cohorts of participants with escalating doses of intravenous nivolumab and ipilimumab administered concurrently every 3 weeks for four doses, followed by nivolumab alone every 3 weeks for four doses (concurrent-regimen group). The combined treatment was subsequently continued every 12 weeks for up to eight doses. When the two drugs were administered together, nivolumab was administered first. Within a cohort, doses of nivolumab and ipilimumab were kept constant. The protocol-specified dose levels in the cohorts were as follows. In the concurrent-regimen group, cohort 1 was designated to receive 0.3 mg of nivolumab per kilogram of body weight and 3 mg of ipilimumab per kilogram; cohort 2, 1 mg of nivolumab per kilogram and 3 mg of ipilimumab per kilogram; cohort 2a, 3 mg of nivolumab per kilogram and 1 mg of ipilimumab per kilogram; cohort 3, 3 mg of nivolumab per kilogram and 3 mg of ipilimumab per kilogram; cohort 4, 10 mg of nivolumab per kilogram and 3 mg of ipilimumab; and cohort 5, 10 mg of nivolumab per kilogram and 10 mg of ipilimumab per kilogram.

SAEs related to the treatment were reported in 49% of participants in the concurrent-regimen group. Common grade 3 or 4 selected AEs that were related to the therapy included hepatic events (in 15% of participants), gastrointestinal events (in 9%), and renal events (in 6%). Isolated cases of pneumonitis and uveitis were observed, a finding that is consistent with previous experience with monotherapy. A total of 11 participants (21%) discontinued therapy owing to treatment-related AEs. In the concurrent-regimen cohorts, across all dose levels, confirmed ORs according to modified WHO criteria were observed in 21 of 52 participants (40%) who had a response that could be evaluated. In addition, four participants had an objective response according to immune-related response criteria and two had an unconfirmed PR. These participants were not included in the calculation of objective response rates. At the maximum doses associated with an acceptable level of adverse vents – that is, nivolumab 1 mg/kg and ipilimumab 3 mg/kg – 53% of participants had objective response, all with tumor reduction of $\geq$80% [80].

Participants with metastatic renal cell cancer were randomized to receive nivolumab 3 mg/kg + ipilimumab 1 mg/kg (arm N3 + I1) or nivolumab 1 mg/kg + ipilimumab 3 mg/kg (arm N1 + I3) IV Q3W for four doses then nivolumab 3 mg/kg IV Q2W until progression/toxicity. The primary objective was to assess safety/tolerability; secondary objective was to assess antitumor activity. Treatment-related AEs were seen in 39/44 pts (89%); 7 pts (16%; N3 + I1: 2; N1 + I3: 5) discontinued due to any grade related AEs. Grade 3–4 related AEs occurred in 19 pts (43%; N3 + I1: 5; N1 + I3: 14), most commonly ↑ lipase (16%, n=7), ↑ ALT (11%, n=5), diarrhea (9%, n=4), colitis (5%, n=2), ↑ amylase (5%, n=2). No grade 3–4 pneumonitis was seen. The ORR was 29% (N3 + I1) and 39% (N1 + I3). Duration of response (DOR) was 4.1+ to 22.1+ wks (all six responses ongoing) in N3 + I1, and 6.1+ to 18.3+ wks (8/9 responses ongoing) in N1 + I3. Responses occurred by first tumor assessment (wk 6) in 67% of responding pts in both N3 + I1 and N1 + I3. SD was seen in 7 (33%) pts (N3 + I1) and 9 (39%) pts (N1 + I3) [81].

Investigators in CheckMate-012 evaluated four administration/dosing schedules for the combination of nivolumab and ipilimumab. In arm A, both agents were administered at a dose of 1 mg/kg every 3 weeks (Q3W, N = 31). In arm B nivolumab was administered at 1 mg/kg every 2 weeks (Q2W) plus ipilimumab 1 mg/kg every 6 weeks (Q6W; N = 40). Arm C and D dosed nivolumab at 3 mg/kg Q2W and ipilimumab 1 mg/kg every 12 weeks (Q12W; N=38) or Q6W (N = 39).

Across the four groups, the patient cohorts had a median age of 62 to 68, stage IV disease in more than 90% of cases, non-squamous histology in more than 80%, and ECOG 0-1 performance status.

All four regimens demonstrated activity, with the arms containing nivolumab at 3 mg/kg showing the best ORR. In arm C, the ORR was 39% and in arm D the ORR was 31%. The ORRs were 25% and 13%, in arm B and arm A, respectively.

Median PFS ranged from 4.9 months in arm B to 10.6 months in arm A. Median PFS was 8 months in arm C and 8.3 months in arm D. The 24-week PFS was 55% in arm A, 63% in arm C, and not yet reached in the other two groups. Median OS was not yet reached in all four arms (follow-up range, 6.2-16.6 months).

In those with ≥1 PD-L1 expression by IHC (n = 77; 68%) the ORR was 8%, 24%, 48%, and 48% in arms A, B, C, and D, respectively. Median PFS across each arm were 11.5 weeks, 21.1 weeks, 34.6 weeks, and not reached and the 24-week PFS rates were 42%, 40%, 74%, and 65%, respectively.

Treatment-emergent grade 3/4 adverse AEs occurred in 28% to 35% of patients in each group but led to discontinuation in just 3% to 10% of cases. All grade treatment-related AEs occurred in 77%, 73%, 74%, and 69% of patients in groups A, B, C, and D, respectively. The safety profile was consistent with previous studies of the combination, and the discontinuation rate associated with AEs was similar to rates observed with nivolumab alone.

The only grade 3/4 AEs that occurred in as many as 10% of patients were hepatic in arm B (10%) and skin-related in arm C and D (13%). Grade 3/4 pulmonary AEs

occurred in no more than 3% of patients in any of the groups. There were no treatment-related deaths in the trial [82].

Given the safety and efficacy profile of nivolumab at 3 mg/kg Q2W and ipilimumab 1 mg/kg every 12 weeks, this schedule will be evaluated in this study of HIV patients with advanced refractory solid tumors.

2.3.3 Clinical data combining of ipilimumab and nivolumab in relapsed refractory classical HL

The combination of nivolumab and ipilimumab in relapsed refractory HL achieved a similar relative response rate, as single agent nivolumab, at 74% [22].

Thus, for the relapsed/refractory HIV-cHL cohort, in light of the lack of evidence that dual blockade is superior to single agent PD-1 blockade, only single agent nivolumab will be used.

2.3.4 HIV: PD1, CTLA-4, Tregs and immune dysfunction causing cancer

Up-regulation of inhibitory receptors such as programmed death 1 (PD 1), T cell immunoglobulin mucin 3 (TIM-3), lymphocyte activation gene 3(LAG-3), and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) is a characteristic feature of exhausted T cells. PD-1, an inhibitory receptor of the CD28 superfamily, is highly expressed on exhausted CD8+ T cells during progressive chronic viral infections and uncontrolled cancer, making it a major factor in T cell exhaustion.

However, in chronic infection, the PD-1 pathway mediates pathogen-specific CD8+ T cell dysfunction as demonstrated in HIV infection [83-85]. For example, the frequency of PD-1+CD8+ T cells is highly elevated in HIV-1 patients where it correlates significantly with viral load and declining CD4+ T cell numbers [83, 76]. PD-1 is also up-regulated on HIV specific CD4+ T cells [84, 87] and inhibits CD4+T cell responses including proliferation. Interestingly, PD-1 levels are significantly reduced in HIV-1 progressors who initiate HAART or in long term non-progressors (LTNPs), suggesting that antigen persistence drives T cells to exhaustion.

Besides PD-1, other inhibitory receptors such as TIM-3, 2B4 (natural killer cell receptor), and LAG-3 are also up-regulated on virus-specific T cells, and the expression of multiple inhibitory receptors correlates with a severely dysfunctional state [88-90]. For example, co-expression of PD-1 and TIM-3 is associated with severely exhausted HIV specific CD8+ T cells [90] and majority of these also co-express PD-1 and 2B4 [91]. CTLA-4 is another inhibitory receptor expressed by activated CD4+ and CD8+ T cells. It has a higher affinity for the B7 ligands (CD80 and CD86) allowing it to out-compete CD28, hence it is a powerful negative regulator of CD28-dependent T cell responses. It is significantly up-regulated on CD4+ T cells during HIV-1 and HCV infections where it correlates positively with disease progression and negatively with antigen-specific IL-2 production [86, 91]. CTLA-4 is abundantly expressed on Treg as it is required for optimum suppressive function.

Increased frequencies of Treg and MDSC are a common feature of persistent chronic viral infections, which is well-documented in infections with HIV [93]. For

instance, it is postulated that by virtue of their activated nature, Treg express higher levels of CCR5 and CXCR4, the co-receptors for HIV-1 thus making them preferential targets for HIV-1 infection [94, 95]. Since Foxp3+ Treg represent a high proportion of CD4+ T cells (up to 50%) found in mucosal lymphoid organs of HIV infected individuals [96], it is plausible that HIV infection can subsequently lead to significant depletion of these cells and impaired immuno-regulatory functions [97].

A number of immuno-regulatory mechanisms triggered to prevent immune activation and inflammation also suppress immune effector functions and sustain chronic virus persistence. For example, during chronic HIV infection, the expansion of Treg [98] with potent suppressive activity within mucosal tissues not only contributes to persistence of HIV, but also reduces immune vigilance and predisposes to HPV and cervical cancer.

Therefore, the profound T cell dysfunction, progressive depletion of CD4+ T cells, B cell hyper-activation, together with the increased immuno-regulatory mechanisms all collude to actively impede tumor immune-surveillance and create a permissive environment for cancer initiation and progression.

2.3.5 In vitro data with anti-PD1 and anti-CTLA-4 in HIV

In vitro antibody blockade of PD-ligand (L)-1 and IL-10Rα results in increases in cytokine expression, including interferon-gamma (IFN-γ) in HIV-1-infected patients [99]. PD-1 blockade using PD-1 antibodies led to an increase in HIV specific CD8+ T cell proliferation demonstrating that HIV-induced T cell exhaustion is reversible *in vitro* [100]. Memory CD4+ T cells expressing high levels of PD-1 contain more proviral DNA than PD-1-low cells and blocking PD-1 may activate HIV-1 replication and thus facilitate the clearance of these infected cells.

T regulatory cells express high levels of CTLA-4 and are implicated in inhibiting immune responses during HIV-1 infection. In vitro experiments using cells from healthy and HIV-1-infected individuals showed that CTLA-4 is up-regulated in the CD4+ T cells from infected patients and inversely correlated with CD4 counts and anti-HIV-1 T cell responses while positively correlating with the percentage of activated CD4+ T cells [101].

2.3.6 Simian studies with anti-PD1 and anti-CTLA-4

Velu and colleagues presented the first *in vivo* study to show enhancement of a virus-specific immune response through PD-1 blockade using a PD-1 antibody [101]. Nine SIV-infected macaques, five in the early phase of infection and four in the chronic phase, received an anti-PD-1 antibody while another 5 SIV-infected macaques received an isotype control antibody. Their results showed an expansion of the Gag-CM9 tetramer specific CD8+ T cells of approximately 2.5 to 11-fold in the treated group. Additionally, an enhancement of Gag-specific CD8+ T cell function was observed after the blockade as measured by the co-expression of IFNγ, interleukin 2 (IL-2), and TNFα. The enhanced immunologic response after anti-PD-1 treatment corresponded with significant reductions in plasma viremia and prolonged survival of the infected macaques. Serum anti-nuclear antibody (ANA) levels were unchanged after treatment suggesting no evidence of autoimmune disease, which had been observed in murine studies of PD-1 gene disruption. The antigen-specific T cell proliferation was significantly elevated until about day 45. Similarly, enhancement of HIV specific CD8+ T cell function peaked at around day 21 and then decreased. These changes reflected a significant reduction in plasma viremia initially, which went back to baseline by day 43 post-treatment. The transient response was associated with a decline in anti-PD-1 antibody between days 14 and 28.

The results of this study of PD-1 blockade in an SIV-macaque model show promise of anti-PD1 antagonists as a novel immunotherapy for HIV. Increasing the immune response to the virus, particularly the Gag-specific polyfunctional CD8+ T cells, which are associated with control of viremia [27] through PD-1 blockade, may allow patients to obtain well-spaced intermittent treatment without being on prolonged and continuous antiretroviral regimens. Administration of PD-1 did not result in toxic side effects or symptoms of autoimmunity. This is consistent with the clinical trial by Berger *et al*. [102], which showed that administration of 0.2 to 6.0 mg/kg of CT-011 (a humanized IgG1 monoclonal PD-1 antibody) was well tolerated in patients with hematologic malignancies.

Francheschini *et al*. [103] showed that PD-L1 negatively regulated Tregs in persons chronically infected with HCV. There have been a number of studies showing ability of Tregs to suppress the immune response to HIV. Blocking PD-1 will not only increase the antiviral function of the CD8+ T cells but may also enhance the function of the antigen-specific Tregs, which may negate the antiviral response. Although this study has suggested that the transient immune response was secondary to declining titers of anti-PD-1 antibody, it is important to look into whether an increase in Treg frequency and function is responsible for this transient response or whether the possible increase was responsible for bystander immunosuppression that prevented autoimmune events from occurring.

These results suggest that beyond its effects on target-specific immune responses, PD-1 blockade may foster immune restoration and/or dampen harmful immune dysregulation.

In contrast, although CTLA-4 expression was found to be up-regulated in CD4 T cells of lymphoid tissues in SIV infection [104], anti-CTLA-4 blockade failed to show a benefit in terms of plasma viral load or survival in acutely or chronically SIV-infected macaques [101]. Whereas an increase in HIV specific CD4 and CD8 T cell responses was noted in one study [105], another study did not show an expansion of SIV-specific CTL and observed an increase in CD4 T cell activation and viral replication at mucosal sites [72]. Further studies are needed to understand these contrasting results between blockades of the PD-1 and CTLA-4 networks, including profiling the receptors, which is being proposed as a translational research component of this clinical trial. Differences between expression and function of the PD-1 and CTLA-4 molecules in the CD4 and CD8 T cell subsets could contribute to these distinct effects. Because CTLA-4 is crucial for regulatory T cell function and findings in HIV infection showed that CTLA-4 is preferentially up-regulated on virus-specific CD4 T cells but not CD8 cells, [105], CTLA-4 blockade might preferentially expand SIV-specific CD4 T cells and increase CD4 activation, thus providing additional targets for viral infection without improvement in CTL function to offset this detrimental effect.

In a preclinical study in rhesus macaques infected with the pathogenic simian immunodeficiency virus SIV_{mac251} and treated with ART. We tested whether vaccination administered together with cytotoxic T lymphocyte associated antigen 4 (CTLA-4) blockade and treatment with the indoleamine 2,3-dioxygenase (IDO) inhibitor 1-methyl-D-tryptophan (D-1mT), decreased immune activation and improved vaccine efficacy. The treatment did not augment vaccine immunogenicity; rather, it dramatically increased ART-related toxicity, causing all treated animals to succumb to acute pancreatitis and hyperglycemic coma. The onset of fulminant diabetes was associated with severe lymphocyte infiltration of the pancreas and complete loss of the islets of Langerhans. The specific pancreatic damage induced by ART, combined with the effect that CTLA-4 and IDO blockade may have on immune homeostasis, might have exacerbated autoimmunity to pancreatic antigens [107].

2.3.7 Clinical experience with nivolumab in patients with Hepatitis C and ipilimumab in a HIV patient

A randomized, modified double-blind placebo-controlled single ascending dose study evaluated the safety, pharmacokinetics, and immunogenicity of nivolumab in HCV-infected participants. In the high dose group, 3/20 participants, or 15%, experienced significant reductions of HCV RNA. Suppression of HCV replication persisted more than eight weeks in most participants. Two participants achieved HCV RNA below the lower limit of quantitation (25 IU/mL), one of whom (a prior null-responder) remained RNA-undetectable 1-year post-study. Transient reductions in CD4+, CD8+ and CD19+ cells, including both naïve and memory CD4+ and CD8+ subsets, were observed at Day 2 without evidence of immune deficit. No clinically relevant changes in immunoglobulin subsets or treatment-related trends in circulating cytokines were noted. One participant experienced an asymptomatic grade 4 ALT elevation coincident with the onset of a 4-log viral load reduction. Six participants exhibited immune-related AEs of mild-to-moderate

intensity, including two cases of hyperthyroidism consistent with autoimmune thyroiditis. This study establishes proof-of-concept that PD-1 blockade with nivolumab can lead to persistent suppression of HCV replication in some patients with chronic infection. The promising results of this study suggest that further exploration of PD-1 pathway blockade is warranted across other chronic viral diseases, possibly in combination with other immunomodulatory or direct-acting antiviral agents [108].

In a case report of a patient with HIV with metastatic melanoma who received ipilimumab, the only drug-related adverse effect was a self-limited grade 2 rash. He achieved an excellent PR in multiple liver lesions, currently ongoing for more than 8 months after completing treatment. There was no adverse effect on HIV viral load or CD4 cell counts [109].2.3.8Dosing revision for nivolumab (September 2016)

In the original version of this protocol, nivolumab was to be administered at a dose of 3 mg/kg every 2 weeks for up to 92 weeks of treatment on dose levels 1 and 2 (1 mg/kg on dose levels -1 and -2). On September 13, 2016, the U.S. Food and Drug Administration modified the dosage regimen for nivolumab (Opdivo®, Bristol-Myers-Squibb Co.) for the currently approved indications for RCC, metastatic melanoma, and NSCLC. The currently approved recommended dosage regimens were modified to 240 mg intravenously (IV) every two weeks.

However for cHL, the FDA approved dosing regimen has remained 3 mg/kg given every 2 weeks until progression or toxicity. Thus for the expansion cohort of HIV-cHL in this protocol, the dose of 3 mg/kg given every 2 weeks will be maintained for three reasons: 1) no DLT was identified in the trial at dose level 1 in the early aspects of this trial, AMC-095, 2) the plethora of clinical data treating non-HIV-cHL at the 3 mg/kg given every 2 weeks, and 3) the FDA approved dose for this indication.

In addition, the nivolumab dosing regimen in combination with ipilimumab for melanoma will remain the same (nivolumab 1 mg/kg IV, followed by ipilimumab on the same day, every 3 weeks for four doses). However, after completion of ipilimumab, the recommended nivolumab dose will be 240 mg every two weeks until disease progression or intolerable toxicity.

The approval was based on population pharmacokinetics analyses and dose/exposure response analyses demonstrating the comparability of the pharmacokinetics exposure, safety, and efficacy of the proposed new dosing regimen with the previously approved regimen.

Based on simulations by the population pharmacokinetics model, FDA determined that the overall exposure at 240 mg every two weeks flat dose is similar (less than 6% difference) to 3 mg/kg every two weeks. These differences in exposure are not likely to have a clinically meaningful effect on safety and efficacy since dose/exposure response relationships appear to be relatively flat in these three indications.

Given the safety and efficacy profile of nivolumab at 240 mg every 2 weeks and ipilimumab 1 mg/kg every 6 weeks, this schedule will be evaluated in this study,

and has been applied with protocol version 5.0.

As of May 2017, three evaluable participants completed the safety evaluation period for dose level 1 (3 mg/kg q 2 weeks, per protocol versions 1-3) on Stratum 1 without DLT. Two participants on this cohort discontinued the study for reasons other than toxicity prior to completing the safety evaluation period and were inevaluable for DLT. Following approval of version 5.0 of the protocol at each center, enrollment on dose level 1 for Stratum 1 began in up to three additional participants at the original dose of 3 mg/kg q 2 weeks with the combination dose level 2 to treat at new dose level 240 mg q 2 weeks. Based on safety of this dose observed in the initial dose level among participants with CD4 cell count above 200, the current experience with single agent nivolumab, and the inclusion of participants in this trial who have exhausted standard chemotherapeutic regimens, an expansion cohort of up to 24 participants with a CD4 count > 200/mm^3 will be initiated with protocol version 7.0 to obtain additional data on the safety and feasibility of this regimen in HIV positive participants with solid tumors.

2.3.9 Dosing revision for nivolumab single agent and combination therapy (April 2019)

In the previous version of this protocol the combination therapy dose was nivolumab 240 mg flat dose q 2 week (IV) with ipilimumab 1 mg/kg q 6 week (IV) for the duration of the study. This dose was found to be tolerable in Stratum 1 Dose Level 2 for participants with a CD4 count above 200 cells/mL. Between May 2017 and February 2018, the FDA updated the label for OPDIVO® to indicate a dosing schedule with only four doses of ipilimumab followed by single agent therapy for advanced RCC and colorectal cancer amd single agent therapy has been updated for many indications to allow 240 mg q 2 week or 480 mg q 4 week, as equal efficacy has been shown for both doses.

This change will be applied to the combination therapy expansion cohort for which tolerability of the original combination therapy has already been established. The new combination therapy will remain the same for four cycles of combination therapy and will then change to single agent therapy with a flat dose of 480 mg q 4 week. This change will bring treatment in line with suggested dosing as well as reducing the burden on study participant by reducing number of visits required.

For single agent therapy in participants with classical Hodgkin's Lymphoma the dose was 3 mg/kg q 2 week. The updated label has been changed for this indication, to a flat dose of nivolumab of either 240 mg q 2 week or 480 mg q 2 week. The protocol dose for the cHL expansion cohort will be updated to 240 mg q 2 week to reflect this change.

Both of these changes will be implemented with version 10.0 of the protocol.

2.4 Correlative Studies (Exploratory)

Unless otherwise noted, specimens will be collected and stored for analysis until funding becomes available.

2.4.1 PD-L1 expression on T cells as a biomarker for response

PD-L1 and PD-L2 expressed on APCs have been shown to induce T cell anergy or apoptosis via PD-1 on T cells, whereas PD-L1 expressed on peripheral tissues directly suppress self-reactive lymphocytes [68, 69]. PD-Ls expressed on tumors regulate the generation of adaptive Tregs, resulting in tumor-induced immune suppression. PD-1 blockade has been shown to enhance the expansion and functional capacity of tumor antigen-specific cytotoxic T cells [72]. Additionally, given the targets differ between PD-1 and ipilimumab, the combination of an anti-PD-1 with ipilimumab might also be the preferred clinical strategy, depending on the tumor expression profile. There are available antibodies to identify the receptor overexpression profile on tumors. Tumor biopsies will be collected by the clinical site for submission of tissue to BMS, depending on the availability of material and funding for analysis. Samples will be tested using the Dako PD-L1 IHC 28-8 pharmDx for Autostainer Link 48 assay.

2.4.2 Role of regulatory T Cells and PD-1 blockage in HIV infection

The effect of blocking of PD-1/PD-L1 on Treg suppressive function has not been clearly defined. In HCV infection, PD-1 expression has been shown to negatively regulate CD4+CD25+FOXP3+ Tregs. [84, 97] Therefore, blocking of PD-1/PD-L1 may potentially increase Treg suppression function thereby further inhibiting HIV-1-specific responses. In contrast, PD-1 has been shown to be important in the peripheral conversion of conventional CD4+ T cells to induced Tregs (iTregs).(96) As such, blocking PD-1 may be beneficial in that it will decrease peripheral Treg frequency leading to a decrease in T cell suppression.

We therefore plan to evaluate changes in the Treg frequency after administration of nivolumab and ipilimumab. We will also assess PD-1 expression in different Treg subsets: CD25+FOXP3+ Tregs, Type 1 regulatory cells (Tr1), as well CD39 expressing Tregs.

We will phenotype T cells with antibodies to CD25 and FoxP3 (Tregs), HLA-DR, CD38 and CCR5 (T cell activation) and CCR7, CD45RA and CD28 (T cell differentiation). Finally, we will use gag peptides and intracellular cytokine staining (ICS) to determine if there are any changes in the frequency of HIV specific CD4+ and CD8+ T cells following administration of anti-CTLA-4 or anti-PD-1.

2.4.3 Herpesvirus load

Approximately 10% of all human CD8 T cells are directed against human cytomegalovirus. Standard plasma and PBMC (latent) viral load will be determined by real-time qPCR for human cytomegalovirus (HCMV), EBV, and Kaposi sarcoma-associated herpesvirus KSHV at the AMC core laboratories as per AMC Standard Operating Procedure (SOP) and expanded to estimate the number of latently infected CD19+ B cells for EBV and KSHV. Samples will be stored for future whole vironome profiling by NextGen sequencing.

2.4.4 Role of regulatory T Cells and PD-1 blockage in herpesvirus infections

As HIV positive participants, and particularly participants with Kaposi sarcoma, typically carry latent herpesviruses, including KSHV, we will collect samples in by methods to support detailed assessments of herpesvirus-directed T cell responses, such as anti-HCMV and anti-KSHV responses as measure by peptide stimulation and ICS or ELISPOT.

2.4.5 HIV viral load and CD4/CD8 T cells

HIV viral load in plasma will be monitored by FDA approved tests with sensitivity to 75 copies/ml or less. CD4/CD8 will be evaluated per institutional standards every 12 weeks during treatment. Tests for the number of integrated proviruses will be conducted by the AMC Core laboratory as per AMC SOP. As HIV viral load measurements are still under development, we will preserve additional material for future assays in specialized labs.

2.4.6 Viral transcription in KS tumor biopsies

Viral transcription in KS tumor samples has been shown to be a sensitive measure of the status of the virus and its response to the cellular microenvironment. It also serves as surrogate marker for the number of tumor cells. This assay will be conducted at the AMC Genomics Core Laboratory per AMC SOP.

2.4.7 HPV genotyping

Anal swabs will be used, when feasible, for polymerase chain reaction (PCR) and reverse line blot analysis to determine the HPV genotype at the AMC Virology Core Laboratory per AMC SOP.

2.4.8 Characterization of activated T cell responses

T cell activation status. For example, leukocytes may then be labeled with CD3/CD8, ICOS1, CDw137, and E6, E7, HCMV, or KSHV MHC tetramers, for analysis by flow cytometry to quantitate and phenotype the percentage of CD8-tetramer double-positive cells. Alternate immune activation markers may be included.

2.4.9 Latent HIV viral load

The latent HIV reservoir will be quantified by resting CD4 T cell outgrowth assays in participants with hematocrit ≥25 and where enough blood (100 ml) is collected, with participant consent. This assay, albeit cumbersome, represents the gold standard in the field. This testing will be collected for all participants meeting the above criteria. The assay will be conducted at the AMC core laboratories per AMC SOP.

Latent HIV reactivation. Changes in HIV plasma RNA by a highly sensitive HIV single copy assay and changes in cell associated HIV unspliced RNA will be measured to see if this treatment leads to HIV reactivation.

Changes in T cell phenotype. Changes in numbers of CD4 and CD8 T cells phenotype will be measured as well as changes in at cell surface markers of activation, immune exhaustion, and functional response to HIV peptides.

2.4.10 Characterization of soluble markers for activated T cell responses

T cell activation status. For example, serum will be analyzed for IL-2, IL-4, IFN-γ, IL-6, TNF-α, CXCL13, IL-10) and immune activation biomarkers (sIL2R-alpha, sCD27, sCD30, sTNFR1, sTNFR2) using luminex beads at the AMC core laboratories as per AMC SOP. Alternate immune activation markers may be included.

3.0 PARTICIPANT SELECTION

A rostered AMC investigator must document that each protocol participant meets all stated eligibility criteria. Participating sites must have documentation that each eligibility requirement is satisfied prior to participant enrollment. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted under any circumstance.

NOTE: Institutions may use this section of the protocol as an eligibility checklist for source documentation if it has been reviewed, signed, and dated before registration/randomization by the study investigator. If used as source documentation, this checklist must be printed, the investigator must check each item to document their assessment that the participant meets each eligibility criterion, and the completed checklist must be maintained in the participant's chart. All questions regarding eligibility should be directed to the study chair.

Participant ID Number: 095 - ___ ___ ___ - ___ ___ ___

Patient's Initials (L, F, M): ___ ___ ___

3.1 Eligibility Criteria

___ 3.1.1 Participants must have histologically confirmed solid tumor malignancy that is metastatic or unresectable and for which standard curative or palliative measures do not exist or are no longer effective. Participants with uncontrolled Kaposi sarcoma are permitted (KS must be increasing despite HAART and HIV suppression for greater than or equal to 2 months, or stable KS despite HAART for greater than or equal to 3 months).

For participants in the 24-participant solid tumor cohort, only those histologies not known to respond to single agent nivolumab (such as pancreas, prostate, and MSS colorectal cancer) will be excluded.

For participants in the relapsed refractory HIV-cHL expansion cohort, participants must have histologically confirmed, relapsed/refractory (defined as relapsed/refractory to one or greater lines of therapy) HIV-associated classical HL.

___ 3.1.2 HIV-1 infection, as documented by any federally approved, licensed HIV rapid test performed in conjunction with screening (or ELISA, test kit, and confirmed by Western blot or other approved test). Alternatively, this documentation may include a record demonstrating that another physician has documented the participant's HIV status based on either: 1) approved diagnostic tests, or 2) the referring physician's written record that HIV infection was documented, with supporting information on the participant's relevant medical history and/or current management of HIV infection.

___ 3.1.3 Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. Scans must have been performed within 4 weeks prior to registration. See Section 9.1.3 or Section 9.3 for HL for the evaluation of measurable disease. Note: For participants with Kaposi sarcoma, the following apply: At least five measurable cutaneous KS lesions or any number of lesions with systemic unresectable disease

with no previous local radiation, surgical, or intralesional cytotoxic therapy that would prevent response assessment.

___ 3.1.4 Prior therapy for metastatic disease permitted. At least 4 weeks must have elapsed since prior chemotherapy or biological therapy, 6 weeks if the regimen included carmustine (BCNU) or mitomycin C; radiotherapy must be completed at least 4 weeks prior to registration.

___ 3.1.5 Age > 18 years, because no dosing or AE data are currently available on the use of ipilimumab in combination with nivolumab in participants <18 years of age, children are excluded from this study.

___ 3.1.6 ECOG performance status ≤ 1 (Karnofsky ≥ 70%, see Appendix III).

___ 3.1.7 Participants not on the HL expansion cohort must have organ and marrow function within the following parameters within 2 weeks prior to enrollment:

Leukocytes: ≥ 2,000/mm^3

Absolute neutrophil count (ANC): ≥ 1,000/mm^3

Platelets: ≥ 75,000/mm^3

Total bilirubin: ≤ 1.5× institutional upper limit of normal (ULN) ≤ 3 x ULN for participants with Gilbert's disease or with atazanavir- or indinavir-induced unconjugated hyperbilirubinemia without AST or ALT elevation and must have a total bilirubin less than 3.0 mg/dL).

Serum lipase and amylase < 1.5 x ULN

AST (SGOT) / ALT (SGPT): ≤ 3 x ULN Creatinine: < 1.5 ULN or Cr Cl >50ml/min

Hemoglobin: ≥ 9g/dL

Serum Albumin ≥2.8g/dL

___ 3.1.8 Participants on the HL expansion cohort must have organ and marrow function within the following parameters within 2 weeks prior to enrollment. Participants may receive GCSF and transfusions to meet these parameters:

- Leukocyte count: no lower limit
- Absolute neutrophil count: ≥ 1,000/mm^3, unless decreased due to bone marrow involvement with lymphoma.
- Platelets: ≥ 75,000/mm^3, unless decreased due to bone marrow involvement with lymphoma.
- Hemoglobin: ≥ 9g/dL unless bone marrow involvement secondary to HL is present
- Total bilirubin: ≤ 1.5× institutional ULN, or ≤ 3 x ULN for participants with Gilbert's disease or with atazanavir- or indinavir-induced unconjugated hyperbilirubinemia without AST or ALT elevation, and must have a total bilirubin less than 3.0mg/dL)

- Serum lipase and amylase < 1.5 x ULN
- AST (SGOT) / ALT (SGPT): ≤ 3 x ULN
- Creatinine: <1.5 x UNL or CrCl > 50ml/min

___ 3.1.9 HIV Viral load should be well suppressed, defined as below the limit of detection of the local assay or below 75 copies/mL) by FDA-approved assays, within 4 weeks prior to registration.

___ 3.1.10 CD4 Counts: For Stratum 1: CD4+ cell count greater than 200 cells/mm^3 obtained within 2 weeks prior to enrollment at any U.S. laboratory that has a clinical laboratory improvement amendments (CLIA) certification or its equivalent.

For Stratum 2: CD4 cell count between 100-200 cells/mm^3 obtained within 2 weeks prior to enrollment at any U.S. laboratory that has a CLIA certification or its equivalent.

Expansion Cohort: CD4 cell count for this cohort will be specified once Stratum 1 and Stratum 2 have completed enrollment (Section 3.3).

Solid Tumor Expansion Cohort: CD4+ cell count greater than 200 cells/mm^3 obtained within 2 weeks prior to enrollment at any U.S. laboratory that has a CLIA certification or its equivalent.

cHL Cohort: CD4 cell count of at least 100 cells/mm^3.

___ 3.1.11 Participants must be PPD negative. Alternatively, the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay (Cellestis Limited, Carnegie, Australia) can be used. An individual is considered positive for *M. tuberculosis* infection if the IFN-γ response to TB antigens is above the test cut-off (after subtracting the background IFN-γ response in the negative control). The result must be obtained within 12 weeks prior to enrollment. PPD positive (or Quantiferon assay positive) participants are permitted if prophylaxis has been completed prior to enrollment.

___ 3.1.12 The effects of nivolumab and ipilimumab on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial are known to be teratogenic, women of childbearing potential (WOCBP)[1] and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. WOCBP should use an adequate method to avoid pregnancy for 6 months) after the last dose of investigational drug. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to enrollment and the start of nivolumab. Women must not be breastfeeding. Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving

[1] Note: Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL.

nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product. Women who are not of childbearing potential (*i.e.*, who are postmenopausal or surgically sterile as well as azoospermic men) do not require contraception.

WOCBP receiving nivolumab will be instructed to adhere to contraception for a period of 6 months after the last dose of investigational product. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product.

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

___ 3.1.13 Participants MUST receive appropriate care and treatment for HIV infection, including antiretroviral medications when clinically indicated, and should be under the care of a physician experienced in HIV management. Participants will be eligible regardless of antiretroviral medication (including no antiretroviral medication) provided there is no intention to initiate therapy, or the regimen has been stable for at least 4 weeks with no intention to change the regimen within 12 weeks following enrollment.

___ 3.1.14 Participants 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 total bilirubin is ≤1.5× institutional ULN, AST (SGOT) and ALT (SGPT) must be ≤3 X institutional upper limit of normal, and HBV DNA < 100 IU/mL (if Hepatitis B positive) within 2 weeks prior to enrollment.

___ 3.1.14 Ability to understand and to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who do not fulfill the criteria as listed in Section 3.1 above, are ineligible. Additionally, the presence of any of the following conditions will exclude a participant from study enrollment:

___ 3.2.1 Participants who have received any other investigational agents within the 4 weeks prior to enrollment. Concurrent radiation therapy is not permitted, except palliative (limited-field) radiation therapy, if all of the following criteria are met:

1) Repeat imaging demonstrates no new sites of bone metastases.

2) The lesion being considered for palliative radiation is not a target lesion.

___ 3.2.2 Participants with known brain metastases or leptomeningeal metastases must be excluded unless they qualify for enrollment as described below because of poor prognosis and concerns regarding progressive neurologic dysfunction that would confound the evaluation of neurologic and other AEs. Participants with brain metastases are permitted if metastases have been treated and there is no magnetic resonance imaging (MRI) evidence of progression for at least 4 weeks or more after treatment is complete and within 4 weeks prior to the first dose of nivolumab

administration.

___ 3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ipilimumab, nivolumab, or other agents used in study, or history of severe hypersensitivity reaction to any monoclonal antibody.

___ 3.2.4 Participants should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 2 weeks of study drug administration. These drugs may interfere with the activity of ipilimumab and nivolumab if administered at the time of the first ipilimumab dose. Inhaled or topical steroids and adrenal replacement doses ≤10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Participants are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if ≥10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (*e.g.*, contrast dye allergy) or for treatment of non-autoimmune conditions (*e.g.*, delayed-type hypersensitivity reaction caused by contact allergen) is permitted. Use of anabolic steroids is permitted.

___ 3.2.5 Participants with clinical or radiographic evidence of pancreatitis are excluded.

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

___ 3.2.7 Participants should be excluded if they have had prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co-stimulation or immune checkpoint pathways. Prior immune modulating therapy including vaccines may be eligible. Prior immune events must be evaluated and the risk for new events, which may represent continued sub clinical disease or a new process at previously damaged site or immune potentiation (*e.g.*, ipilimumab followed by IL2 causing bowel perforation, ipilimumab followed by IDO inhibitor resulting in clinical hypophysitis). *Please keep in mind that inflammatory events may occur weeks to months following the last dose of ipilimumab and possibly nivolumab.*

Assessment of potential effects of prior therapy should include:

Immune status

Organ damage

Risk of autoimmunity

Immunopotentiation

___ 3.2.8 The participant has not recovered to baseline or CTCAE ≤ Grade 1 from toxicity due to all prior therapies except ≤ Grade 2 alopecia, neuropathy, and other non-clinically significant AEs.

___ 3.2.9 The participant has a primary brain tumor.

___ 3.2.10 Participant has ≥ grade 2 diarrhea (participants with grade 1 diarrhea are eligible provided stool for ova/parasites and stool cryptosporidium studies are negative).

___ 3.2.11 Opportunistic infection within the last 3 months.

___ 3.2.12 Autoimmune disease: Participants 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 participants 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 participants 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. Participants with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Participants with rheumatoid arthritis and other arthropathies, Sjögren's syndrome and psoriasis controlled with topical medication and participants with positive serology, such as 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.

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

___ 3.2.13 Participants who have had evidence of *C. difficile* infection, 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.

___ 3.2.14 cHL cohort only: history of allogeneic transplant.

Physician Signature: _____ Date: _____

(Optional unless this section is used as an eligibility checklist)

3.3 Criteria for Solid Tumor Expansion and Lymphoma Cohorts

Inclusion and exclusion criteria for the solid tumor cohorts are the same as above, with the following rule for CD4 count based on tolerability in Phase I. If, participants with lymphocyte T CD4 count between 100-200/mm^3 (Stratum 2) are shown to tolerate treatment in the Phase I dose de-escalation portion at the same dose level as those with CD4 counts >200/mm^3 (Stratum 1), participants in the expansion cohort with CD4 counts ≥100/mm^3 are permitted. Otherwise, the expansion is open to all solid tumor patients except those whose tumors are known not to respond to nivolumab (pancreas, prostate and MSS colon cancer). For the relapsed refractory HIV-cHL cohort, participants with CD4

count ≥ 100/mm^3 are permitted.

3.4 Number of Participants to be Enrolled

3.4.1 Proposed sample size

This study will enroll a minimum of 4 participants and a maximum of 96 participants (see Section 10.2).

3.4.2 Accrual rate

Approximately two participants per month for Phase I, Stratum 1; approximately one participant per month for Phase I, Stratum 2. Approximately 2-3 participants per month for the combination therapy solid tumor expansion cohort and approximately 2-3 participants per month for the single agent solid tumor cohort (excluding those histologies not known to respond to single agent nivolumab, i.e., pancreas, prostate, MSS colorectal cancer). The cHL cohort will enroll approximately 3-4 patients per month.

3.5 Participant Enrollment Procedures

Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered for study participation with the AMC Operations and Data Management Center (ODMC) before they may enroll participants.

After an informed consent form has been signed by the participant and it has been determined that the participant is eligible, the participant must be registered on-line via the AMC AdvantageEDC^{SM} Internet Data Entry System (AdvantageEDC). Enrollment and data collection will occur via the AMC Internet Data Entry System.

The participating site will ensure a participant meets all eligibility criteria prior to completing the protocol-specific eligibility checklist in AdvantageEDC for enrollment. Participants will be enrolled on-line via AdvantageEDC no more than 1 week prior to the initiation of treatment (enrollment 1 day prior to or on the day of treatment is strongly encouraged). It may take up to three business days for sites to receive nivolumab. Once the eligibility checklist is submitted, a system-generated confirmation email will be sent to the enroller upon successful completion of the participant enrollment. If the on-line system is inaccessible, the site should notify the AMC ODMC (via email at amcpm@emmes.com or via phone at 301-251-1161) for further instructions.

4.0 TREATMENT PLAN

4.1 Treatment Design

Protocol agents will be administered on an outpatient basis. Reported AEs and potential risks for ipilimumab and nivolumab are described in Section 6.0. Appropriate dose modifications for ipilimumab and nivolumab are described in Section 5.0, Appendix X. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Participants will be studied in separate strata. Stratum 1 will enroll participants with lymphocyte T CD4+ count above 200/mm^3. Stratum 2 will enroll participants with lymphocyte T CD4+ count between 100-200/mm^3. Enrollment on Stratum 2 will occur sequentially, initially in Stratum 1 at dose level 1, and then in Stratum 2 after Stratum 1 has completed and an MTD has been determined. Once the MTD is determined in each stratum, the MTD will then be studied in a dose expansion cohort of up to 12 participants. If the MTDs differ between the two strata, expansion cohorts of six participants each will be enrolled. An additional cohort of 12 participants with cHL and lymphocyte T CD4+ count $\geq$ 100/mm^3 will be treated with single agent nivolumab. Lastly, an additional 24 participants with solid tumors will be treated with single agent nivolumab.

4.2 Dose De-Escalation Cohorts: Stratum 1 and Stratum 2

For Stratum 1, the study is a phase I dose de-escalation trial with a rolling six design to establish the MTD for single agent nivolumab and then the MTD for combination of ipilimumab and nivolumab. Since there are only two dose levels within each single and combination therapy, the design requires the full cohort of six participants to be enrolled unless two of the first three participants in that cohort experience a DLT, or if two or more participants in that cohort experience a DLT. In Stratum 1, two agents will be studied, initially nivolumab as a single agent (starting at Dose Level 1) and then the combination of ipilimumab and nivolumab (starting at Dose Level 2 or -2). After evaluating single agent nivolumab dosing, the next dose level will be 2 if dose level 1 is the MTD for single agent nivolumab or dose level -2 if dose level -1 is the single agent MTD. If 2 DLTs are experienced at Dose Levels 1 or 2, then one dose de-escalation (Dose Levels of -1 or -2, respectively) will be permitted according to the dose de-escalation schedule. Fixed doses will be administered according to the dosing schema in the treatment plan. The Phase I MTD for single agent or combination therapy is the dose level at which $\leq$1/6 participants experience DLT, with the next higher dose having $\geq$2 participants encountering DLT (unless the MTD is the highest pre-specified dose level). The safety evaluation period is 6 weeks at a given dose level. No dose escalation will be allowed in an individual participant, and participants who have dose limiting AEs or disease progression will discontinue therapy. During the period for evaluating DLT, participants who withdraw from the study or do not complete the required cycles of treatment owing to reasons other than drug-related AEs can be replaced. Participants will have blood drawn before each cycle for routine safety laboratory monitoring. All participants will be followed every 2 weeks for treatment administration, exam, toxicity, response, and compliance assessment for a minimum of 8 weeks and up to 92 weeks of treatment.

Table 1: Dose De-Escalation Schedule Stratum 1 and 2

Dose Level	Nivolumab* q2 wk x 46 doses	Ipilimumab**
Level -1	1 mg/kg	-
Level 1	3 mg/kg	-
Level 2	240 mg	1 mg/kg q 6 wk x 16 doses (day 1 of nivolumab cycles 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46)
Level -2	1 mg/kg	1 mg/kg q 12 wk x 8 doses (day 1 of nivolumab cycles 1, 7, 13, 19, 25, 31, 37, and 43)

*Doses are stated as exact dose in units (e.g., mg/m^2, mcg/kg, etc.) rather than as a percentage.

**When the two drugs are administered, nivolumab will be administered first. Doses of intravenous nivolumab will be administered every 2 weeks up to 46 doses. Ipilimumab will be administered every 6 weeks on dose level 2 or every 12 weeks on dose level -2.

***Treatment will continue until documented disease progression, discontinuation due to toxicity, withdrawal of consent, or the study ends, up to 46 doses of nivolumab (with ipilimumab, if receiving).

NOTE: After evaluating single agent nivolumab dosing, the next dose level will be 2 if dose level 1 is the MTD for single agent nivolumab and dose level -2 if dose level -1 is the single agent MTD. It is also possible to de-escalate from dose level 2 to -2. Stratum 2 participants will not be allowed to escalate beyond the MTD for Stratum 1; one dose level de-escalation of the nivolumab and ipilimumab combination or to single agent nivolumab will be allowed based on the doses in the schema in the treatment plan.

NOTE: Participants treated on the cHL cohort and participants on the solid tumor expansion cohort of 24 participants will be treated at 240 mg.

These rules are based on concurrently enrolling up to six participants on the initial dose level (Dose Level 1). However, accrual will be suspended before enrolling the 4th–6th participants on a cohort to until safety has been evaluated in the first three participants, unless at least two participants on that cohort have completed the safety evaluation period without DLT.

1. If, after completing the first six weeks for single agent nivolumab (Dose Level 1), none or one participant out of six exhibits a POSSIBLY, PROBABLY or DEFINTELY, study treatment-related DLT (i.e., ≤1/6 participants with DLT at that Dose Level 1; see Section 4.4), then the next cohort of participants will be treated at Dose Level 2.

2. However, if a second participant develops a study treatment POSSIBLY, PROBABLY or DEFINTELY related DLT, even if it is before there are six total participants on that level, then the single agent MTD has been exceeded and no additional participants should be added to this dose. A total of six participants should then be treated on the next lowest dose level (Dose Level -1) to ensure its tolerability and to better define the MTD. Thus, the MTD is defined as the highest assigned dose level at which ≤1/6 participants have a DLT. If ≥2 participants on level -1 experience a POSSIBLY, PROBABLY or DEFINTELY related DLT, the MTD will have been exceeded and no additional participants will be added at level -1 or receive combination therapy levels 2 or -2. If Dose Level -1 is the single agent MTD, then the next cohort of participants will be treated at Dose Level -2.

3. If, after completing first 6 weeks for combination of ipilimumab and nivolumab (Dose Level 2 or -2), 0/6 or ≤1/6 exhibits a POSSIBLY, PROBABLY or DEFINTELY, study treatment-related DLT then that dose level is the combination-agent MTD. If a second participant develops a study treatment POSSIBLY, PROBABLY or DEFINTELY related DLT, even if it is before there are six total participants on that level, then the combination-agent MTD has been exceeded and no additional participants should be added to this dose. A total of six participants should then be treated on the next lowest dose level (Dose Level -2 if not already at the lowest dose) to ensure its tolerability and to better define the MTD. If ≥2 participants on level -2 experience a POSSIBLY, PROBABLY or DEFINTELY related DLT, the MTD will have been exceeded and no additional participants will be added at level -2.

4.2.1 Dosing for Stratum 1

The study treatment consists of up to 46 intravenous (IV) infusions of nivolumab on day 1 of each 2-week cycle for the dose level -1 and 1 cohorts. For dose level 2, IV infusions of nivolumab are given on day 1 of each 2-week cycle and ipilimumab on day 1 every 6 weeks, i.e., every third cycle of nivolumab (cycles 1, 4, 7, etc.). For dose level -2, IV infusions of nivolumab are given on day 1 of each 2-week cycle, and ipilimumab is given on day 1 every 12 weeks, i.e., every sixth cycle of nivolumab (cycles 1, 7, 13, etc.).

Participants with an overall response of SD, PR, CR, or unconfirmed PD (refer to Section 9.1 for Response Evaluation Criteria in Solid Tumors (RECIST) Criteria at the scheduled response visits at week 8 and all subsequent visits day 1 of cycles 6 and 9 will be eligible to continue to receive study drug treatments provided that they also have an Eastern Cooperative Oncology Group (ECOG) performance status score of ≤ 2, and did not have a DLT or other safety risk requiring discontinuation of the study drugs. Before dosing can continue after the day 1 of cycles 5 and each subsequent visit at which response is assessed (every 12 weeks thereafter), the results of the scheduled response assessments must be reviewed to determine, and document continued eligibility prior to administration of the study drugs.

Participants with an overall response of SD, PR, CR, or unconfirmed PD at the end of the first four cycles will continue treatment for up to a total of 46 cycles (refer to Section 9.1 for RECIST Criteria). Participants with confirmed PD after the first four cycles of therapy will discontinue the study drugs. Participants without evidence of confirmed progression after the first four cycles, with either a poor performance status (ECOG > 2) or toxicity requiring discontinuation of the study drugs will continue treatment until confirmed PD, requiring initiation of a new treatment.

4.2.2 Dosing Stratum 2

After review of AEs in Stratum 1, this will be followed by evaluation of nivolumab and ipilimumab in Stratum 2. Stratum 2 single agent dosing will begin after the MTD for Stratum 1 single agent therapy has been confirmed (dose level 1 or -1). After the Stratum 1 combination therapy MTD is determined (dose level 2 or -2), Stratum 2 will begin evaluation of combination therapy at that dose level. Up to six participants will be concurrently enrolled at the Stratum 1 MTD, following the dose de-escalation rules and treatment plan outlined above in Section 4.2 and 4.2.1. Stratum 2 participants will not be allowed to escalate beyond the MTD for Stratum 1; one dose level de-escalation of the nivolumab and ipilimumab combination or to single agent nivolumab will be allowed based on the doses in the schema in the treatment plan. The Phase I MTD is the dose level at which ≤1/6 participants experience DLT with the next higher dose having ≥2 participants encountering DLT (unless the MTD is the highest pre-specified dose level). No dose escalation will be allowed in an individual participant, and participants who have dose limiting AEs or disease progression will discontinue therapy. During the period for evaluating DLT, participants who withdraw from the study or do not complete the required cycles of therapy owing to reasons other than drug-related AEs can be replaced. During the first four cycles of treatment, participants will have blood drawn weekly for routine safety laboratory monitoring. Participants will then be followed every 2 weeks for treatment administration, exam, toxicity, response, and compliance assessment for a minimum of 8 weeks, and up to 92 weeks.

4.2.3 Expansion Cohort in Solid Tumors and Lymphoma

An additional 24 participants with incurable solid tumors will be treated at the MTD, according to the treatment schedule defined above for each stratum. If the MTDs differ between the two strata, expansion cohorts of six participants each will be enrolled.

Another 24 participants with incurable solid tumors will be treated at single agent nivolumab 240 mg every 2 weeks. Only those histologies that are not known to respond to single agent nivolumab will be excluded (i.e., pancreas, prostate, MSS colorectal cancer, unless results from another clinical trial showing non-response in another tumor type become available in the future).

Participants with HL will be treated at the FDA approved flat dose of 240mg given every 2 weeks.

Participants with an overall response of SD, PR, CR, or unconfirmed PD at the end of the first four cycles of nivolumab will continue treatment for up to a total of 46 cycles of nivolumab (with ipilimumab if receiving dose level 2 or -2) (refer to Section 9.1 for RECIST Criteria and Section 9.3 for the 2017 response evaluation criteria in lymphoma (RECIL) Criteria for evaluating measurable disease in HIV-cHL).

Participants with a confirmed PD will discontinue the study drugs.

4.3 Treatment Plan

4.3.1 Nivolumab

For Dose Levels 1 and -1 in Stratum 1

1 cycle = 14 days

Treatment: A maximum of 46 cycles of nivolumab will be given every 2 weeks.

Dose Level 1: Nivolumab 3mg/kg q 2 wk x 46 doses

Dose Level -1: Nivolumab 1mg/kg q 2 wk x 46 doses

Nivolumab will be given every two weeks (±2 days) when given as a single agent as per the dose de-escalation table in Section 4.2 and will be modified in cohorts of up to six participants (as outlined in Section 4.2) based upon the number of participants who have a DLT event at each dose level (as defined in Section 4.2) during the first cycle of therapy. Participants may be dosed no less than 12 days from the previous dose of drug.

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

Nivolumab is to be administered as a 60-minute IV infusion, using a volumetric pump with a 0.2 to 1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery, but the total drug concentration of the solution cannot be below 0.35 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

For the solid tumor expansion cohort, a dose of 240mg of single agent nivolumab will be administered every 2 weeks for 46 doses as described above, as a 60-minute IV infusion.

For the cohort of HL, a dose of 240mg flat dose of single agent nivolumab will be administered every 2 weeks for 46 doses as described above, as a 60-minute IV infusion.

For nivolumab and ipilimumab combination studies see Section 4.3.2.

4.3.2 Nivolumab and ipilimumab

For Dose Levels 2 and -2 in Stratum 1

1 cycle = 14 days

Nivolumab treatment: A maximum of 46 cycles of nivolumab will be given every 2 weeks

Ipilimumab treatment: A maximum of 16 cycles of ipilimumab given every 6 weeks (on day 1 of every third nivolumab cycle (cycles 1, 4, 7, etc.)) for Dose Level 2, and a maximum of eight cycles of ipilimumab given every 12 weeks, (on day 1 of every sixth nivolumab cycle (cycles 1, 7, 13, etc.)) for Dose Level -2.

Dose Level 2:

- Nivolumab 240 mg q 2 wk x 46 doses
- Ipilimumab 1mg/kg q 6 wk x 16 doses (day 1 of nivolumab cycles 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46)

Dose Level -2:

- Nivolumab 1 mg/kg q 2 wk x 46 doses
- Ipilimumab 1mg/kg q 12 wk x 8 doses (day 1 of nivolumab cycles 1, 7, 13, 19, 25, 31, 37, and 43)

Toxicity management for the combined agents follows the same template guidelines and algorithms that are provided in Appendix X for single agent nivolumab.

Follow the same infusion timing guidelines: nivolumab over 60 minutes and ipilimumab over 90 minutes.

When both nivolumab and ipilimumab are to be administered on the same day, separate infusion bags and filters must be used for each infusion. Nivolumab is to be administered first. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion.

Nivolumab cycles may be repeated every 14 days (± 2 days). Cycles in which nivolumab and ipilimumab are administered, per the dosing schedule for the assigned dose level 2 or -2, no earlier than 12 days after the last cycle of nivolumab.

4.3.3 Stratum 2 treatment

Stratum 2 dosing will begin based on the single agent therapy MTD determined in Stratum 1. Stratum 2 will not be allowed to escalate beyond the MTD for Stratum 1. An expansion cohort will be treated at the MTD for Stratum 1. If the MTDs differ between the two strata, expansion cohorts of six participants each will be enrolled.

4.3.4 Expansion cohorts

For the solid tumor expansion cohort, a dose of 240 mg of single agent nivolumab will be administered every 2 weeks for 46 doses as described above, as a 60-minute IV infusion. Inclusion criteria for CD4 count will be managed as noted in Section 4.1.

1 nivolumab cycle = 14 days

A maximum of 46 cycles of nivolumab will be given every 2 weeks. If the combination is tolerated, ipilimumab will be given according to the treatment instructions for dose level 2 or -2 as above, depending on the MTD for that stratum, in a separate solid tumor expansion cohort.

For the combination solid tumor expansion cohort, the dosing will be 12 intravenous (IV) infusions of nivolumab, 240 mg, on day 1 of each 2-week cycle and ipilimumab on day 1 every 6 weeks with four total ipilumimab cycles, i.e., every third cycle (cycles 1, 4, 7, 10). After completion of the 12th cycle of nivolumab, the nivolumab dose will change to a flat dose of 480 mg, on day 1 of

each 4-week cycle without ipilimumab. Participants with an overall response of SD, PR, CR, or unconfirmed PD at week 8 will continue treatment (Refer to Section 9.1 for RECIST Criteria) for up to 84 weeks. For participants with HIV-cHL, single agent nivolumab will be given as a flat dose of 240 mg every 2 weeks as described in Section 4.3.1.

4.3.5 Study procedures by visit and treatment cycle

Note that results of all safety laboratory test (that is, all chemistry and all hematology results) must be obtained and reviewed before ipilimumab administration, as applicable. All laboratory results must be within the established range before ipilimumab is administered. All laboratory samples for the first four cycles of nivolumab must be collected within a window of up to 3 days before administration of nivolumab and/or ipilimumab. Laboratory evaluations using a local laboratory must be performed and the result examined by the investigator before administration of each dose of nivolumab and/or ipilimumab.

4.4 Definition of DLT

Nivolumab has the potential to be synergistic with ipilimumab and augment the rate and duration of clinical responses. It also, however, has the potential to augment the frequency and severity of previously characterized ipilimumab-related immune-related adverse events (irAE), as well as to generate new study drug-related irAEs or toxicities. It will be important to differentiate between irAEs expected from ipilimumab toxicity as opposed to an increase in frequency or a worsening of severity of irAEs as an indicator of unacceptable toxicity related to the combination.

The MTD to define the optimal dose. The MTD itself is determined by DLT, i.e., toxicity/severity that limits the possibility to treat a participant at the planned dose. DLT is traditionally defined as any grade 3-4 non-hematological or grade 4 hematological toxicity at least possibly related to the treatment, occurring during the first cycle of treatment. DLTs will be defined by drug-related AEs that meet the criteria below as evaluated by NCI Common Terminology Criteria for AEs version 5 (CTCAE v5.0), unless clearly unrelated to study drugs (e.g., disease progression) defined based on DLTs that occur during the first 6 weeks for single agent nivolumab (Dose Levels 1 and -1) and 6 weeks for the nivolumab and ipilimumab combination (Dose Levels 2 and -2).

Note: DLT and other toxicities are discussed among the Study Chair, Co-Chairs, the Phase I Committee Chair, the AMC Medical Monitor, and the AMC Biostatistician. The discussions are the basis for the decisions regarding dose de-escalation according to the rules of the protocol and opening of Stratum 2 to enrollment.

- Permanently stopping protocol treatment during the DLT period (defined as 6 weeks from the first dose) for either arm is considered a DLT.
- Any drug-related death is a DLT.
- Grade 3 or 4 events requiring permanent discontinuation of nivolumab or ipilimumab, including those that will require long term, high dose corticosteroids.

Management and dose modifications associated with the above AEs are outlined in Section 5.0 and Appendix X.

4.5 Dose Adjustments, Dose Delays, and Missed Doses

There will be no dose adjustments allowed except for weight changes (± 10% from the baseline weight measurement) at visits that require weight measurements. If it is determined that a dose level combination exceeds the MTD, participants tolerating that dose level combination will receive subsequent doses at the next lower dose level combination.

During treatment for all dose levels, if there is a dose delay between 1 and 5 days, the procedures at the original scheduled visit should be performed as soon as possible. If the delay is more than 5 days, the visit and dose will be considered missed; the procedures at the next scheduled visit should be performed, and subsequent visits will follow every 2 weeks.

If treatment is delayed for > 35 days for participants on dose levels 1 or -1, or > 42 days for participants on dose levels 2 or -2 (> 56 days for participants on a steroid taper), the participant must be permanently discontinued from study therapy, except as specified in Section 4.7 (Duration of Therapy).

4.6 General Concomitant Medication and Supportive Care Guidelines

4.6.1 Supportive care

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

See Appendix X for supportive care considerations for nivolumab and nivolumab and ipilimumab combination administration.

If the confirmed viral load of a participant is > 1,000, the treating physician is advised to make changes to the ART regimen, preferably after consultation with an HIV specialist. If the participant is not receiving ART at the time of study initiation and his/her viral load becomes detectable during study participation, ART initiation may be recommended, at an HIV specialist's direction for the participant's medical care.

Participants requiring chemotherapy or radiation therapy during the study will be taken off study treatment. Any exceptions must be discussed with the study chair. Palliative (limited-field) radiation therapy is permitted if all of the following criteria are met:

1) Repeat imaging demonstrates no new sites of bone metastases.
2) The lesion being considered for palliative radiation is not a target lesion.

4.6.2 Other medications

Participants MUST receive medically appropriate care and treatment for HIV infection, including antiretroviral medications when clinically indicated. Suspension or modification of an antiretroviral regimen during study participation will be permitted (after 8 weeks from study entry) at the discretion of the physician providing HIV care, provided the change and its rationale are carefully documented.

Recommended prophylaxis for opportunistic infections is listed in Appendix VIII. Participants 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. Only prescription medications will be recorded in the case report forms. Bisphosphonates started prior to screening activities or initiated during the course of the study to control bone pain may be used with caution.

As the study will identify DLTs of this treatment regimen during the initial cycle of therapy, 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.

4.6.3 Prohibited therapies

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

Where possible, routine vaccination for influenza, pneumococcal pneumonia should be given prior to the start of therapy but may be administered during treatment when clinically indicated. Vaccination should be given when there is enough separation to distinguish any vaccine reactions from drug toxicity. There is no experience using live attenuated vaccination during ipilimumab therapy, so that live vaccine should be used cautiously during treatment.

Concomitant systemic or local anti-cancer medications or treatments are prohibited in this study while receiving ipilimumab treatments.

Participants 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
- 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).

4.6.4 Precautions

Combination therapy may result in unexpected toxicity especially in novel combinations with other immune modifying agents. A striking example in macaques is presented in Vaccari*, et al.* 2012.

Please note that while unproven, there is a suggestion that autoimmune events, including hepatitis, may occur more frequently at sites of metastases or prior injury.

Caution is advised when considering treatment with high dose IL-2 in participants who have previously been administered ipilimumab, particularly in participants who experienced ipilimumab-related diarrhea/colitis. Colonoscopy or sigmoidoscopy with biopsy may be advisable prior to IL-2 administration once the participant is no longer receiving ipilimumab.

Participants who have received ipilimumab may potentially develop autoimmune disease with subsequent therapy including the appearance of colitis, hypophysitis, or adrenal insufficiency.

4.7 Duration of Therapy

In the absence of treatment delays due to AE(s), treatment may continue 92 weeks of nivolumab (with ipilimumab, if receiving dose level 2 or -2) until one of the following criteria applies:

- The development of progression (confirmed PD by RECIST Criteria or PD by the KS evaluation criteria in Section 9.2 or per Section 9.3, utilizing the consensus RECIL (2017). Participants will remain on study treatment until progression is confirmed,
- Pregnancy
  - All WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed, or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.
  - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a study participant.
- Intercurrent illness that prevents further administration of treatment.
- Unacceptable AE(s) which include the following (see also Section 6.0 and specific algorithms in Appendix X):
  - Any grade 4 events except as noted below.
  - Grade 3 drug-related autoimmune or inflammatory events including uveitis, pneumonitis, diarrhea, colitis, neurologic AEs, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation except as noted below:
  - Any other grade 3 non-skin, drug-related AE lasting >7 days including fatigue.
  - Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality,

not associated with underlying organ pathology that does not require treatment except for electrolyte replacements **does not** require treatment discontinuation.

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

- Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
  - Participants requiring > two dose delays for the same type of event should go off protocol therapy.
  - Any AE, laboratory abnormality, or intercurrent illness, which, in the judgment of the investigator, presents a substantial clinical risk to the participant with continued study drug dosing.
- Any participants who require additional immune suppressive treatment beyond steroids should go off study treatment.
- In participants with HIV-cHL, any participant who requires consolidative therapy with high dose chemotherapy followed by stem cell transplantation will be permitted to go off study therapy.
- Withdrawal of informed consent (participant's decision to withdraw for any reason).
- General or specific changes in the participant's condition, or any clinical AE, laboratory abnormality or intercurrent illness, which, in the opinion of the investigator, indicate that continued treatment with study therapy is not in the best interest of the participant.
- Termination of the study by sponsor.
- Participants treated with systemic steroids, except for a short course of tapered steroids for grade 3 rash and physiologic replacement doses, should be off study.
  - Participants who have been treated with steroids and who do not have an immunologically based event (e.g., infectious pneumonia rather than lymphocytic pneumonitis) may be restarted on treatment if asymptomatic after 2 weeks off all steroids.
  - Tumor assessments should continue as per protocol even if dosing is interrupted.

4.8 Duration of Follow-Up

Participants will be followed for 16 weeks or 112 days (based on five half-lives) after removal from study treatment, or until death, whichever occurs first. Participants removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE.

4.9 Criteria for Removal from Treatment

Participants will be removed from study when any of the applicable criteria, including PD, AEs, participant withdrawal, or inability to follow study protocol as listed in Section 4.7.

The reason for study removal and the date the participant was removed must be documented in the Case Report Form.

4.10 Criteria to Resume Treatment

Restarting applies only to grade 2 events and some grade 3 events (skin rash and thyroiditis). The protocol chairs emphasize stopping treatment and starting steroids earlier to obtain resolution with the possibility for restarting rather than waiting for higher grade events.

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

- Participants 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 participant should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol the treatment should resume at the earliest convenient point that is within the six-week delay period.

If treatment is delayed for > 35 days for participants on dose levels 1 or -1, or > 42 days for participants on dose levels 2 or -2 (> 56 days for participants on a steroid taper), the participant must be permanently discontinued from study therapy, except as specified in Section 4.7 (Duration of Therapy).

4.11 Treatment of Nivolumab-Related and Ipilimumab Combination Infusion Reactions

Since ipilimumab and nivolumab contain only human immunoglobulin or immunoglobulin protein sequences, these agents are 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 a SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE version 5.0 guidelines.

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

4.11.1 For Grade 1 symptoms

Mild reaction: infusion interruption not indicated; intervention not indicated.

Remain at bedside and monitor participant 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 participant 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.

4.11.2 For Grade 2 symptoms

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

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the participant with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor participant 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 participant 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 participant until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedication's 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.

4.11.3 For Grade 3 or Grade 4 symptoms

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

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

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the participant 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. Participant 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 participant 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).

5.0 DOSING DELAYS/DOSE MODIFICATIONS

5.1 Dose Modifications for Nivolumab and Nivolumab and Ipilimumab Combination

Participants with an overall response of SD, PR, CR, or unconfirmed PD (refer to Section 9.1 for RECIST Criteria, Section 9.2 for KS response evaluation, and Section 9.3 for the 2017 RECIL Criteria for response evaluation) at the scheduled response visits at week 8 and all subsequent visits day 1 of cycles 6 and 9 will be eligible to continue to receive study drug treatments provided that they also have an ECOG performance status score of ≤ 2, and did not have a DLT or other safety risk requiring discontinuation of the study drugs, as defined below.

Please refer to Appendix X to the protocol for toxicity management algorithms, which include specific treatment guidelines. Where discrepancies occur between Section 5.1 and Appendix X, use the instructions in Section 5.1. These algorithms should be followed unless there are specific clinical circumstances for which the treating physician indicates variations or alternative treatment are needed.

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

Table 2: Dose Modification Tables for Nivolumab and Nivolumab and Ipilimumab Combination

All Other Events*	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	No change in dose.
Grade 2	Hold** until ≤ Grade 1 OR baseline. Resume at same dose level.
Grade 3	Hold** until ≤ Grade 1 continue at investigator discretion.
Grade 4	Off protocol therapy.
* Not immunologically mediated (excluding clinically insignificant lab abnormalities, see Section 6.4.1) **Participants being treated on the HL expansion cohort meeting the inclusion criteria will hold therapy only if a worsening in ≥ 1 grade beyond baseline eligibility requirements occurs on the day of therapy. Participants may resume treatment when AEs are resolved or have returned to baseline. Participants requiring more than two dose delays for the same event should go off protocol therapy.	
Recommended management: As clinically indicated.	
All Other Events**	**Management/Next Dose for Nivolumab and Combination**
≤ Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1 OR baseline. When resolved ≤ Grade 1 and off steroids for at least 1 week, resume at same dose level.
Grade 3	Off protocol therapy (exceptions noted in 5.4). May resume nivolumab monotherapy if resolved to Grade 1 or less (off physiologic

All Other Events*	Management/Next Dose for Nivolumab and Combination
	replacement doses of corticosteroids) by end of week 8. If AE occurs with nivolumab monotherapy, then off protocol therapy.
Grade 4	Off protocol therapy.
** Immunologically mediated.	
Recommended management: As clinically indicated.	

Cardiac *	Management/Next Dose for BMS-936558 (Nivolumab) + Ipilimumab Cardiac Toxicities
≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia, or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation
Grade ≥2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac ECHO. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. 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.	

Skin Rash and Oral Lesions	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	No change in dose.* Manage as clinically indicated.
Grade 2	Hold* until 1≤ Grade resolved^. Consider steroid treatment > 7 days. Resume at same dose level.

Skin Rash and Oral Lesions	Management/Next Dose for Nivolumab and Combination
Grade 3	Hold* until ≤ Grade 1. Resume at same level at investigator discretion.
Grade 4	Off protocol therapy.
*Participants 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: AE management guidelines (Appendix X).	

Liver Function (AST, ALT, Bilirubin)	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	Continue protocol therapy.
Grade 2	Hold until UNL or baseline. Resume at same dose level. Consider steroid treatment if > 7 days.
Grade 3	Discontinue protocol therapy if occurring before cycle 5. Initiate IV or PO steroids. May begin nivolumab monotherapy if returns to grade 1 off steroids by week 8 (no earlier than week 6). Hold protocol therapy if occurring after cycle 5. Initiate IV or PO steroids. If not returned to grade 1 off steroids by 6 weeks from missed dose, then off protocol therapy.
Grade 4	Off protocol therapy. Initiate IV steroids.
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.	
Recommended management: see Hepatic AE management algorithm (Appendix X).	

Renal Toxicity	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level. Steroid treatment if > 7 days.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off protocol therapy.
Recommended management: see Renal AE management algorithm (Appendix X).	

Diarrhea/Colitis	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	Hold until Grade 0 (if Grade 1) or baseline^. No change in dose.
Grade 2	Hold until Grade 0 or baseline. Steroid treatment if > 7 days
Grade 3	Off protocol therapy, may begin nivolumab monotherapy if resolved to grade 0 (or baseline) by week 16. If occurs during nivolumab monotherapy, then participant is off protocol therapy.
Grade 4	Off protocol therapy
See GI AE Algorithm for management of symptomatic colitis. Participants with ≤ grade 2 toxicity resolving to baseline within 7 days in the absence of steroids may resume with omitted dose and have treatment schedule postponed 1 week. Participants with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Participants who require steroids should be taken off study treatment if occurring before cycle 5. Please evaluate pituitary function prior to starting steroids, if possible, without compromising acute care. Evaluation for all participants for additional causes includes *C. diff*, acute and self-limited infectious and food borne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: see GI AE management algorithm (Appendix X).	

Pancreatitis Amylase/Lipase	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	Continue protocol therapy if asymptomatic.
Grade 2	Continue protocol therapy if asymptomatic.
Grade 3	Continue protocol therapy if asymptomatic at investigator's discretion.
Grade 4	If asymptomatic, hold therapy until ≤ grade 2, then resume at same dose level
Participants 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 participants who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm (Appendix X).	

Pneumonitis	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	Hold dose pending evaluation and resolution to ≤ grade 0 or baseline including baseline pO_2. Resume no change in dose after pulmonary and/or ID consultation

Pneumonitis	Management/Next Dose for Nivolumab and Combination
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. Otherwise, off protocol therapy
Grade 4	Off protocol therapy
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for participants who do not respond to antibiotics and have no causal organism identified including influenza. Most participants 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. Participants with new lung nodules should be evaluated for sarcoid like granuloma.	
Recommended management: See Pulmonary Adverse Event Management Algorithm (Appendix X).	

Other GI N-V	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	No change in dose.
Grade 2	Hold pending evaluation for gastritis duodenitis and other immune AEs 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 participants should go off protocol therapy if occurring before cycle 5. May resume nivolumab monotherapy if resolved to ≤ grade 1 (off of > replacement steroids for 2 weeks by week 8)
Grade 4	Off protocol therapy.
Participants with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune-related events.	

Fatigue	Management/Next Dose for Nivolumab and Combination
≤ 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 AE 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 and Combination
≤ Grade 1	Hold dose pending evaluation and observation. ^ If symptoms do not progress and processing below excluded may resume with no change in dose.*
Grade 2	Hold dose pending evaluation and observation. ^ Hold until ≤ Grade 1.* Off protocol therapy if treatment with steroids is required. ^ Resume at same dose level for peripheral isolated n. VII (Bell's palsy)^
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
*Participants 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 Neurologic Adverse Event Management Algorithm (Appendix X).	

Endocrine Hypophysitis Adrenal Insufficiency	Management/Next Dose for Nivolumab and Combination
≤ Grade 1	Asymptomatic TSH elevation. Add hormone replacement as clinically indicated. Continue therapy.
Grade 2	Hold until participants are on a stable replacement hormone regimen. Resume at same dose level.
Grade 3	Hold until patients are on stable replacement hormone regimen. Resume at same dose level. If diagnosed with hypophysitis should hold therapy if occurring before cycle 5 but may begin nivolumab monotherapy when returned to grade 1 (no earlier than 6 week and by end of up to an 8-week delay).
Grade 4	Off protocol therapy
Note all participants 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 participants with thyroiditis may be retreated on replacement therapy. Participants must be evaluated to rule out pituitary disease prior to initiating thyroid replacement. Participants with symptomatic hyperthyroidism can be given a short course of a beta blocker (e.g., propranolol). Hyperthyroidism usually converts to hypothyroid within 4 weeks.	
Recommended management: See Endocrine Management Algorithm (Appendix X).	

Fever	Management/Next Dose for Nivolumab and Combination
≤ 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 treatment.
Participants with fever should be evaluated as clinically appropriate. Participants may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever.	
See Section 4.12, infusion reactions.	

HIV Viral Load Increase	Management/Next Dose for Nivolumab and Combination
>1,000 on two consecutive readings	Strongly consider modifying HAART therapy, preferably after consulting an HIV specialist.

Treatment with steroids may be initiated at any time if clinically indicated; recommended delayed dosing for evaluation is not mandatory.

Participants on single agent therapy requiring a delay of > 35 days must be permanently discontinued from study therapy, except as specified in Section 4.10 (Criteria to Resume Treatment).

Participants on combination therapy requiring a delay of > 42 days (> 56 days for participants on high dose steroids with required 4 weeks minimal taper and 2-week observation) should go off protocol therapy.

Participants requiring > two dose delays for the same event should go off protocol therapy.

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

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

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

5.2 Other Guidance

These original ipilimumab guidelines are for investigator information. Treatment management decisions should be based on the information in Section 5.1, treatment algorithms in Appendix X, and further information in the IB.

5.2.1 Treatment of infusion reactions associated with ipilimumab

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in participants. However, it is possible that infusion

of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. No prophylactic pre-medication should be given unless indicated by previous experience in an individual participant. Reactions should be treated based upon the following recommendations.

- For mild symptoms (*e.g.*, localized cutaneous reactions such as mild pruritus, flushing, rash):
    - Decrease the rate of infusion until recovery from symptoms, remain at bedside, and monitor participant.
    - Complete the ipilimumab infusion at the initial planned rate.
    - Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and participants may receive additional doses with close monitoring.
    - Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses of ipilimumab.
- For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mmHg):
    - Interrupt ipilimumab.
    - Administer diphenhydramine 50 mg IV.
    - Monitor participant closely until resolution of symptoms.
    - Corticosteroids may abrogate any beneficial immunologic effect but may be administered at the discretion of the treating physician.
    - Resume ipilimumab infusion after recovery of symptoms.
    - At the discretion of the treating physician, ipilimumab infusion may be resumed at one-half the initial infusion rate, then increased incrementally to the initial infusion rate.
    - If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab should be administered that day.
    - The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
    - At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.
- For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure <80 mm Hg, or angioedema):
    - Immediately discontinue infusion of ipilimumab and disconnect infusion

tubing from the participant.

- Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.
- Participants should be monitored until the investigator is comfortable that the symptoms will not recur.
- No further ipilimumab will be administered.

- In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

5.2.2 Treatment of ipilimumab-related isolated drug fever

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology. If a participant experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a participant experiences recurrent isolated drug fever following premedication and post-dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the participant's level of discomfort with the event and use clinical judgment to determine if the participant should receive further ipilimumab.

5.2.3 Monitoring and management of immune-mediated adverse reactions

Immune-mediated enterocolitis

The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia, with or without fever. However, inflammation may occur in any part of the GI tract including esophagitis and gastritis. Fatalities due to GI perforation have been reported in clinical trials of ipilimumab. Participants should be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of ipilimumab therapy should be evaluated to exclude infectious or alternate etiologies. In clinical trials, immune-related colitis was associated with evidence of mucosal inflammation, with or without ulcerations, and lymphocytic infiltration.

Monitor participants for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus, or blood in stool, with or without fever) and bowel perforation (such as peritoneal signs and ileus). In symptomatic participants, rule out infectious etiologies and consider endoscopic evaluation to establish etiology and for persistent or severe symptoms. C. difficile toxin has been detected in several participants with colitis and may be an independent entity or may co-exist with ipilimumab induced inflammatory colitis.

Withhold ipilimumab dosing for any participants with enterocolitis pending

evaluation; administer anti-diarrheal treatment and, if persistent evaluate with colonoscopy and initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

Permanently discontinue ipilimumab in participants with severe enterocolitis and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In clinical trials, rapid corticosteroid tapering has resulted in recurrence or worsening symptoms of enterocolitis in some participants.

Participants have been treated with anti-TNF agents for persistent colitis not responding to steroids.

Please note autoimmune pancreatitis may cause abdominal pain and should be included in all evaluations. Enteritis may occur occasionally with other autoimmune events including hepatitis, pancreatitis, and endocrine insufficiency, which should be evaluated as clinically indicated.

Immune-mediated hepatitis and pancreatitis

Hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Fatal hepatic failure has been reported in clinical trials of ipilimumab. Serum transaminase, bilirubin, and lipase levels must be evaluated before each dose of ipilimumab as early laboratory changes may be indicative of emerging immune-related hepatitis/ pancreatitis and elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms. Increase in LFT or total bilirubin should be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or other medications, and monitored until resolution. Liver biopsies from participants who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Monitor LFTs (hepatic transaminase and bilirubin levels, lipase) and assess participants for signs and symptoms of hepatotoxicity/ pancreatitis before each dose of ipilimumab. In participants with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution. Withhold ipilimumab in participants with grade 2 hepatotoxicity.

Permanently discontinue ipilimumab in participants with grade 3-5 hepatotoxicity/pancreatitis and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When LFTs show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month. Across the clinical development program for ipilimumab, mycophenolate treatment has been administered in participants who have persistent severe hepatitis despite high dose corticosteroids.

Immune-mediated dermatitis

Skin immune-related AEs presented mostly frequently as a rash and/or pruritus. Some participants reported vitiligo associated with ipilimumab administration. Fatal TEN has been reported in clinical trials of ipilimumab.

Monitor participants for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

Permanently discontinue ipilimumab in participants with Stevens-Johnson syndrome, TEN, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month. Withhold ipilimumab dosing in participants with moderate to severe signs and symptoms.

For mild-to-moderate dermatitis, such as grade 2 localized rash and pruritus, treat symptomatically. For persistent grade 2, grade 3, or greater, topical steroids may be administered. Administer topical or systemic corticosteroids as indicated if there is no improvement of symptoms within 1 week.

Immune-related neurological events

Fatal Guillain-Barré syndrome has been reported in clinical trials of ipilimumab. Participants may present with muscle weakness and myasthenia gravis, cranial nerve palsy (n VII Bell's palsy), and aseptic meningitis, encephalopathy. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 days should be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, nerve entrapment, and medications should be excluded as causes.

Withhold ipilimumab dosing in participants with any evidence of neuropathy pending evaluation.

Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Permanently discontinue ipilimumab in participants with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Institute medical intervention as appropriate for management of neuropathy and other neurologic events. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies.

Immune-mediated endocrinopathies

Ipilimumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thryroiditis with positive antibodies) and adrenal glands, hypophysitis, hypopituitarism, and resulting thyroid and adrenal insufficiency, low ADH, prolactin, FSH, LH. Hyperthyroid with Graves' disease and positive antibody has been reported. Participants may present with subtle and nonspecific symptoms. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, and electrolyte disturbances including hyponatremia and hypotension. Adrenal crisis as a cause of the participant's symptoms should be excluded. Based on the available data with known outcome, most of the participants symptomatically improved with hormone replacement therapy. Long term hormone replacement therapy with HC

and synthroid will typically be required for participants developing hypophysitis/hypopituitarism after treatment with ipilimumab. Some participants have regained partial function following steroid treatment.

Monitor participants for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. Headache is often the first symptoms of hypophysitis. Participants may present with fatigue, headache, mental status changes, loss of libido, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms, which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated and drug withheld pending evaluation. Participants may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

Endocrine evaluation, including TSH, should be performed at baseline prior to initial treatment

Monitor thyroid function tests and clinical chemistries at the start of treatment for baseline function and anti-thyroid antibodies. Endocrine function will be evaluated every 12 weeks by monitoring TSH, free T4, TPO, morning ACTH, and morning cortisol if ACTH is abnormal. The package insert for ipilimumab includes a recommendation for monitoring TSH prior to each infusion; as an early indication for pituitary dysfunction and hypophysitis, clinical monitoring of symptoms may be equally or more sensitive as an initial presentation. In a limited number of participants, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland.

Withhold ipilimumab dosing in participants symptomatic for hypophysitis. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent and initiate appropriate hormone replacement therapy.

Other immune-mediated adverse reactions, including ocular manifestations

Ocular inflammation, manifested as grade 2 or grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few participants (<1%) and occasionally occurred in the absence of clinically apparent GI symptoms. Other presumed immune-related AEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which were individually reported for <1% of participants.

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of ipilimumab-treated participants in Study 1: nephritis, pneumonitis, pulmonary granuloma resembling sarcoidosis, meningitis, pericarditis, uveitis, iritis, ITP, neutropenia, and hemolytic anemia.

Across the clinical development program for ipilimumab, the following likely immune-mediated adverse reactions were also reported with less than 1% incidence: myocarditis, angiopathy, temporal arteritis, vasculitis, polymyalgia rheumatica, conjunctivitis, blepharitis, episcleritis, scleritis, leukocytoclastic

vasculitis, erythema multiforme, psoriasis, pancreatitis, arthritis, and autoimmune thyroiditis.

Permanently discontinue ipilimumab for clinically significant or severe immune-mediated adverse reactions. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to participants who develop uveitis, iritis, or episcleritis. Permanently discontinue ipilimumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Overall, immune-related AEs commonly started within 3 to 10 weeks from first dose, were successfully managed in most instances by omitting doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as mentioned above, and detailed in this section. Immune-related AEs generally resolved within days to weeks in the majority of participants.

6.0 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 6.1) and the characteristics of an observed AE (Section 6.2) will determine whether the event requires expedited (via CTEP-AERS) **in addition** to routine reporting (via AdvantageEDC).

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE Version 5.0. A copy of the CTCAE Version 5.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Comprehensive AEs and Potential Risks Lists (CAEPRs)

6.1.1 CAEPR for nivolumab (BMS-936558, 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 Nivolumab.

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 2.5, June 10, 2023[1]

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 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 3)***
		Blood and lymphatic system disorders - Other (lymphatic dysfunction)	
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2] Pericarditis	
ENDOCRINE DISORDERS			

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Adrenal insufficiency[3]		
	Hypophysitis[3]		
	Hyperthyroidism[3]		
	Hypothyroidism[3]		
EYE DISORDERS			
		Blurred vision	
		Dry eye	
		Eye disorders - Other (diplopia)[3]	
		Eye disorders - Other (Graves ophthalmopathy)[3]	
		Eye disorders - Other (optic neuritis retrobulbar)[3]	
		Eye disorders – Other (Vogt-Koyanagi-Harada)[3]	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Enterocolitis	
		Gastritis	
		Mucositis oral	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		***Injection site reaction (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatobiliary disorders - Other (immune-related hepatitis)	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Autoimmune disorder[3]	
		Cytokine release syndrome[5]	
		Immune system disorders - Other (GVHD in the setting of allotransplant)[3,6]	
		Immune system disorders - Other (sarcoid-granuloma, sarcoidosis)[3]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		
INVESTIGATIONS			
	Alanine aminotransferase increased[3]		***Alanine aminotransferase increased[3] (Gr 3)***
	Aspartate aminotransferase increased[3]		***Aspartate aminotransferase increased[3] (Gr 3)***
	Blood bilirubin increased[3]		***Blood bilirubin increased[3] (Gr 2)***

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	CD4 lymphocytes decreased		***CD4 lymphocyte decreased (Gr 4)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	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)	
		Myositis	
		Rhabdomyolysis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy[3]	
		Facial nerve disorder[3]	
		Guillain-Barré syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)[3]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
		Reversible posterior leukoencephalopathy syndrome[3]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury[3]	
		Renal and urinary disorders - Other (immune-related nephritis)	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia (BOOP))[3]	

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme[3]	
	Pruritus[3]		***Pruritus[3] (Gr 2)***
	Rash maculo-papular[3]		***Rash maculo-papular[3] (Gr 2)***
		Skin and subcutaneous tissue disorders -Other (bullous pemphigoid)	
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)[3]		
	Skin hypopigmentation[3]		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	

[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]Nivolumab 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.

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

[5]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopenia.

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

[7]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.

AEs reported on Nivolumab trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Nivolumab caused the AE:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis

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; Periorbital edema

GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Flatulence; Gastrointestinal disorders - Other (mouth sores); 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 - Blood lactate dehydrogenase increased; GGT increased; Investigations - Other (protein total decreased); 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; Photosensitivity; Rash acneiform; Skin and subcutaneous tissue disorders - Other (rosacea)

VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Vasculitis

Note: Nivolumab in combination with other agents could cause an exacerbation of any AE currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

6.1.2 CAEPR for ipilimumab (MDX-010, NSCs 732442 and 720801)

The 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 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 2678 patients.* Below is the CAEPR for Ipilimumab (MDX-010).

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 2.10, March 29, 2019[1]

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
		Blood and lymphatic system disorders - Other (acquired hemophilia)	
CARDIAC DISORDERS			
	Atrial fibrillation		
		Myocarditis[2]	
		Pericardial effusion	
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2] Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
	Testosterone deficiency[2]		
EYE DISORDERS			
	Eye disorders - Other (episcleritis)[2]		
	Uveitis[2]		
GASTROINTESTINAL DISORDERS			
	Abdominal pain Colitis[2]		***Colitis (Gr 3)***
		Colonic perforation[3]	
	Constipation		
Diarrhea			***Diarrhea (Gr 3)***
	Enterocolitis		
	Esophagitis		
		Ileus	
Nausea			***Nausea (Gr 3)***

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Pancreatitis[2]		
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
		General disorders and administration site conditions - Other (systemic inflammatory response syndrome [SIRS])	
		Multi-organ failure	
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (hepatitis)[2]		
IMMUNE SYSTEM DISORDERS			
	Autoimmune disorder[2]		
		Immune system disorders - Other (GVHD in the setting of allotransplant)[4]	
INFECTIONS AND INFESTATIONS			
		Infections and infestations - Other (aseptic meningitis)[2]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
		Lymphocyte count decreased	
	Neutrophil count decreased		
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		
	Hyperglycemia		
		Metabolism and nutrition disorders - Other (exacerbation of pre-existing diabetes mellitus)	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Arthritis		
		Generalized muscle weakness	
	Musculoskeletal and connective tissue disorder - Other (polymyositis)[2]		
NERVOUS SYSTEM DISORDERS			
		Ataxia	
	Facial nerve disorder		
	Guillain-Barré syndrome[2]		
	Headache		
	Myasthenia gravis[2]		

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Nervous system disorders - Other (immune-mediated encephalitis)[2]	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
	Trigeminal nerve disorder		
PSYCHIATRIC DISORDERS			
		Psychiatric disorders - Other (mental status changes)	
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
	Renal and urinary disorders - Other (granulomatous tubulointerstitial nephritis)		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis		
		Respiratory failure	
		Respiratory, thoracic, and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)	
		Respiratory, thoracic, and mediastinal disorders - Other (lung infiltration)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	
	Pruritus		***Pruritus (Gr 3)***
Rash maculo-papular			***Rash maculo-papular (Gr 3)***
	Skin and subcutaneous disorders - Other (Sweet's syndrome)		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
	Urticaria		
VASCULAR DISORDERS			
	Hypotension		

[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]Ipilimumab can result in severe and fatal immune-mediated adverse events probably due to T-cell activation and proliferation. These can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune thyroiditis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, and adrenal insufficiency), ocular manifestations (e.g., uveitis, iritis, conjunctivitis, blepharitis, and episcleritis), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome. The majority of these reactions manifested early during treatment; however, a minority occurred weeks to months after discontinuation of ipilimumab especially with the initiation of additional treatments.

[3]Late bowel perforations have been noted in patients receiving MDX-010 (ipilimumab) in association with subsequent IL-2 therapy.

[4]Complications including hyperacute graft-versus-host disease (GVHD), may occur in patients receving allo stem cell transplant (SCT) after receiving Ipilimumab (MDX-010). These complications may occur despite intervening therapy between receiving Ipilimumab (MDX-010) and allo-SCT.

[5]In rare cases diplopia (double vision) has occurred as a result of muscle weakness (Myasthenia gravis).

[6]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[7]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on ipilimumab (MDX-010) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ipilimumab (MDX-010) caused the AE:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Blood and lymphatic system disorders - Other (pure red cell aplasia)[2]; Febrile neutropenia

CARDIAC DISORDERS - Conduction disorder; Restrictive cardiomyopathy

EYE DISORDERS - Extraocular muscle paresis[5]; Eye disorders - Other (retinal pigment changes)

GASTROINTESTINAL DISORDERS - Colonic ulcer; Dyspepsia; Dysphagia; Gastrointestinal disorders - Other (gastroenteritis); Gastrointestinal hemorrhage[6]; Proctitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain

HEPATOBILIARY DISORDERS - Hepatic failure[2]

IMMUNE SYSTEM DISORDERS - Allergic reaction

INFECTIONS AND INFESTATIONS - Infection[7]

INVESTIGATIONS - Creatinine increased; Investigations - Other (rheumatoid factor); Lipase increased; Platelet count decreased; Serum amylase increased; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Joint range of motion decreased; Myalgia; Pain in extremity

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Dizziness; Dysphasia; Ischemia cerebrovascular; Seizure

PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia

RENAL AND URINARY DISORDERS - Proteinuria

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Cough; Dyspnea; Laryngospasm

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Skin hypopigmentation

VASCULAR DISORDERS - Flushing; Hypertension; Vascular disorders - Other (temporal arteritis)

Note: Ipilimumab (BMS-734016; MDX-010 Transfectoma-derived) in combination with other agents could cause an exacerbation of any AE currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

6.2 Classification of AEs by Severity and Relationship to Study Drug Administration

6.2.1 AE: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

6.2.2 Life threatening Adverse Event: Any AE that places the participant or participant, in view of the Investigator, at immediate risk of death from the reaction.

6.2.3 Serious Adverse Event (SAE): Any AE occurring at any dose that results in any of the following outcomes: Death, a life threatening AE, inpatient hospitalization, or prolongation of existing hospitalization, a persistent or significant disability/ incapacity, or a congenital anomaly/birth defect.

6.2.4 Please note for hospitalization – All hospitalizations (or prolongation of existing hospitalization) for medical events equivalent to CTCAE Grade 3, 4, 5 must be reported regardless of the requirements for Phase of study, expected or unexpected, and attribution. For example, do not report an admission for pharmacokinetic sampling, but do report an admission for a myocardial infarction.

6.2.5 Toxicity: Toxicity is a term NOT clearly defined by regulatory organizations. Toxicity has been described as an AE that has an attribution of possibly, probably, or definitely related to investigational treatment. To minimize confusion the NCI would recommend that the term toxicity NOT be utilized for AE reporting purposes. The CTCAE continues to use the term ‘toxicity’ because of familiarity.

6.2.6 Unexpected Adverse Event: Any AE that is not listed in available sources including the package insert, the Investigator’s Brochure, or the protocol.

6.2.7 CTEP Adverse Event Reporting System (CTEP-AERS): An electronic system for expedited submission of AE reports.

6.2.8 Attribution: The determination of whether an AE is related to a medical treatment or procedure. Attribution categories:

Definite – The AE is clearly related to the investigational agent.

Probable – The AE is likely related to the investigational agent.

Possible – The AE may be related to the investigational agent.

Unlikely – The AE is doubtfully related to the investigational agent.

Unrelated – The AE is clearly NOT related to the investigational agent.

6.3 Expedited AE Reporting

6.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page (**http://ctep.cancer.gov**). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (**http://ctep.cancer.gov**). These requirements are briefly outlined in the table below (Section 6.3.3).

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

6.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

6.3.3 Expedited Reporting Guidelines

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

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

Death due to PD should be reported as **Grade 5 "General disorders and administration site conditions - Disease Progression."** Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An AE is considered serious if it results in **ANY** of the following outcomes:

- Death
- A life threatening AE
- 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 participant or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

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

NOTE: Protocol-specific exceptions to expedited reporting of SAEs are found in the SPEER portion of the CAEPR.

Expedited AE reporting timelines are defined as:

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

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

- Expedited 24-hour notification followed by complete report within 5 calendar days for:
- All Grade 3, 4, and Grade 5 AEs
- Expedited 10 calendar day reports for:
- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

6.4 Routine AE Reporting

All AEs that occur, regardless of attribution to the study agent, **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

6.4.1 Clinical laboratory abnormalities

Clinical laboratory abnormalities will be considered AEs if determined to be clinically significant by the investigator. In assessing laboratory results, an abnormal laboratory value will be considered clinically significant if it is characterized by one or more of the following criteria:

1. Is not proved erroneous result by repeat testing, if performed
2. Is judged by the investigator to have a causal relationship to the investigational agent
3. Requires clinical intervention or monitoring, such as: close observation, more frequent follow-up assessments, further diagnostic intervention, treatment/therapeutic intervention, or protocol therapy dose modification

4. Is associated with clinical signs or symptoms, which may suggest a disease and/or organ toxicity, or may represent a new condition or worsening of a baseline condition

5. Is associated with a SAEs, or is otherwise judged by the Investigator to be of significant clinical impact

In general, a laboratory abnormality that is not clinically significant will be consistent with CTCAE grade 1 (mild) or 2 (moderate) severity, as categorized by the relevant severity description in the Investigations System Organ Class (SOC) or Metabolism and Nutrition Disorders SOC. Investigators may not designate laboratory abnormalities that are consistent with grade 3 or greater severity as not clinically significant.

All laboratory values deemed clinically significant will be subsequently reported as AEs unless the result is a sign of a clinical diagnosis that is reported as an AE.

6.5 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 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.

6.6 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.

7.0 PHARMACEUTICAL INFORMATION

A list of the AEs and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 6.1.

7.1 Nivolumab (NSC 748726)

Amino Acid Sequence: Four polypeptide chains, which include two identical heavy chains with 440 amino acids and two 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), and water for injection. Dilute solutions of hydrochloric acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5).

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

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

Storage: Vials of nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing, and shaking. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours

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 over 30 minutes. Do not administer as an IV push or bolus injection. See Section 4.3 for dosing instructions.

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

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

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

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

7.2 Ipilimumab (NSC 732442)

Chemical Name or Amino Acid Sequence: Four polypeptide chains, two identical heavy chains with 447 amino acids and two identical light chains consisting of 215 amino acids.

Other Names: Anti-CTLA-4 monoclonal antibody, MDX-010, Yervoy™

Classification: Human monoclonal antibody

M.W.: 147,991 Daltons

Mode of Action: Ipilimumab is specific for the CTLA-4 antigen expressed on a subset of activated T cells. CTLA-4 interaction with the B7 molecule, one of its ligands expressed on professional antigen-presenting cells, can down-regulate T cell response. Ipilimumab is, thought to act by blocking the interaction of CTLA-4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T cell activation. The CTLA-4/B7 creates the interaction.

Description: Ipilimumab is a fully human immunoglobulin ($IgG_1\kappa$) with two manufacturing processes – ongoing trials have been using substances manufactured using Process B. New clinical trials will be using ipilimumab that is manufactured by Process C. The Process C has been developed using a higher producing sub-clone of the current Master Cell Bank, and modified cell culture and purification steps.

How Supplied: Bristol-Myers-Squibb (BMS) supplies Ipilimumab to the DCTD/NCI. Ipilimumab injection, 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, nonpyrogenic, single-use, isotonic aqueous solution that may contain particles.

Each vial is a Type I flint glass vial with gray butyl stoppers and sealed with aluminum seals.Table 3: Ipilimumab Composition

Component	Process C
	200mg/ vial[a]
Ipilimumab	213 mg
Sodium Chloride, USP	249 mg
TRIS-hydrochloride	134.3 mg
Diethylenetriamine pentacetic acid	1.67 mg
Mannitol, USP	426 mg
Polysorbate 80 (plant-derived)	4.69 mg
Sodium Hydroxide	QS to pH 7
Hydrochloric acid	QS to pH 7
Water for Injection	QS: 42.6 mL
Nitrogen[b]	Processing agent

[a] Includes 2.6 mL overfill.

[b] Nitrogen is used to transfer the bulk solution through the pre-filled and sterilizing filters into the aseptic area.

In 2023, PMB will transition to a 50 mg/10 mL (5 mg/mL) vial, which will replace the 200 mg vial. The 50 mg vial is packaged in a 10-cc Type I flint tubing glass vial, stoppered with a 20-mm gray butyl rubber stopper, and sealed with a 20-mm aluminum flip-off seal. Each vial includes a 0.7-mL overfill for vial, needle, and syringe (VNS) holdup.

Preparation: Ipilimumab is given undiluted or further diluted in 0.9% NaCl Injection, USP or 5% Dextrose Injection, USP in concentrations between 1 mg/mL and 4 mg/mL. Ipilimumab is stable in a polyvinyl chloride (PVC), non-PVC/non DEHP (di-(2-ethylhexyl) phthalate) IV bag or glass container up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

Storage: Store intact vials refrigerated at (2° to 8° C), protected from light. Do not freeze.

The storage and use conditions recommended for Ipilimumab also apply to the Placebo for Ipilimumab Injection.

Stability: Shelf-life surveillance of the intact vials is ongoing. Solution as described above is stable up to 24 hours refrigerated at (2° to 8° C) or at room temperature/ room light.

CAUTION: Ipilimumab does not contain antibacterial preservatives. Use prepared IV solution immediately. Discard partially used vials.

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

The placebo for ipilimumab injection is also a ready-to-use solution, which is administered in a similar fashion as described above for the product.

Method of Administration: Can use a volumetric pump to infuse ipilimumab at the protocol-specific dose(s) and rate(s) via a PVC IV infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (0.2 micron to 1.2 micron).

Patient Care Implications: Monitor participants for immune-related AEs, e.g., rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis, and hypothyroidism. If you suspect toxicity, refer to the protocol guidelines for ruling out other causes.

7.3 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 participant 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. In general, sites may order initial agent supplies when a participant is being screened for enrollment onto the study.

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. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB at any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

7.4 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 PMB at 240-276-6575). 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.

7.5 Useful Links and Contacts

CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/

NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov

PMB policies and guidelines:

http://ctep.cancer.gov/branches/pmb/agent_management.htm

PMB OAOP application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx

CTEP IAM account: https://eapps-ctep.nci.nih.gov/iam/

CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov

PMB email: PMBAfterHours@mail.nih.gov

PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.0 CLINICAL AND LABORATORY EVALUATIONS

A Study Calendar of the evaluations described below is provided in Appendix I.

8.1 Pre-Study Evaluations (Visit -1)

Pre-study evaluations may be performed (or existing results obtained for the purpose of documenting eligibility) after the participant provides informed consent.

8.1.1 Complete medical history, to include CDC HIV risk categories and history of AIDS-defining conditions, documentation of HIV infection with duration of HIV and AIDS diagnoses, history of opportunistic illnesses, history of prior treatment for the study malignancy, history of any other prior malignancies and treatment, and history of other autoimmune conditions per Section 3.2.12.

8.1.2 Complete physical examination, including ECOG score or KPS (Appendix III), vital signs, weight, and height.

8.1.3 AE assessment (from any prior therapies). Recovery to CTCAE ≤ grade 1 from toxicity from prior therapies is required for all AEs except alopecia, neuropathy, and other non-clinically significant AEs. AEs from prior therapies present at baseline should be documented in the source only. Assessment of effects of prior therapy should include evaluation of immune status, organ damage, risk of autoimmunity, and immunopotentiation.

8.1.4 List of all concurrent medications taken for the 3 months prior to enrollment, including ART regimen.

8.1.5 Laboratory studies within 2 weeks prior to enrollment (unless otherwise noted), including:

a) CBC with differential and platelet count: Hematology labs are to include hemoglobin, hematocrit, red blood cell count, WBC, ANC, platelets (direct platelet count), and total and differential CBC counts.

b) Serum chemistry analysis, to include albumin, urea or BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, HCO_3 (CO_2; venous blood), calcium, phosphorous.

c) Serum amylase and lipase

d) EKG. EKG and echocardiogram (ECHO) for any patients with a history of congestive heart failure (CHF) or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated. For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, echocardiogram.

e) PPD (or Quantiferon assay) within 12 weeks prior to enrollment if known PPD negative. If PPD positive, the participant must complete prophylaxis before enrollment.

f) HIV viral load will be performed using an assay with a limit of detection of 75 copies/ml or less within 4 weeks prior to enrollment

g) CD4/CD8 count within 2 weeks prior to enrollment

h) Hepatitis B antigen, and Hepatitis C antibody tests within 2 weeks prior to enrollment

i) Pregnancy test will be performed (and results obtained) within 72 hours of enrollment (WOCBP)

j) Immunologic labs consisting of: rheumatoid factor, C-reactive protein, and ANA, SPEP, quantitative immunoglobulins and free light chains to be completed within 4 weeks prior to enrollment.

k) Endocrine labs consisting of: TSH, free T4, TPO, morning ACTH, and morning cortisol if ACTH is abnormal.

8.1.6 Radiologic evaluation, including studies to assess all measurable or non-measurable sites of disease must be done within 4 weeks prior to registration.

For participants with known KS, chest X-ray to determine pulmonary involvement. Pulmonary KS must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy in the judgment of the investigator. KS participants with a positive chest X-ray or symptoms suggestive of pulmonary disease must have a chest CT performed before study entry.

Participants with KS must also have tumor assessments with a photographic record performed within 7 days before initiating study treatment. Tumor assessments and photographs should be performed as outlined in the AMC's Kaposi's Sarcoma (KS) Tumor Assessment Manual of Procedures (MOP).

8.1.7 Participants with HL: PET/CT scans are required to be completed within 4 weeks of starting nivolumab. A combined FDG-PET/CT or FDG-PET + CT scan with and without IV contrast is required for this study. If a participant has an allergy to IV contrast, a non-IV contrast CT scan will be permitted. To ensure consistency between all centers, FDG-PET/CT scans at diagnosis should be conducted no more than 28 days before enrollment.

If the CT scan of a PET/CT hybrid is performed with both oral and IV contrast with contrast enhancement in the arterial and/or portal venous phase, with at least a 2-slice CT, and is acquired with at least 80 mAs and CT scans are obtained with contiguous sections (with a maximum of 5 mm slice thickness), then the pre-treatment PET/CT scan alone will suffice for participants enrolled on this trial. If no contrast is used in the FDG-PET/CT, an additional CT scan with contrast is required of the neck, chest, abdomen, and pelvis.

8.1.8 Bone marrow biopsy for histology and determination of percent bone marrow involvement for HL participants. If bone marrow involvement by lymphoma has already been documented after bone marrow biopsy performed more than 6 weeks prior to registration, the biopsy does not have to be repeated.

8.1.9 Optional donation to the AIDS and Cancer Specimen Resource (ACSR). (See Appendix V for ACSR Informed Consent Form and Appendix IV for ACSR Specimen Preparation and Shipping Instructions).

8.1.10 Biological sample submissions for correlative studies

The collection procedures for correlative studies are described in analyte-specific appendices to this protocol and based on validated AMC SOPs. The guiding principles are to be consistent across past and future AMC trials, coordinating with other clinical trials with these agents. They involve the AMC Biorepository as the central laboratory to manage all samples for this study.

a) Blood draw:

- Collect two 6 ml red top tubes for assessment of circulating levels of pro-inflammatory molecules (Appendix XV).
- Collect twelve 8.5 mL yellow top (ACD) tubes for the HIV latent reservoir assay and viral outgrowth assay for participants with hematocrit ≥ 25 who provide consent for this optional sample. The sample will be collected at baseline (within 7 days before starting treatment and must be collected after enrollment in AdvantageEDC). ***These samples will be shipped directly to the AMC Biorepository via priority overnight service for processing.*** See Appendix XVI for collection and shipping instructions. Material from these samples will also be used for herpesvirus load and latent virus studies (Appendix XIV).
- If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 mL yellow top tubes will be collected for herpesvirus load and latent virus studies (Appendix XIV and Appendix XVI).

b) The collection, shipping, and storage of 2x2 mm biopsies in RNALater and formalin is described in Appendix XIV (optional for KS cases only, with participant consent).

c) The collection, shipping, and storage of anal swabs is described in Appendix XVII (optional for anal cancer cases only, with participant consent).

d) Collect four 4 micron thick unstained slides from the initial diagnostic biopsy and store at the AMC Biorepository for PDL-1 testing at the AMC Pathology Core Lab..

8.2 Cycle 1, Day 1

Unless noted otherwise below, the evaluations may be performed up to three days prior to treatment initiation. Pre-visit requirements may be combined with the pre-dose Cycle 1 Day 1 requirements provided that the evaluation is within the stated window for visits, and all required pre-study evaluation results have been evaluated by an investigator prior to enrollment in AdvantageEDC.

8.2.1 Review of medical history since pre-study visit

8.2.2 Physical examination (vital signs, weight, performance status)

8.2.3 Review of changes in concurrent medications

8.2.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count within 2 days prior to treatment

b) Serum chemistries

c) Serum amylase and lipase

d) Serum or urine pregnancy test for WOCBP within 72 hours prior to treatment

8.2.5 Biological sample submissions for correlative studies (as defined in Section 8.1.10) must be collected at any time prior to starting treatment on Day 1. The samples below must be collected at the time points provided below.

a) Blood draw: Five 8.5mL ACD tubes for assays (HIV DNA, HIV RNA, single copy assays (SCA), and phenotypic/ICS for all participants with hematocrit ≥ 25 to be collected within 2 days before the Day 1 dose, and Day 1 of Cycle 1 post-dose. (Appendix XVI). The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days and should be collected at least 12 hours apart. The post-dose sample must be drawn between 12 and 36 hours after the infusion has been completed. ***Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection.*** The samples will be shipped to the **AMC Biorepository** via priority overnight service for processing if this cannot occur on site.

8.2.6 First dose of study drug(s) (if receiving). Vital signs will be obtained before the infusion and every 15 minutes from the start of the infusion until 60 minutes post-completion. When slowing or restarting an infusion due to an infusion reaction, vital signs should be monitored every 15 minutes or as directed by the Investigator until the infusion is completed and/or the participant is stabilized. See Section 4.11 for dosing information and instructions regarding management of nivolumab infusion reactions, and Section 5.1 for management of ipilimumab infusion reactions.

8.2.7 AE assessment

8.3 Cycle 1, Day 8

Unless noted otherwise below, the evaluations may be performed up to three days prior.

8.3.1 Review of medical history since last visit

8.3.2 Physical examination (vital signs, weight, performance status)

8.3.3 Review of changes in concurrent medications

8.3.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count within 2 days prior to treatment

b) Serum chemistries

c) Serum amylase and lipase

8.3.5 AE assessment

8.3.6 Blood draw: Five 8.5 mL yellow ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) for all participants with hematocrit ≥ 25 to be collected (Appendix XVI). ***Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection.*** The samples will be shipped to the AMC Biorepository via priority overnight service for processing if this cannot occur on site.

8.4 Cycles 2-4, Day 1

Unless noted otherwise below, the evaluations may be performed up to three days prior to treatment.

8.4.1 Review of medical history since last visit

8.4.2 Physical examination (vital signs, weight, performance status)

8.4.3 Review of changes in concurrent medications

8.4.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count within 2 days prior to treatment

b) Serum chemistries

c) Serum amylase and lipase

d) Serum or urine pregnancy test for WOCBP within 72 hours prior to treatment

8.4.5 AE assessment

8.4.6 For participants with KS, tumor, and response assessment with photographic documentation at cycle 3 before receiving treatment, as outlined in the KS Tumor Assessment MOP. Response of KS to treatment is described in Section 9.2.

8.4.7 Dose of nivolumab. Vital signs will be monitored as per Section 8.2.6). Prophylactic preinfusion medications should be given if infusion reactions were observed during prior infusions.

8.4.8 Blood draw: At cycle 4 only, collect five 8.5 mL yellow ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) for all participants on dose levels 1 (including 240 mg q 2 weeks) or 2 with hematocrit ≥ 25, to be collected at the following time points: pre-dose on Day 1, post-dose on Day 1, and on day 8 (Appendix XVI). The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days and should be collected at least 12 hours apart. The post-dose sample must be drawn between 12 and 36 hours after the infusion has been completed. ***Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection.*** The samples will be shipped to the **AMC Biorepository** via priority overnight service for processing if this cannot occur on site.

8.5 Cycles 5-46, Day 1

Unless noted otherwise below, the evaluations may be performed up to three days prior to treatment.

8.5.1 Review of medical history since last visit

8.5.2 Physical examination (vital signs, weight, performance status)

8.5.3 Review of changes in concurrent medications

8.5.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count within 2 days prior to treatment

b) Serum chemistries (odd # cycles only 5, 7, 9, etc.)

c) Serum amylase and lipase (odd # cycles only 5, 7, 9, etc.)

d) Serum or urine pregnancy test for WOCBP at Cycle 5 Day 1 and every 12 weeks thereafter

e) HIV viral load and CD4/CD8 count at Cycle 5 Day 1 and every 12 weeks thereafter: Viral load studies will be performed using an assay with a limit of detection of 75 copies/ml or less. Repeat Hepatitis B antigen and/or Hepatitis C antibody if positive at baseline.

f) Immunologic labs at Cycle 5 Day 1 and every 12 weeks thereafter after consisting of: rheumatoid factor, C-reactive protein, ANA, SPEP, immunoglobulins, and free light chains (FLC).

g) Endocrine labs at Cycle 5 Day 1 and every 12 weeks thereafter consisting of: TSH, free T4, TPO, morning ACTH, and morning cortisol if ACTH is abnormal

8.5.5 AE assessment

8.5.6 Biological sample submissions for correlative studies. Samples will be collected as defined in Section 8.1.10, unless otherwise noted.

a) Collect two 6 ml red top tubes for assessment of circulating levels of pro-inflammatory molecules before cycle 5. Collection, shipping, and storage methods are described in Appendix XV.

b) Collect 2x2 mm biopsies in RNALater and formalin before cycle 5 as described in Appendix XIV (optional for KS cases only, with participant consent).

c) Collect anal swabs before cycle 5 as described in Appendix XVII (optional for anal cancer cases only, with participant consent).

d) Blood draw: Five 8.5mL ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) for participants on dose levels 1 (including 240 mg q 2 weeks) or 2 with hematocrit ≥ 25, **to be collected pre-dose on Day 1, post-dose on Day 1, and on Day 8 of Cycles 7, 10, 13, 19, 22, 25, 28, 31, 34, 37, 40, 43 and 46 (Appendix XIV).** The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days and should be collected at least 12 hours apart. The post-dose sample must be drawn between 12 and 36 hours after the infusion has been completed. Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection. The samples will be shipped to the AMC Biorepository via priority overnight service for processing if this cannot occur on site.

Five 8.5 mL ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) for participants on dose levels -1 or -2 with hematocrit ≥ 25, **to be**

collected pre-dose on Day 1, post-dose on Day 1, and on Day 8 of Cycles 7, 13, 19, 25, 31, 37, and 43 (Appendix XIV). The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days and should be collected at least 12 hours apart. The post-dose sample must be drawn between 12 and 36 hours after the infusion has been completed. ***Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection.*** The samples will be shipped to the **AMC Biorepository** via priority overnight service for processing if this cannot occur on site.

e) Blood draw: Twelve 8.5mL ACD tubes for assays (latent reservoir and VOA) for all participants who consent to this optional draw with hematocrit ≥ 25 to be collected **on day 1 of cycle 16.** ***These tubes will be shipped directly to the* AMC Biorepository *for processing*** (Appendix XVI).

f) If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 yellow top tubes will be collected for herpesvirus load and latent virus studies on day 1 of cycle 16 (Appendix XIV and Appendix XVI).

8.5.7 Radiologic assessment of response before cycle 5, and thereafter every 12 weeks. All imaging studies or CT scans should be performed in an identical way to the baseline scan with the same scanner, same scan direction, and consistent arm pointing. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response. If tumor flare is suspected, the participant must undergo a second scan within a month of the first scan and the case must be discussed with the principal investigator. If progression is documented at that time, participant must come off study. For all cohorts and disease types if a scheduled response assessment is missed, an assessment must be completed and documented prior to drug administration for the next cycle.

For participants with KS, tumor, and response assessment with photographic documentation before odd numbered cycles (5, 7, 9, etc.), as outlined in the KS Tumor Assessment MOP. Response of KS to treatment is described in Section 9.2. Scans will not be required for participants with KS unless clinically indicated.

In participants with HIV-cHL, F-FDG-PET/CT scans will be done at cycles 9 and 13 (weeks 17 and 25). At cycle 24 (week 49), a F-FDG-PET/CT scan will be required for participants who do not have two consecutive negative scans before this time point. A combined FDG-PET/CT or FDG-PET + CT scan with and without IV contrast is required for this study. If a participant has an allergy to IV contrast, a non-IV contrast CT scan will be permitted. If the CT scan of a PET/CT hybrid is performed with both oral and IV contrast with contrast enhancement in the arterial and/or portal venous phase, with at least a 2-slice CT, and is acquired with at least 80 mAs and CT scans are obtained with contiguous sections (with a maximum of 5 mm slice thickness), then the PET/CT scan alone will suffice. If no contrast is used in the FDG-PET/CT, an additional CT scan with contrast is required of the neck, chest, abdomen, and pelvis, see Section 8.1.7.

8.5.8 Dose of nivolumab (or nivolumab and ipilimumab). Prophylactic preinfusion medications should be given if infusion reactions were observed during prior infusions. Vital signs will be monitored pre-infusion and every 30 minutes (+/- 10 minutes) during dosing, and 1-hour post-completion for participants with prior infusion reactions. Participants with no prior infusion reactions may have vital signs monitor pre- and post-infusion only.

8.6 Combination Therapy Solid Tumor Expansion Cohort: Cycle 5-23 Day 1

Unless noted otherwise below, the evaluations may be performed up to three days prior to treatment.

8.6.1 Review of medical history since last visit

8.6.2 Physical examination (vital signs, weight, performance status)

8.6.3 Review of changes in concurrent medications

8.6.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count within 2 days prior to treatment

b) Serum chemistries Serum amylase and lipase

c) Serum or urine pregnancy test for WOCBP at Cycle 5 Day 1 and every 12 weeks thereafter

d) HIV viral load and CD4/CD8 count at Cycle 5 Day 1 and every 12 weeks thereafter: Viral load studies will be performed using an assay with a limit of detection of 75 copies/ml or less. Repeat Hepatitis B antigen and/or Hepatitis C antibody if positive at baseline.

e) Immunologic labs at Cycle 5 Day 1 and every 12 weeks thereafter after consisting of: rheumatoid factor, C-reactive protein, ANA, SPEP, immunoglobulins, and FLC.

f) Endocrine labs at Cycle 5 Day 1 and every 12 weeks thereafter consisting of: TSH, free T4, TPO, morning ACTH, and morning cortisol if ACTH is abnormal

8.6.5 AE assessment

Biological sample submissions for correlative studies. Samples will be collected as defined in Section 8.1.10, unless otherwise noted.

a) Collect two 6 ml red top tubes for assessment of circulating levels of pro-inflammatory molecules before cycle 5. Collection, shipping, and storage methods are described in Appendix XV.

b) Collect anal swabs before cycle 5 as described in Appendix XVII (optional for anal cancer cases only, with participant consent).

c) Blood draw: Five 8.5mL ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) with hematocrit ≥ 25, **to be collected pre-dose on Day 1, post-dose on Day 1, and on Day 8 of Cycles 7, 10, (Appendix XIV).** The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days and should be collected at least 12 hours apart. The post-dose sample must be

drawn between 12 and 36 hours after the infusion has been completed. **Every effort should be made to process samples for viable PBMC and plasma on site within 6 hours of collection**. The samples will be shipped to the AMC Biorepository via priority overnight service for processing if this cannot occur on site.

d) Blood draw: Twelve 8.5mL ACD tubes for assays (latent reservoir and VOA) for all participants who consent to this optional draw with hematocrit ≥ 25 to be collected **on day 1 of cycle 15.** ***These tubes will be shipped directly to the*** **AMC Biorepository** ***for processing*** (Appendix XVI).

e) If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 yellow top tubes will be collected for herpesvirus load and latent virus studies on day 1 of cycle 15 (Appendix XIV and Appendix XVI).

8.6.7 Radiologic assessment of response before cycle 5, and thereafter every 12 weeks. All imaging studies or CT scans should be performed in an identical way to the baseline scan with the same scanner, same scan direction, and consistent arm pointing. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response. If tumor flare is suspected, the participant must undergo a second scan within a month of the first scan and the case must be discussed with the principal investigator. If progression is documented at that time, participant must come off study. If a scheduled response assessment is missed, an assessment must be completed and documented prior to drug administration for the next cycle.

8.7 Treatment Discontinuation Evaluations

End of study assessments will be done at 6 weeks (± 1 week) and at 16 weeks (± 1 week) of the last dose of study treatment, unless otherwise noted.

Participants with ongoing toxicities should be seen more often as clinically indicated. The schedule of clinical follow-up for these participants will be at the discretion of the treating physicians and according to established Standard of Care. AEs Assessment on the study, including assessment of immune-mediated AEs and SAEs, will continue for all participants until 90 days after the last study drug administration.

8.6.1 Review of medical history since last visit

8.6.2 Physical examination (vital signs, weight, performance status)

8.6.3 Review of changes in concurrent medications

8.6.4 Laboratory studies (as defined in Section 8.1.5)

a) CBC with differential and platelet count

b) Serum chemistries

c) Serum amylase and lipase

d) Serum or urine pregnancy test for WOCBP

e) HIV viral load, performed using an assay with a limit of detection of 75 copies/ml or less, CD4/CD8, and repeat Hepatitis B antigen and/or Hepatitis C antibody if positive at baseline

f) Immunologic labs consisting of rheumatoid factor, C-reactive protein, and ANA

g) Endocrine labs consisting of: TSH, free T4, TPO, morning ACTH, and morning cortisol if ACTH is abnormal

h) For participants with HIV-cHL and with bone marrow involvement at screening, a bone marrow biopsy will be required to confirm complete remission.

8.6.5 AE assessment

8.6.6 Biological sample submissions for correlative studies (as defined in Section 8.1.10), within 6 weeks (± 1 week) of the last dose of study treatment only

a) Collect two 6 ml red top tubes for assessment of circulating levels of pro-inflammatory molecules (Appendix XV).

b) The collection, shipping, and storage of 2x2 mm biopsies in RNALater and formalin is described in Appendix XIV (optional for KS cases only, with participant consent).

c) The collection, shipping, and storage of anal swabs is described in Appendix XVII (optional for anal cancer cases only, with participant consent).

d) Blood draw: Twelve 8.5mL ACD tubes for assays (HIV DNA, HIV RNA, SCA, and Pheno/ICS) for all participants who consent to this optional draw with hematocrit ≥ 25. ***These tubes will be shipped directly to the*** **AMC Biorepository** ***for processing*** (Appendix XVI).

e) If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 yellow top tubes will be collected for herpesvirus load and latent virus studies (Appendix XIV).

8.6.7 Radiologic assessment of response, as applicable per Section 9.0.

For participants with KS, tumor, and response assessment with photographic documentation, as outlined in the KS Tumor Assessment MOP. Response of KS to treatment is described in Section 9.2. Scans will not be required for participants with KS unless clinically indicated.

In participants with HIV-cHL, F-FDG-PET/CT scans will be done at treatment completion. A combined FDG-PET/CT or FDG-PET + CT scan with and without IV contrast is required for this study. If a participant has an allergy to IV contrast, a non-IV contrast CT scan will be permitted. If the CT scan of a PET/CT hybrid is performed with both oral and IV contrast with contrast enhancement in the arterial and/or portal venous phase, with at least a 2-slice CT, and is acquired with at least 80 mAs and CT scans are obtained with contiguous sections (with a maximum of 5 mm slice thickness), then the PET/CT scan alone will suffice. If no contrast is

used in the FDG-PET/CT, an additional CT scan with contrast is required of the neck, chest, abdomen, and pelvis, see Section 8.1.7.

9.0 MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria. For the purposes of this study, participants should be evaluated for response after cycle 4, and then re-evaluated every 12 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 4 weeks following initial documentation of an objective response. Participants with KS will be evaluated for response before odd numbered cycles (5, 7, 9, etc.). Scans will not be required for participants with KS unless clinically indicated. Response of KS to treatment is described in Section 9.2. In participants with HIV-cHL, F-FDG-PET/CT scans will be done at cycles 9 and 13 (weeks 17 and 25). At cycle 24 (week 49), a F-FDG-PET/CT scan will be required for participants who do not have two consecutive negative scans before this time point. Refer to Section 9.3 for the 2017 RECIL Criteria for evaluating measurable disease in HIV-cHL.

9.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be evaluated for response after completing four cycles of therapy and re-evaluated for response every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

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

For progression to visceral disease in participants with KS, see Section 9.2.4, Noncutaneous Progression.

9.1.1 Definitions

Evaluable for Toxicity: All participants will be evaluable for toxicity from the time of their first treatment with nivolumab and/or ipilimumab.

Evaluable for Objective Response: Only those participants 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 participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Participants 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.

9.1.2 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

by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with 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. Such lesions should be considered measurable only if evidence of disease progression has been observed in this site. Disease present only in a previously irradiated field should have biopsy confirmation.

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

Non-measurable Disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, 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 participant, these are preferred for selection as target lesions.

Target Lesions: All measurable lesions up to a maximum of two lesions per organ and five 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 five target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

9.1.3 Methods for Evaluation of Measurable Disease

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

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

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

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

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

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

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

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

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 CR or surgical resection is an endpoint.

Tumor Markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria, which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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 SD is mandatory to differentiate between response or SD (an effusion may be a side effect of the treatment) and PD.

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:

Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion. However, as this may not necessarily represent a new tumor lesion for individuals with ongoing HIV replication, new lesions on FDG-PET MUST be confirmed by CT scan to document PD.

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.

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.

9.1.4 Response Criteria

9.1.4.1 Evaluation of Target Lesions

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

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

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

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

9.1.4.2 Evaluation of Non-Target Lesions

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

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

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

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

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

9.1.4.3 Evaluation of Best Overall Response

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

Table 4: Response Evaluation for Participants with Measurable Disease (i.e., Target Disease)

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

Table 5: Response Evaluation for Participants with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

9.1.5 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 PD is objectively documented (taking as reference for PD 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 PD 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.

9.2 Evaluation of Response for Participants with Kaposi Sarcoma

Response and progression will be evaluated for participants with KS using the ACTG response criteria for KS. For the purposes of this study, participants should be re-evaluated for response every 4 weeks. See Appendix I for the KS Tumor Assessment schedule. Refer to the AMC KS Tumor Assessment MOP for details regarding tumor assessment and photographic documentation.

Evaluable for Toxicity: All participants will be evaluable for toxicity from the time of their first dose of study drug.

Evaluable for Objective Response: Only those participants who have measurable disease present at baseline and have received study drug will be considered evaluable for response.

Response status will be classified as complete response **(CR),** partial response **(PR)**, stable disease (**SD**), or progressive disease (**PD**).

9.2.1 Complete response (CR) is defined as the absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks. Participants in whom pigmented (brown or tan) macular skin lesions persist after apparent complete response, biopsy of at least one representative lesion is required in order to document the absence of malignant cells. Participants without visceral disease are not required to undergo radiographic measurements. In participants known to have had visceral disease, an assessment at restaging with appropriate endoscopic or radiographic procedures should be made.

9.2.2 Partial response (PR) is defined as no new lesions (skin or oral), or new visceral sites of involvement (or the appearance or worsening of tumor-associated edema or effusions); AND

- A 50% or greater decrease in the number of all previously existing lesions lasting for at least 4 weeks; OR
- Complete flattening of at least 50% of all previously raised lesions (i.e., 50% of all previously nodular or plaque-like lesion become macules); OR
- A 50% decrease in the sum of the products of the largest perpendicular diameters of the marker lesions

Note: Participants with residual tumor-associated edema or effusion who otherwise meet the criteria for complete response will be classified as having a partial response.

9.2.3 Stable disease (SD) is defined as any response not meeting the criteria for CR, PR, or PD.

9.2.4 Progressive disease (PD) is defined as follows:

For participants with ≤ 50 cutaneous lesions

- ≥ 25% increase in the sum of perpendicular diameters of the indicator lesions, OR
- ≥ 25% increase in the total lesion count, or a minimum of five new lesions, whichever is greater, OR
- ≥ 25% increase in the number of raised lesions (minimum of five new raised lesions if there are very few raised lesions, for example ≤ 8), whichever is greater.

Note: There are body sites where disease is particularly difficult to evaluate, and a few new lesions may be counted in spite of the fact that a participant is not actually progressing. For example, lesions of the foot, particularly those which are flat, are difficult to evaluate because their intensity may be variable based on how much edema is present, how much the person walked the day before, how long their feet have been in a dependent position prior to the physical exam, etc.

For participants with >50 cutaneous lesions

- 25% increase in the sum of the perpendicular diameters of the indicator lesions, OR

- 25% increase in the total number of lesions in the prospectively defined anatomic sites containing representative numbers of lesions, OR
- A total of five new lesions in anatomic sites which were previously documented as having no evidence of cutaneous disease on the whole-body diagram, OR
- 25% increase in the number of raised lesions (minimum of five raised lesions if there are very few raised lesions, for example <8) whichever is greater. Photographic documentation of "gross" or significant progression, particularly in areas that were not being followed, will be of particular value.

In order to classify a response as PR, the participant must have at least a PR in either the cutaneous or noncutaneous sites of disease, and no evidence of progression as defined in the above criteria. In order to classify a response as a CR, the participant must have a CR in both the cutaneous (if applicable) and noncutaneous (if applicable) sites of disease and no evidence of progression as defined by the above criteria.

Noncutaneous Progression

Progressive disease includes new visceral sites of involvement or progression of visceral disease or the development of new or increasing tumor-associated edema or effusion lasting at least 1 week, which interferes with the participant's normal activities. Progressive visceral disease, for measurable and evaluable disease, should be analogous to non-KS response criteria (RECIST; see Section 9.1).

9.2.5 Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.

9.2.6 Time to response is defined as time from the first dose of chemotherapy until documentation of first response.

9.2.7 Time to progression is defined as time from initiation of chemotherapy to documentation of first progression.

9.2.8 Response duration is defined as the time from first documentation of response to documentation of first progression.

9.3 Evaluation of Response for Participants with HIV-Associated Hodgkin Lymphoma

9.3.1 Definition of Response

The response definitions used for this study are the 2017 International Working Group Consensus RECIL 2017) [23]. This is similar to the Lugano criteria of 2014 where response is determined by a combination of Deauville score and CT scan criteria; however, only three lymph node targets are required in this evaluation criteria [23]. See table below.

9.3.2 Complete Response (CR)

1. Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.

2a. Typically FDG-avid lymphoma: In participants with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual

mass of any size is permitted as long as it is PET negative. PET negative is defined as a Deauville score of less than 4.

2b. Variably FDG-avid lymphomas/FDG-avidity unknown: In participants without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (1.0 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to 1.0 cm in their short axis after treatment.

3. The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.

4. If the bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry.

9.3.3 Partial Response (PR) requires all of the following

1. At least a less than or equal to a 30% decrease in sum of the product of the longest diameters (SLD) of up to three of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least two perpendicular dimensions; if possible, they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.

2. No increase should be observed in the size of other nodes, liver, or spleen.

3. Splenic and hepatic nodules must regress by 30% in their SLD or, for single nodules, in the greatest transverse diameter.

4. With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable, and no measurable disease should be present.

5. Participants who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders. When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, participants should be considered partial responders.

6. No new sites of disease should be observed.

7. Typically FDG-avid lymphoma: for a participant with no pretreatment PET

scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.

8. Variably FDG-avid lymphomas/FDG-avidity unknown: if a pretreatment PET scan was negative, CT criteria should be used.

9.3.4 Minor Response (MR)

Minor Response (MR) is defined as the following A participant is considered to have MR when he or she fails to attain the criteria needed for a CR or PR, less than or equal to10% decrease or less than or equal to 20% increase in the sum of longest diameters of the target lesions. Typically FGD-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.

9.3.5 Stable Disease (SD) is defined as the following A participant is considered to have SD when he or she fails to attain the criteria needed for a CR or PR, <10% decrease or less than or equal to 20% increase in the sum of longest diameters of target lesions. Typically FGD-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post- treatment CT or PET.

Variably FDG-avid lymphomas/FDG-avidity unknown: if the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

9.3.6 Relapsed Disease (RD) (after CR)/Progressive Disease (PD) (after PR, SD)

Lymph nodes should be considered abnormal if the long axis is more than 1.0 cm regardless of the short axis. >20% increase in the sum of longest diameters of target lesions. For small lymph nodes measuring <15mm post-therapy, a minimum absolute increase of 5mm and the long diameter should exceed 15mm or the appearance of a new lesion will enough define the participant with RD or PD.

If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0. Lymph nodes 1.0 x 1.0 cm will not be considered as abnormal for relapse or PD.

Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In participants with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

9.3.7 *Recurrent Disease* is defined as the appearance of tumor following documentation of a complete remission.

9.3.8 *Time to Response* is defined as time from the first dose of chemotherapy until documentation of first response.

9.3.9 *Time to Progression* is defined as time from initiation of chemotherapy to documentation of first progression.

9.3.10 *Response Duration* is defined as the time from first documentation of response to documentation of first progression.

Table 6. Response Categories Based on Assessment of Target Lesions

% Change in sum of diameters of target lesions from nadir					
	CR	**PR**	**MR**	**SD**	**PR**
% change from baseline	• Complete disappear-ance of all target le-sions and all nodes with long axis <10mm • ≥30% decrease in the sum of longest diameters of target lesions (PR) with normaliza-tion of FDG-PET	≥30% decrease in the sum of longest diameters of lesions but not a CR	≥10% decrease in the sum of longest diameters of target lesions but not a PR (<30 %)	<10% decrease or ≤20% increase in the sum or the longest diameters of target lesions	• >20% increase in the sum of longest diameters of target lesions • For small lymph nodes measuring <15mm post-therapy, a minimum absolute increase of 5mm and the long diameter should exceed 15mm • Appearance of a new lesion
FDG-PET	Normalization of FDG-PET (Deauville score 1-3)	Positive (Deauville score 4-5)	Any	Any	Any
Bone marrow involvement	Not involved	Any	Any	Any	Any
New lesions	No	No	No	No	Yes or No
CR, complete response; CT, computerized tomography; FDG-PET, [18F]2-fluoro-2-deoxy-D-glucose; MR, minor response; PD, progression of disease; PR, partial response; SD, stable disease.					

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

This is a phase I dose de-escalation trial of ipilimumab and nivolumab in advanced HIV-associated solid tumors implemented as a rolling six design that first establishes the MTD for single agent nivolumab and then the MTD for combination of ipilimumab and nivolumab. Since there are only two dose levels within each single and combination therapy, the design requires the full cohort of six participants be enrolled unless two or more experience a DLT. The design is stratified by lymphocyte T CD4+ count (above 200/mm^3 for Stratum 1 and 100-200/mm^3 for Stratum 2). Please see Section 4.2 and Section 4.3 of the Treatment Plan for the dose levels of ipilimumab and nivolumab, and Section 4.4 for the definition of DLT. After the combination therapy MTD has been determined, an expansion cohort in HIV-associated solid tumors will be enrolled. An expansion cohort in solid tumors, excluding those histologies not known to respond to single agent nivolumab (i.e. pancreas, prostate, MSS colorectal cancer) with CD4 counts > 200/mm^3 will also be enrolled and treated with single agent nivolumab. An additional cohort of participants with relapsed refractory cHL will be treated with single agent nivolumab. No intra-participant dose escalations will be allowed. Overall study duration including completion of evaluation of the final participant is projected to be 5 years.

10.1.1 Primary endpoints

Dose De-Escalation Strata: To demonstrate safety and feasibility of ipilimumab and nivolumab at the standard doses of drug in solid tumor participants with HIV infection given the possibility of increased toxicity based on immune activation, co-morbidity, or interference with HAART therapy. The purpose for this would be to provide appropriate experience and guidelines, if necessary, to allow participants with HIV infections to participate in ongoing trials.

10.1.2 Analysis of primary objective

Please see Section 4.2 of the Treatment Plan for the dose de-escalation decision rule. In summary, the primary goal in this phase I study is to determine the safety and feasibility of ipilimumab and nivolumab in combination therapy in participants with advanced HIV-associated solid tumors, and to determine the maximum tolerated dose (MTD) in this participant population. A rolling design will be used, where successive cohorts of two to six participants will be enrolled into specified dose levels. Dosing from nivolumab alone to nivolumab in combination with ipilimumab will occur according to the scheme in the treatment plan; the safety evaluation period is 6 weeks. The MTD for single agent or combination therapy is the starting dose level at which ≤1/6 participants experience DLT with the next higher dose having at least ≥2 participants encountering DLT (unless the MTD is the highest pre-specified dose level). DLTs for this protocol are defined in Section 4.4. Given the potential susceptibility of participants with CD4+ counts ≤200/mm^3, the dose de-escalation will initially occur in participants with counts >200/mm^3 (Stratum 1), and then participants in Stratum 2 (100-200/mm^3 CD4 counts) will be evaluated starting at the MTD determined in Stratum 1 after reviewing the safety profile for Stratum 1. For Stratum 2, participants will be enrolled starting at the Stratum 1 DLT in cohorts of 2-6 participants. Stratum 2 participants will not be

allowed to escalate beyond the MTD for Stratum 1; one dose level de-escalation will be allowed based on the criteria in the treatment plan.

If a DLT occurs in none or one participant in Stratum 1 at a particular dose level, a total of six participants will be enrolled to that cohort. If a second participant experiences a DLT, the MTD will have been exceeded. If 2 DLTs are experienced at the first cohort, one dose de-escalation (Dose Level -1) will be permitted according to the study schema in the treatment plan. Similarly, if 2 DLTs are experienced at dose level 2, a dose de-escalation (Dose Level -2) will be permitted. Thus, the MTD is the dose level at which ≤1/6 participants experience DLT with the next higher dose having ≥2 participants encountering DLT (unless the MTD is the highest pre-specified dose level) during the safety evaluation period.

To better estimate the preliminary efficacy of treatment, an expansion cohort of 12 participants will be treated at the MTD for single agent nivolumab or combination therapy with ipilimumab and nivolumab (depending on the results of the Phase I portion). If the MTDs differ between the two strata then there will be two expansion cohorts of 18 participants with a CD4 count >200 cells/mL and six participants with a CD4 count between 100 and 200 cell/mL. Also, to better estimate preliminary safety (secondary objective) in HIV infected individuals, a cohort of 24 participants with solid tumors (excluding histologies not known to respond to single agent nivolumab) and a cohort of 12 participants with cHL will be treated with single agent nivolumab.

All participants will be evaluable for toxicity from the time of nivolumab (which is administered first when there is concurrent treatment with ipilimumab). The details of toxicity grading and reporting may be found in Section 6.0. Toxicity data will be presented by type and severity for each dose group and overall; the incidence of toxicity related dose reductions and treatment discontinuations will be summarized.

10.2 Sample Size/Accrual Rate

For phase I, Stratum 1, a minimum of four to a maximum of 18 participants will be enrolled. For phase I, Stratum 2, a minimum of zero to a maximum of 18 participants will be enrolled. An expansion cohort of 24 participants will be treated at the combination therapy MTD, and an expansion cohort of 24 participants with solid tumors, excluding those histologies not known to respond to single agent nivolumab (i.e. pancreas, prostate, MSS colorectal cancer) will be treated with single agent nivolumab. Also, 12 participants with relapsed refractory cHL will be treated with single agent nivolumab. Therefore, in total, this study will enroll a minimum of four participants and a maximum of 96 participants.

Considering precision, 12 participants in the additional cHL cohort would yield an exact 95% confidence interval of 38% to 92% when the ORR is 70%, and 24 participants in the additional solid tumor cohort would yield an exact 95% confidence interval of 48% to 87%, such that these estimates would be considered preliminary.

Accrual will be approximately two participants per month for phase I, Stratum 1; approximately one participant per month for phase I, Stratum 2; and approximately 2-3 participants per month for each of the two solid tumor expansion cohorts. And, 3-4 participants per month for the cHL cohort.

10.3 Stratification Factors

Participants will be studied in separate strata. Stratum 1 will first enroll participants with lymphocyte T CD4+ count above 200/mm^3. After Stratum 1 single agent therapy completes, Stratum 2 will enroll participants with lymphocyte T CD4+ count between 100-200/mm^3. Stratum 2 will be evaluated starting at the single agent therapy MTD determined in Stratum 1, after reviewing the safety profile for Stratum 1. Stratum 2 participants will not be allowed to escalate beyond the MTD for Stratum 1; one dose level de-escalation will be allowed based on the doses in the schema in the treatment plan.

10.4 Analysis of Secondary Endpoints

10.4.1 Preliminary objective response rates

The proportion of participants achieving ORs (by RECIST criteria or KS response criteria, which includes RECIST for visceral disease, or by RECIL for cHL) and their corresponding 95% confidence intervals (calculated using exact Binomial) will be reported separately for solid tumor and cHL according to treatment (combination therapy and single agent) using response criteria designated in Section 9.0. Participants in the expansion cohort and those treated at any Dose Level in the dose de-escalation cohorts will be included. Disease-specific outcomes will be estimated for histologies with > 5 participants. For participants who fail therapy, the proportion with loco-regional vs. distant progression will be summarized. Descriptive statistics will also be compiled for DOR.

10.4.2 Immune function and other parameters

Descriptive statistics will be generated to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on immune function (HIV viral load, CD4 and CD8 cells).

Depending on the number of dose levels tested, immune status and HIV viral load will be summarized overall and according to dose level at pre-study and end of study. In an explorative fashion, change in these parameters from pre-study to the end of study will be examined using a nonparametric Wilcoxon signed-rank test.

Due to the small number of participants, no adjustments for multiple testing are planned.

10.5 Exploratory Objectives

All exploratory objectives will addressed in batch at the completion of enrollment/study as applicable. Some measures are only applicable to disease subsets.

10.5.1 Understand the immune response to agent in the context of ART, of altered immune function, and repertoire due to prior HIV infection

10.5.1.1 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab, on intratumor immune cells by IHC such as PD1, PDL-1, and others.

10.5.1.2 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on circulating cytokine markers by multiplex assay.

10.5.2 To understand the response of human tumor viruses (HPV, EBV, and KSHV) to agent

10.5.2.1 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus loads (EBV, KSHV, CMV) in plasma.

10.5.2.2 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent herpesvirus (EBV, KSHV, CMV) in PBMC.

10.5.2.3 To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on herpesvirus specific CD8 and CD4 T cells in PBMC.

10.5.2.4 In cases of KS, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on viral transcription in tumor biopsies.

10.5.2.5 In cases of anal cancer, to evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HPV types in anal swabs, when feasible.

10.5.3 Understand the response of HIV to agent

10.5.3.1. To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on latent HIV loads in PBMC using outgrowth assay.

10.5.3.2. To evaluate the effects of single agent nivolumab, and the combination of ipilimumab and nivolumab on HIV reactive T cells.

10.5.3.3. Descriptive statistics will be generated for the above measures. Changes from pre-study to end of study will be explored using nonparametric Wilcoxon signed-rank test.

11.0 ROLE OF DATA MANAGEMENT

11.1 CRF Instructions

Access to the internet data entry system for this study, AdvantageEDC^{SM}, and instructions for recording of study data on CRFs will be provided by the AMC ODMC at **www.AIDSCancer.org**. Participating institutions are responsible for submitting data and/or data forms via AdvantageEDC in accordance with the AMC Data Entry Guide and specific form instructions, within the timelines specified by the AMC's Standards of Procedure for Site Performance Measures.

11.2 Data Quality

It is the responsibility of the AMC ODMC to assure the quality of data for the study (See Appendix VI, AMC Data and Safety Monitoring Plan). This role extends from protocol development to generation of the final study database.

11.3 Data Monitoring

This study will be monitored in compliance with AMC policies and by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and participant-specific CDUS data will be submitted electronically to CTEP on a quarterly basis. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (**http://ctep.cancer.gov/reporting/cdus.html**).

The AMC ODMC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.0 ETHICAL AND REGULATORY CONSIDERATIONS

12.1 IRB Approval and Informed Consent

The principles of Institutional Review Board (IRB) approval and informed consent described in the Food and Drug Administration (FDA) regulations (21 CFR Part 50 and 56) and Department of Health and Human Services (DHHS) regulations for the Protection of Human Participants regulations (45 CFR Part 46) must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The sponsor's designee (AMC ODMC) must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before participant enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the AMC ODMC. The IRB must review the research project at least once every 365 days during the duration of the project. Continuing approval of the project must also be given in writing and provided to the AMC ODMC.

Records of all study review and approval documents must be kept on file by the Investigator and are participant to inspection during or after completion of the study. AEs must be reported to the IRB according to local procedures. The IRB should receive notification of completion of the study and final report within 3 months of study completion and termination. The Investigator will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

Written informed consent will be obtained from the participant. The nature, significance, and risks associated with the study must be explained to the participant. The informed consent will describe the purpose of the study, the procedures to be followed, the risks, and benefits of participation, all risks of the investigational agent(s) and/or study participation as listed in the model informed consent form, and all other elements of informed consent as required by regulation. A copy of the consent form will be given to the participant to keep.

In addition, any institution(s) conducting research according to the guidelines of this protocol is required to adhere to local and national laws and regulations governing the confidentiality and disclosure of health information.

12.2 Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by CTEP and the Investigator before implementation. All amendments require approval by the IRB/IEC of the treating institution. A copy of the written approval of the IRB/IEC must be sent to the ODMC.

12.3 Women and Minorities

This study is being conducted by the NCI-sponsored AIDS Malignancy Consortium (AMC). As part of their contractual obligations, each participating site within the AMC and the AMC as a whole is required to assure that the participation of women and minority participants reflects the percentage representation of these populations in their geographic region and, for the AMC, the United States as a whole. As such, it is expected that the

representation of participants on this trial will reflect the constitution of the respective populations.

Table 7: Accrual Targets

Ethnic Category	Sex/Gender				
	Females		**Males**		**Total**
Hispanic or Latino	9	+	24	=	33
Not Hispanic or Latino	23	+	40	=	63
Ethnic Category: Total of all participants	32	+	64	=	96
Racial Category					
American Indian or Alaskan Native	0	+	1	=	1
Asian	0	+	2	=	2
Black or African American	23	+	37	=	60
Native Hawaiian or other Pacific Islander	0	+	1	=	1
White	9	+	23	=	32
Racial Category: Total of all participants	**32**	**+**	**64**	**=**	**96**
	(A1 = A2)		(B1 = B2)		(C1 = C2)

13.0 REFERENCES

1. Rubinstein PG, Aboulafia DM, Zloza A. Malignancies in HIV/AIDS: from epidemiology to therapeutic challenges. AIDS. 2014 Feb;28(4):453-65.
2. Carbone A, Vaccher E, Gloghini A, Pantanowitz L, Abayomi A, de Paoli P, Franceschi S. Diagnosis and management of lymphomas and other cancers in HIV-infected patients. Nat Rev Clin Oncol. 2014;11(4):223.
3. Pantanowitz L, Schlecht HP, Dezube BJ. The growing problem of non-AIDS-defining malignancies in HIV. Curr Opin Oncol 2006; 18: 469-478.
4. Bower M, Collins S, Cottrill C, Cwynarski K, Montoto S, Nelson M, Nwokolo N, Powles T, Stebbing J, Wales N, Webb A, AIDS Malignancy Subcommittee. British HIV Association guidelines for HIV-associated malignancies 2008. HIV Med. 2008;9(6):336.
5. Deeken JF, Tjen-A-Looi A, Rudek MA, Okuliar C, Young M, Little RF, Dezube BJ. The rising challenge of non-AIDS-defining cancers in HIV-infected patients. Clin Infect Dis. 2012;55(9):1228.
6. Ramsdell F, Fowlkes BJ. Maintenance of in vivo tolerance by persistence of antigen. Science (1992) 257(5073):1130–410.1126/science.257.5073.1130.
7. Utzschneider DT, Legat A, Fuertes Marraco SA, Carrie L, Luescher I, Speiser DE, et al. T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. Nat Immunol (2013) 14(6):603–1010.1038/ni.2606.
8. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. Cell (2009) 138(1):30–5010.1016/j.cell.2009.06.036.
9. Critchlow CW, Surawicz CM, Holmes KK, Kuypers J, Daling JR, Hawes SE, et al. (1995). Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. AIDS 9:1255-1262.
10. Frisch M, Glimelius B, van den Brule AJ, Wohlfahrt J, Meijer CJ, Walboomers JM, Goldman S, Svensson C, Adami HO, Melbye M. Sexually transmitted infection as a cause of anal cancer. N Engl J Med 1997; 337: 1350-1358.
11. Palefsky JM, Holly EA, Ralston ML, Da Costa M, Bonner H, Jay N, et al. (2001b). Effect of highly active antiretroviral therapy on the natural history of anal squamous intraepithelial lesions and anal human papillomavirus infection. J Acquir Immune Defic Syndr 28:422-428.
12. Bedani PL, Risichella IS, Strumia R, Cavazzini L, Gilli P, Calastrini C, Verzola A, Stabellini G. Kaposi's sarcoma in renal transplant recipients: pathogenetic relation between the reduced density of Langerhans cells and cyclosporin-A therapy. J Nephrol. 1999 May-Jun;12(3):193-6. PubMed PMID:10440518.
13. Maurer T, Ponte M, Leslie K. HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. N Engl J Med. 2007 Sep 27;357(13):1352-3.
14. Bihl F, Berger C, Chisholm JV 3rd, Henry LM, Bertisch B, Trojan A, Nadal D, Speck RF, Flepp M, Brander C, Mueller NJ; Swiss HIV Cohort Study. Cellular immune responses

and disease control in acute AIDS-associated Kaposi's sarcoma. AIDS. 2009 Sep 10;23(14):1918-22. doi: 10.1097/QAD.0b013e3283300a91. PubMed PMID: 19609199.

15. Lepone L, Rappocciolo G, Knowlton E, Jais M, Piazza P, Jenkins FJ, Rinaldo CR. Monofunctional and polyfunctional CD8+ T cell responses to human herpes virus 8 lytic and latency proteins. Clin Vaccine Immunol. 2010 Oct;17(10):1507-16.

16. Skinnider BF, Elia AJ, Gascoyne RD, Patterson B, Trumper L, Kapp U, et al. Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood 2002,99:618-626.

17. Skinnider BF, Mak TW. The role of cytokines in classical Hodgkin lymphoma. Blood 2002:4283-4297.

18. Armand P. Immune checkpoint blockade in hematologic malignancies. Blood. 2015;125:3393-400.

19. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl JMed. 2015;372(4): 311-319.

20. Moskowitz CH, Ribrag V, Michot J-M, et al. PD-1 blockade with the monoclonal antibody pembrolizumab (MK-3475) in patients with classical Hodgkin lymphoma after brentuximab vedotin failure: preliminary results from a phase 1b study (KEYNOTE-013) [abstract]. Blood. 2014

21. Younes A, Santoro A, Shipp M, Zinzani PL, Timmerman JM, Ansell S et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol. 2016;17:1283-94.

22. Ansell S , Gutierrez ME , Shipp MA , Gladstone D , Moskowitz A, Borello I et al. A Phase 1 Study of Nivolumab in Combination with Ipilimumab for Relapsed or Refractory Hematologic Malignancies (CheckMate 039). Blood 2016 128:183.

23. Younes A, Hilden P, Coiffier B, Hagenbeek A, Salles G, Wilson W et al. International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). Ann Oncol. 2017 Apr 3. [Epub ahead of print].

24. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature (2006) 443(7109):350–410.1038/nature05115.

25. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med (2006) 12(10):1198–20210.1038/nm1106-1329b.

26. Zhang JY, Zhang Z, Wang X, Fu JL, Yao J, Jiao Y, et al. PD-1 up-regulation is correlated with HIV-specific memory CD8+ T-cell exhaustion in typical progressors but not in long-term nonprogressors. Blood (2007) 109(11):4671–810.1182/blood-2006-09-044826.

27. Maier H, Isogawa M, Freeman GJ, Chisari FV. PD-1:PD-L1 interactions contribute to the functional suppression of virus-specific CD8+ T lymphocytes in the liver. J Immunol (2007) 178(5):2714–20.

28. Golden-Mason L, Palmer B, Klarquist J, Mengshol JA, Castelblanco N, Rosen HR. Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. J Virol (2007) 81(17):9249–5810.1128/JVI.00409-07.

29. Zhang Z, Zhang JY, Wherry EJ, Jin B, Xu B, Zou ZS, et al. Dynamic programmed death 1 expression by virus-specific CD8 T cells correlates with the outcome of acute hepatitis B. Gastroenterology (2008) 134(7):1938–4910.1053/j.gastro.2008.03.037.

30. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. J Exp Med (2006) 203(10):2281–9210.1084/jem.20061496.

31. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat Immunol (2007) 8(11):1246–5410.1038/ni1515.

32. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007;117: 1137-1146.

33. Drake CG, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. Adv Immunol. 2006;90:51-81.

34. Investigator Brochure. (2014). Nivolumab, BMS-936558, MDX1106. Version 13, 21 July 2014. Bristol-Myers Squibb. Lawrenceville, NJ.

35. Wolchok, J.D., H. Kluger, M.K. Callahan, et al. (2013). Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 369:122-133.

36. Woo, S.R., M.E. Turnis, M.V. Goldberg, et al. (2012). Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res. 72:917-927.

37. Taube, J.M., R.A. Anders, G.D. Young, et al. (2012). Colocalization of inflammatory response with B7-H1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 4:127ra137.

38. Thompson, R.H., M.D. Gillett, J.C. Cheville, et al. (2004). Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. 101:17174-17179.

39. Thompson, R.H., M.D. Gillett, J.C. Cheville, et al. (2005). Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. Cancer. 104:2084-2091.

40. Thompson, R.H., S.M. Kuntz, B.C. Leibovich, et al. (2006). Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res. 66:3381-3385.

41. Ohigashi, Y., M. Sho, Y. Yamada, et al. (2005). Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res. 11:2947-2953.

42. Wu, C., Y. Zhu, J. Jiang, et al. (2006). Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem. 108:19-24.

43. Dong, H., and L. Chen. (2003). B7-H1 pathway and its role in the evasion of tumor immunity. J Mol Med (Berl). 81:281-287.

44. Nomi, T., M. Sho, T. Akahori, et al. (2007). Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 13:2151-2157.

45. Zitvogel, L., A. Tesniere, and G. Kroemer. (2006). Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol. 6:715-727.

46. Sznol, M., H.M. Kluger, F.S. Hodi, et al. (2013). Survival and long-term follow-up of safety and response in patients (pts) with advanced melanoma (MEL) in a phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538). J Clin Oncol. 31:ACRA9006.

47. Brahmer, J.R., L. Horn, S.J. Antonia, et al. (2013). Survival and long-term follow-up of the phase I trial of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with previously treated advanced non-small cell lung cancer (NSCLC). J Clin Oncol. 31:A8030.

48. Drake, C.G., D.F. McDermott, M. Sznol, et al. (2013). Survival, safety, and response duration results of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in a phase I trial in patients with previously treated metastatic renal cell carcinoma (mRCC): Long-term patient follow-up. J Clin Oncol. 31:A4514.

49. Rizvi, N.A., S.J. Antonia, L.Q.M. Chow, et al. (2013). A phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive non-small cell lung cancer (NSCLC) patients (pts). J Clin Oncol. 31:A8072.

50. Topalian, S.L., F.S. Hodi, J.R. Brahmer, et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 366:2443-2454.

51. Ipilimumab Investigator Brochure. (2014). BMS-734016, MDX010. Version 17, 10 March 2014. Bristol-Myers Squibb. Wallingford, CT.

52. Curran, MA, Montalvo W, Yagita H, et al. (2010). PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A. 107: 4275-80.

53. Hammers, HJ, Plimack ER, Infante JR, et al. (2014). Phase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (mRCC). J Clin Oncol. 32:A4504.

54. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. Nov 1995;3(5):541-547.

55. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science. Nov 10 1995;270(5238):985-988.

56. Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. Immunity. Dec 1997;7(6):885 895.

57. Tchekmedyian S, Glaspy J, Korman A, et al. MDX-010 (human anti-CTLA4): a phase I trial in malignant melanoma. In: Programs and abstracts of The American Society of Clinical Oncology Annual Meeting. Orlando, Florida, May 18–21, 2002 [abstract: 56].

58. Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A 2003;100:8372–7.

59. Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 2010;11:155–64.

60. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.

61. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364:2517–26.

62. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000; 192:1027-1034.

63. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99:12293-12297

64. Parekh VV, Lalani S, Kim S, Halder R, Azuma M, Yagita H, et al. PD-1/PD-L blockade prevents anergy induction and enhances the anti-tumor activities of glycolipid- activated invariant NKT cells. J Immunol. 2009;182:2816-2826.

65. Talay O, Shen CH, Chen L, Chen J. B7-H1 (PD-L1) on T cells is required for T-cell-mediated conditioning of dendritic cell maturation. Proc Natl Acad Sci USA. 2009;106:2741-2746.

66. Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3þCD4þ regulatory T cells. Proc Natl Acad Sci U S A. 2008;105:9331-9336.

67. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005;65:1089-1096.

68. Azuma T, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. Blood. 2008; 111:3635.

69. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Ann Rev Immunol.2005;23:515.

70. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. 2006;27:195-201.

71. Velu, V., K. Titanji, B. Zhu, S. Husain, A. Pladevega, L. Lai, T. H. Vanderford, L. Chennareddi, G. Silvestri, G. J. Freeman, et al. 2009. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature 458: 206–210.

72. Boasso, A., M. Vaccari, A. Hryniewicz, D. Fuchs, J. Nacsa, V. Cecchinato, J. Andersson, G. Franchini, G. M. Shearer, and C. Chougnet. 2007. Regulatory T-cell markers, indoleamine 2,3-dioxygenase, and virus levels in spleen and gut during progressive simian immunodeficiency virus infection. J. Virol. 81: 11593–11603.

73. Brahmer JR, Drake CG, Wollner I, et al: Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 28:3167-3175, 2010

74. Topalian SL, Hodi FS, Brahmer JR, et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366:2443-2453, 2012.

75. Sznol, M., H.M. Kluger, F.S. Hodi, et al. (2013). Survival and long-term follow-up of safety and response in patients (pts) with advanced melanoma (MEL) in a phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538). J Clin Oncol. 31:ACRA9006

76. Drake, C.G., D.F. McDermott, M. Sznol, et al. (2013). Survival, safety, and response duration results of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in a phase I trial in patients with previously treated metastatic renal cell carcinoma (mRCC): Long-term patient follow-up. J Clin Oncol. 31:A4514.

77. Rizvi, N.A., S.J. Antonia, L.Q.M. Chow, et al. (2013). A phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive non-small cell lung cancer (NSCLC) patients (pts). J Clin Oncol. 31:A8072.

78. Topalian, S.L., F.S. Hodi, J.R. Brahmer, et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 366:2443-2454.

79. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.

80. Wolchok JD, Kluger H, Callahan MK et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013 Jul 11;369(2):122-33. doi: 10.1056/NEJMoa1302369. Epub 2013 Jun 2.

81. Hammers HJ., Plimack ER, Infante JR., Ernstoff MS et al. Clin Oncol 32:5s, 2014 (suppl; abstr 4504) Phase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (mRCC).

82. Rizvi NA, Gettinger SN, Goldman MD, et al. Safety and Efficacy of First-Line Nivolumab (NIVO; Anti-Programmed Death-1 [PD-1]) and Ipilimumab in Non-Small Cell Lung Cancer (NSCLC). Presented at: 16th World Conference on Lung Cancer; September 6-9; Denver, CO. Abstract 786.

83. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature (2006) 443(7109):350–410.1038/nature05115

84. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat Med (2006) 12(10):1198–20210.1038/nm1106-1329b [

85. Zhang JY, Zhang Z, Wang X, Fu JL, Yao J, Jiao Y, et al. PD-1 up-regulation is correlated with HIV-specific memory CD8+ T-cell exhaustion in typical progressors but not in long-term nonprogressors. Blood (2007) 109(11):4671–810.1182/blood-2006-09-044826

86. D'Souza M, Fontenot AP, Mack DG, Lozupone C, Dillon S, Meditz A, et al. Programmed death 1 expression on HIV-specific CD4+ T cells is driven by viral replication and associated with T cell dysfunction. J Immunol (2007) 179(3):1979–87

87. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, Miura T, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat Immunol (2007) 8(11):1246–5410.1038/ni1515

88. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol (2009) 10(1):29–3710.1038/ni.1679

89. Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, Long BR, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. J Exp Med (2008) 205(12):2763–7910.1084/jem.20081398

90. Jin HT, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, et al. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. Proc Natl Acad Sci U S A (2010) 107(33):14733–810.1073/pnas.1009731107

91. Yamamoto T, Price DA, Casazza JP, Ferrari G, Nason M, Chattopadhyay PK, et al. Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. Blood (2011) 117(18):4805–1510.1182/blood-2010-11-317297

92. Nakamoto N, Cho H, Shaked A, Olthoff K, Valiga ME, Kaminski M, et al. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. PLoS Pathog (2009) 5(2):e1000313.10.1371/journal.ppat.1000313

93. Manches O, Munn D, Fallahi A, Lifson J, Chaperot L, Plumas J, et al. HIV-activated human plasmacytoid DCs induce Tregs through an indoleamine 2,3-dioxygenase-dependent mechanism. J Clin Invest (2008) 118(10):3431–910.1172/JCI34823

94. Moreno-Fernandez ME, Zapata W, Blackard JT, Franchini G, Chougnet CA. Human regulatory T cells are targets for human immunodeficiency virus (HIV) infection, and their susceptibility differs depending on the HIV type 1 strain. J Virol (2009) 83(24):12925–3310.1128/JVI.01352-09 [PMC free article] [PubMed] [Cross Ref]

95. Jiang Q, Zhang L, Wang R, Jeffrey J, Washburn ML, Brouwer D, et al. FoxP3+CD4+ regulatory T cells play an important role in acute HIV-1 infection in humanized Rag2-/-gammaC-/- mice in vivo. Blood (2008) 112(7):2858–6810.1182/blood-2008-03-145946

96. Nilsson J, Boasso A, Velilla PA, Zhang R, Vaccari M, Franchini G, et al. HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS. Blood (2006) 108(12):3808–1710.1182/blood-2006-05-021576

97. Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, et al. CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med (2004) 200(3):331–4310.1084/jem.20032069

98. Weiss L, Donkova-Petrini V, Caccavelli L, Balbo M, Carbonneil C, Levy Y. Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV- infected patients. Blood (2004) 104(10):3249–5610.1182/blood-2004-01-0365

99. Porichis F, Hart MG, Zupkosky J, Barblu L, Kwon DS, McMullen A, Brennan T, Ahmed R, Freeman GJ, Kavanagh DG, Kaufmann DE: Differential impact of PD-1 and/or IL-10 blockade on HIV-1- specific CD4 T cell and antigen-presenting cell functions. J Virol 2013, 88:2508-18.

100. Trautmann L, Janbazian L, Chomont N, et al. Upregulation of PD-1 expression on HIV-specific CD8 + T cells leads to reversible immune dysfunction. Nat Med Oct; 2006 12(10):1198–1202. [PubMed:16917489]

101. Hryniewicz A, Boasso A, Edghill-Smith Y, Vaccari M, Fuchs D, Venzon D, Nacsa J, Betts MR, Tsai WP, Heraud JM, Beer B, Blanset D, Chougnet C, Lowy I, Shearer GM, Franchini G: CTLA-4 blockade decreases TGF-beta, IDO, and viral RNA expression in tissues of SIVmac251-infected macaques. Blood 2006, 108: 3834-42.

102. Velu V, Titanji K, Zhu B, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature Mar 12;2009 458(7235):206–210. [PubMed: 19078956]

103. Berger R, Roten-Yehudar R, Slama G, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res May;2008 14(10):3044–3051. [PubMed: 18483370]

104. Franceschini D, Paroli M, Francavilla V, et al. PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. J Clin Invest Mar; 2009 119(3):551–564. [PubMed: 19229109]

105. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. 2006;27:195-201.

106. Hryniewicz, A., A. Boasso, Y. Edghill-Smith, M. Vaccari, D. Fuchs, D. Venzon, J. Nacsa, M. R. Betts, W. P. Tsai, J. M. Heraud, et al. 2006. CTLA-4 blockade decreases TGF-_, indoleamine 2,3- dioxygenase, and viral RNA expression in tissues of SIVmac251-infected macaques. Blood 108: 3834–3842.

107. Cecchinato, V., E. Tryniszewska, Z. M. Ma, M. Vaccari, A. Boasso, W. P. Tsai, C. Petrovas, D. Fuchs, J. M. Heraud, D. Venzon, et al. 2008. Immune activation driven by CTLA-4 blockade augments viral replication at mucosal sites in simian immunodeficiency virus infection. J. Immunol. 180: 5439–5447.

108. Vaccari M, Boasso A, Fenizia C, Fuchs D, Hryniewicz A, Morgan T, Weiss D, Doster MN, Heraud JM, Shearer GM, Franchini G. Fatal pancreatitis in simian immunodeficiency virus SIV(mac251)-infected macaques treated with 2',3'-dideoxyinosine and stavudine following cytotoxic-T-lymphocyte-associated antigen 4 and indoleamine 2,3-dioxygenase blockade. J Virol. 2012 Jan;86(1):108-13. doi: 10.1128/JVI.05609-11. Epub 2011 Oct 19. PubMed PMID:22013040; PubMed Central PMCID: PMC3255892.

109. Gardiner D, Lalezari J, Lawitz E, DiMicco M, Ghalib R, Reddy KR, Chang KM, Sulkowski M, Marro SO, Anderson J, He B, Kansra V, McPhee F, Wind-Rotolo M, Grasela D, Selby M, Korman AJ, Lowy I. A randomized, double-blind, placebo-controlled assessment of BMS-936558, a fully human monoclonal antibody to programmed death-1 (PD-1), in patients with chronic hepatitis C virus infection. PLoS One. 2013 May 22;8(5):e63818. doi: 10.1371/journal.pone.0063818. Print 2013. PubMed PMID: 23717490; PubMed Central PMCID: PMC3661719.

110. Burke MM, Kluger HM, Golden M, Heller KN, Hoos A, Sznol M. Case Report: Response to Ipilimumab in a Patient With HIV With Metastatic Melanoma J Clin Oncol. 2011 Nov 10;29(32):e792-4. doi: 10.1200/JCO.2011.36.9199. Epub 2011 Oct 11

111. Zang X, et al. (Tumor associated endothelial expression of B7-H3 predicts survival in ovarian carcinomas. (Translated from eng) Mod Pathol 23(8):1104-1112 (in eng).

112. Zang X, et al. (2007) B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. Proc Natl Acad Sci U S A 104(49):19458-19463.

113. Wei L, et al. (Identification and testing of reference genes for Sesame gene expression analysis by quantitative real-time PCR. Planta 237(3):873-889.

114. Zhao R & al. e (2013) HHLA2 is a member of the B7 family and inhibits human CD4 and CD8 T cell function. Proc Natl Acad Sci U S A 2013 Jun 11;110(24):9879-84

115. Loi S, et al. (Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 31(7):860-867

116. Eggena MP, Barugahare B, Jones N, Okello M, Mutalya S, Kityo C, Mugyenyi P, Cao H. Depletion of regulatory T cells in HIV infection is associated with immune activation. Journal of immunology. 2005;174(7):4407-14. PubMed PMID: 15778406.

117. Ndhlovu LC, Loo CP, Spotts G, Nixon DF, Hecht FM. FOXP3 expressing CD127lo CD4+ T cells inversely correlate with CD38+ CD8+ T cell activation levels in primary HIV-1 infection. Journal of leukocyte biology. 2008;83(2):254-62. doi: 10.1189/jlb.0507281. PubMed PMID: 17982112.

118. Kinter A, McNally J, Riggin L, Jackson R, Roby G, Fauci AS. Suppression of HIV-specific T cell activity by lymph node CD25+ regulatory T cells from HIV-infected individuals. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(9):3390-5. doi: 10.1073/pnas.0611423104. PubMed PMID: 17360656 PMCID: PMC1805624.

119. Angin M, King M, Altfeld M, Walker BD, Wucherpfennig KW, Addo MM. Identification of HIV-1-specific regulatory T-cells using HLA class II tetramers. Aids. 2012;26(16):2112-5. doi: 10.1097/QAD.0b013e328358cc75. PubMed PMID: 22874519 PMCID: PMC3825094.

120. Macatangay BJ, Szajnik ME, Whiteside TL, Riddler SA, Rinaldo CR. Regulatory T cell suppression of Gag-specific CD8 T cell polyfunctional response after therapeutic vaccination of HIV-1-infected patients on ART. PloS one. 2010;5(3):e9852. doi: 10.1371/journal.pone.0009852. PubMed PMID: 20352042 PMCID: PMC2844424.

121. Chen X, Fosco D, Kline DE, Meng L, Nishi S, Savage PA, Kline J. PD-1 regulates extrathymic regulatory T-cell differentiation. European journal of immunology. 2014. doi: 10.1002/eji.201344423. PubMed PMID: 2497512.

122. Radziewicz H, Dunham RM, Grakoui A. PD-1 tempers Tregs in chronic HCV infection. The Journal of clinical investigation. 2009;119(3):450-3. PubMed PMID: 19306502 PMCID: PMC2648682.

APPENDIX I: SCHEDULES OF EVALUATIONS

Schedule of Evaluations

Tests and Observations	Pre-Study Visit -1	Cycles 1-4					Cycles 5-46, Day 1		Off Study[k]
		C1 D1	C1 D8	C2 D1	C3 D1	C4 D1	Odd cycles (5, 7, 9 etc)	Even cycles (6, 8, 10 etc)	
Informed consent	X								
Medical History, Physical Exam, PPD[1], Vital Signs, Weight, Performance Status[b,c]	X	X	X	X	X	X	X	X	X
Height	X								
AE Assessment[i]	X	X	X	X	X	X	X	X	X
Concurrent meds	X							All visits	
EKG[7]	X								
Laboratory Studies [b]									
CBC w/diff, plts [a, d]	X	X	X	X	X	X	X	X	X
Serum chemistry [e]	X	X	X	X	X	X	X		X
Amylase/Lipase	X	X	X	X	X	X	X		X
HBV, HCV[f]	X						Before Cycle 5 and then every 12 weeks		X
Serum or Urine Pregnancy Test[e]	X	X		X	X	X	Before Cycle 5 and then every 12 weeks		X
HIV viral load[f] and CD4/CD8 count	X						Before Cycle 5 and then every 12 weeks		X
Immunologic Labs[g]	X						Before Cycle 5 and then every 12 weeks		X
Endocrine Labs[h]	X						Before Cycle 5 and then every 12 weeks		X
Radiologic Evaluation/ Response Assessment[j]	X						Before Cycle 5 and then every 12 weeks		X
KS Tumor Assessment[l]	X				X		X		X
ACSR Donation (optional with consent)	X							See Appendix IV	

Tests and Observations	Pre-Study Visit -1	Cycles 1-4					Cycles 5-46, Day 1		Off Study[k]	
		C1 D1	C1 D8	C2 D1	C3 D1	C4 D1	Odd cycles (5, 7, 9 etc)	Even cycles (6, 8, 10 etc)		
Additional Evaluations for cHL Cohort Participants ONLY										
PET/CT	X						At Cycle 9 (week 17) and Cycle 13 (week 25)			
F-FDG							At Cycle 24 (week 49)			
Bone marrow biopsy[m]	X									X

Therapeutic Parameters

1. Prestudy PPD testing (only if known to be negative) is only required within the last 12 weeks before enrollment. The Quantiferon assay is an acceptable alternative to PPD testing. If PPD positive, prophylaxis must be complete before enrollment.
2. Prestudy scans and x-rays used to assess all measurable or non-measurable sites of disease must be done ≤ 4 weeks prior to registration.
3. Prestudy CBC (with differential and platelet count) should be done ≤ 2 weeks prior to registration.
4. All required prestudy chemistries, as outlined in Section 8.1.5, should be done ≤ 2 weeks prior to registration – unless specifically required within 48 hours before starting treatment as per protocol.
5. Baseline Hepatitis B and C testing and viral load studies (HIV, if indicated Hepatitis B and C studies) should be done ≤ 2 weeks prior to registration.
6. See Table below regarding biologic sample submissions.
7. EKG. EKG and echocardiogram (ECHO) for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated. For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, echocardiogram.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

a. CBCs (with differential and platelet count) which includes WBC, ANC, platelets, Hgb, and Hct required for protocol therapy must be done within 2 weeks of enrollment for eligibility, and < 48 hours prior to the treatment cycle. See 8.2 for instructions on performing these evaluations once prior to eligibility and treatment.

b. All study procedures including, blood samples collected for pretreatment laboratory tests may be collected and analyzed no more than 2 days prior to dosing. Chemistry results must be reviewed and confirm that participant's LFTs and other safety labs still meet inclusion criteria prior to administration of nivolumab dose. Baseline pregnancy exam must be performed within 72 hours of beginning nivolumab dosing and determined to be negative. They should be seen and evaluated more often if clinically indicated for the management of toxicities, at the discretion of the treating physician investigator. Hormonal studies and immunologic labs are required for monitoring at the specified time points and as clinically indicated. The results of these tests (hormonal studies and immunologic labs) are not required for dosing unless there are clinical indications and/or associated AEs as described under Section 5.0 and Appendix X, Dose and Schedule Modifications for ipilimumab and nivolumab.

c. During C1-4 on days when a participant will receive a nivolumab and an ipilimumab infusion, vital signs will be obtained before the nivolumab infusion and every 15 minutes until 60 minutes post-completion of the ipilimumab infusion. On days when a participant will only receive a nivolumab infusion, vital signs will be obtained before the BMS nivolumab infusion, every 15 minutes during the infusion, and at 15, 30, 45, and 60 minutes post-infusion. When slowing or restarting an infusion due to an infusion reaction, vital signs should be monitored every 15 minutes or as directed by the Investigator until the infusion is completed and/or the participant is stabilized. If a participant has an infusion reaction with nivolumab, the ipilimumab infusion can be given (without prophylactic medications) if the infusion reaction resolves within 3 hours. For scheduling purposes after a nivolumab infusion reaction, the ipilimumab infusion may be given the next day. Prophylactic preinfusion medications should be given prior to all subsequent nivolumab. For subsequent infusions, vital signs should be collected prior to dosing. For subsequent infusions, vital signs should be collected prior to dosing, every 30 minutes (-/+ 10 minutes) during dosing, and 1 hour post-dosing. For cycles 5 and beyond, for participants receiving nivolumab alone, who have never had a documented infusion reaction, pre and post-dosing vital signs may be obtained.

d. Hematology labs to include hemoglobin, hematocrit, red blood cell count, white blood cell count, platelets (direct platelet count), as well as total and differential CBC counts. These labs must be done and reviewed before ipilimumab and nivolumab infusion. These labs are required to be done throughout follow-up regardless if the participant goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed.

e. Serum chemistry laboratory analysis includes albumin, urea or BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, HCO3 (CO2; venous blood), calcium, phosphorous. These labs must be done 2 weeks before enrollment for eligibility purposes, and within 48 hours before drug infusion (See 8.2). These labs are required to be done throughout follow-up regardless if the participant goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 4 weeks prior to registration. Serum or urine pregnancy test must be done within 72 hours prior to Day 1 of cycles 1-5 and then every 12 weeks thereafter.

f. At screening, testing should be performed for HIV antibody (refer to eligibility criteria 3.1.2), hepatitis C antibody, and Hepatitis B

antigen utilizing local standard informed consent procedures prior to this laboratory collection. Viral load for HIV and if positive for Hepatitis C/Hepatitis B should repeated prior to cycle 5 and every 12 weeks thereafter till participant off study. Refer to eligibility criteria at 3.1.12 and 3.1.13.

g. The following immunologic labs are to be done at baseline (within 4 weeks of starting the study drug) prior to cycle 5 and then every 12 weeks: rheumatoid factor, C-reactive protein and ANA, SPEP, quantitative immunoglobulins and FLC. These labs must be repeated prior to cycle 5 and then every 12 weeks after that until the participant goes off study.
h. Endocrine labs: To be done at the indicated visits. These include TSH, free T4, TPO, morning ACTH, morning cortisol if ACTH is abnormal.
i. All AEs must be collected whether they occur on treatment or non-treatment weeks and must be submitted utilizing the corresponding AE Forms, covering all time periods specified on the forms. AE assessment must include assessment of immune-mediated AEs.
j. Scans will be performed at baseline, before cycle 5, and thereafter every 12 weeks. It is critical that all imaging studies or CT scans be performed in an identical way to the baseline scan with the same scanner, same scan direction, and consistent arm pointing. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response. If tumor flare is suspected, the participant must undergo a second scan within a month of the first scan and the case must be discussed with the principal investigator. If progression is documented at that time, participant must come off study. If a participant has a history of brain metastasis, a brain MRI at baseline is required. Participants with KS will be evaluated for response before odd numbered cycles (5, 7, 9, etc.). Scans will not be required for participants with KS unless clinically indicated.

   For participants with relapsed refractory HIV-cHL, F-FDG-PET /CT scans will be done at baseline, and weeks 17 and 25. At week 49, an F-FDG-PET/CT scan is required for patients who did not have two consecutive negative scans before this time point. If F-FDG-PET/CT scans are done without contrast, a full scan with and without contrast will be required unless there is an allergy to IV contrast. CT with and without contrast will follow the same schedule as stated above. For patients with bone marrow involvement at screening, a bone marrow biopsy was required to confirm complete remission.
k. Participants with ongoing toxicities should be seen more often as clinically indicated. AEs Assessment on the study will continue for all participants until 90 days after the last study drug administration. End of Study Assessment will be done within 6 weeks (±1 week) and at 16 weeks (± 1 week) of the last dose of study treatment.
l. For participants with KS, tumor assessments with photographic record must be performed within 7 days prior to initiating treatment. Tumor assessments and photographs should be performed as outlined in the AMC Kaposi's Sarcoma (KS) Tumor Assessment MOP. KS tumor assessment will occur every 4 weeks during study participation.
m. For participants with classical HL who had bone marrow involvement at baseline only.

Biological Sample Submissions for Correlative Studies

Specimens are to be submitted as outlined in Section 8. All samples will be submitted to the AMC Biorepository unless noted otherwise. All blood tubes are to be filled completely.

	Visit -1	C1 Pre D1 Post D1 D8	C4 Pre D1 Post D1 D8	Pre C5 D1	C7 and Pre D1 Post D1 D8	C10 Pre D1 Post D1 D8	C13 Pre D1 Post D1 D8	C16 D1	C19, C25, C31, C37, and C43 Pre D1 Post D1 D8	C22, C28, C34, C40, and C46 Pre D1 Post D1 D8	After Completion of Therapy/ Off Study[9]
MANDATORY											
Two 6 mL red top tubes	X			X							X
Twelve 8.5 mL ACD tubes[1,3] OR two 8.5 mL ACD tubes[1] Ship to AMC Biorepository	X							X			X
Five 8.5 mL ACD tubes[2, 3, 7] Ship to AMC Biorepository		X	X[10]		X	X[10]	X		X	X[10]	
Four 4 micron thick unstained slides from initial biopsy[6,8]	X										
ADDITIONAL BIOPSIES DEPENDING ON TUMOR TYPE Submit from participants who answer *"Yes"* to *"I agree to participate in the laboratory research studies that are being done as part of this clinical trial."*											
Tumor Tissue Biopsy if participants have KS[4] OR Anal swab for participants with anal cancer[5]	X			X							X

1. If consenting to participate in the HIV latent reservoirs study and hematocrit ≥ 25. To be shipped unprocessed. If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 yellow top tubes will be collected for herpesvirus load and latent virus studies (Appendix XIV and Appendix XVI).
2. To be processed on site for viable PBMC and plasma within 6 hours of collection. If processing cannot be completed on site, specimens should be shipped via priority overnight service.

3. Only to be completed for participants with hematocrit ≥ 25.
4. Two 2x2 mm biopsies in RNALater and formalin as described in Appendix XIV. The AMC Genomics Core Laboratory will provide kits for 2x2 punch biopsy collection and shipping samples.
5. Anal swabs as described in Appendix XVII.
6. Representative tumor tissue from the initial diagnostic biopsies or repeat diagnostic biopsy prior to initiating treatment and related pathology reports are to be submitted within one (1) month following registration or collection as outlined in Appendix XIV.
7. Collect within 2 days prior to day 1 of each cycle for pre-dose samples, and on day 1 for post-dose samples. The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days, and should be collected at least 12 hours apart. All samples on day 8 of a given cycle may be collected up to 3 days prior to day 8.
8. A minimum of four unstained sections (or three unstained sections plus one H&E), adhered to positively charged slides, and cut at a thickness of four microns are preferred for testing. Slides should be stored refrigerated if possible.
9. Samples are to be collected within 6 weeks (± 1 week) of the last dose of study treatment only.
10. For dose levels 1 (including 240 mg q 2 weeks) and 2 only.

Schedule of Evaluations for participants on combination therapy solid tumor expansion cohort

Tests and Observations	Pre-Study Visit -1	Cycles 1-4					Cycles 5-13, Day 1		Cycles 14-22	Off Study[k]
		C1 D1	C1 D8	C2 D1	C3 D1	C4 D1	Odd cycles (5, 7, 9, 11, 13)	Even cycles (6, 8, 10, 12)		
Informed consent	X									
Medical History, Physical Exam, PPD[1], Vital Signs, Weight, Performance Status[b,c]	X	X	X	X	X	X	X	X	X	X
Height	X									
AE Assessment[i]	X	X	X	X	X	X	X	X	X	X
Concurrent meds	X							All visits		
EKG[7]	X									
Laboratory Studies [b]										
CBC w/diff, plts [a, d]	X	X	X	X	X	X	X	X	X	X
Serum chemistry [e]	X	X	X	X	X	X	X		X	X
Amylase/Lipase	X	X	X	X	X	X	X		X	X
HBV, HCV[f]	X						Before Cycle 5 and then every 12 weeks			X
Serum or Urine Pregnancy Test[e]	X	X		X	X	X	Before Cycle 5 and then every 12 weeks			X
HIV viral load[f] and CD4/CD8 count	X						Before Cycle 5 and then every 12 weeks			X
Immunologic Labs[g]	X						Before Cycle 5 and then every 12 weeks			X
Endocrine Labs[h]	X						Before Cycle 5 and then every 12 weeks			X
Radiologic Evaluation/ Response Assessment[j]	X						Before Cycle 5 and then every 12 weeks			X
KS Tumor Assessment[l]	X				X		X		X	X
ACSR Donation (optional with consent)	X							See Appendix IV		

Therapeutic Parameters

1. Prestudy PPD testing (only if known to be negative) is only required within the last 12 weeks before enrollment. The Quantiferon assay is an acceptable alternative to PPD testing. If PPD positive, prophylaxis must be complete before enrollment.
2. Prestudy scans and x-rays used to assess all measurable or non-measurable sites of disease must be done ≤ 4 weeks prior to registration.
3. Prestudy CBC (with differential and platelet count) should be done ≤ 2 weeks prior to registration.
4. All required prestudy chemistries, as outlined in Section 8.1.5, should be done ≤ 2 weeks prior to registration – unless specifically required within 48 hours before starting treatment as per protocol.
5. Baseline Hepatitis B and C testing and viral load studies (HIV, if indicated Hepatitis B and C studies) should be done ≤ 2 weeks prior to registration.
6. See Table below regarding biologic sample submissions.
7. EKG. EKG and echocardiogram (ECHO) for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated. For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, echocardiogram.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

a. CBCs (with differential and platelet count) which includes WBC, ANC, platelets, Hgb, and Hct required for protocol therapy must be done within 2 weeks of enrollment for eligibility, and < 48 hours prior to the treatment cycle. See 8.2 for instructions on performing these evaluations once prior to eligibility and treatment.

b. All study procedures including, blood samples collected for pretreatment laboratory tests may be collected and analyzed no more than 2 days prior to dosing. Chemistry results must be reviewed and confirm that participant's LFTs and other safety labs still meet inclusion criteria prior to administration of nivolumab dose. Baseline pregnancy exam must be performed within 72 hours of beginning nivolumab dosing and determined to be negative. They should be seen and evaluated more often if clinically indicated for the management of toxicities, at the discretion of the treating physician investigator. Hormonal studies and immunologic labs are required for monitoring at the specified time points and as clinically indicated. The results of these tests (hormonal studies and immunologic labs) are not required for dosing unless there are clinical indications and/or associated AEs as described under Section 5.0 and Appendix X, Dose and Schedule Modifications for ipilimumab and nivolumab.

c. During C1-4 on days when a participant will receive a nivolumab and an ipilimumab infusion, vital signs will be obtained before the nivolumab infusion and every 15 minutes until 60 minutes post-completion of the ipilimumab infusion. On days when a participant will only receive a nivolumab infusion, vital signs will be obtained before the BMS nivolumab infusion, every 15 minutes during the infusion, and at 15, 30, 45, and 60 minutes post-infusion. When slowing or restarting an infusion due to an infusion reaction, vital signs should be monitored every 15 minutes or as directed by the Investigator until the infusion is completed and/or the participant is stabilized. If a participant has an infusion reaction with nivolumab, the ipilimumab infusion can be given (without prophylactic medications) if the infusion reaction resolves within 3 hours. For scheduling purposes after a nivolumab infusion reaction, the ipilimumab infusion may be given the next day. Prophylactic preinfusion medications should be given prior to all subsequent nivolumab. For subsequent infusions, vital signs should be collected prior to dosing. For subsequent infusions, vital signs should be collected prior to dosing, every 30 minutes (-/+ 10 minutes) during dosing, and 1 hour post-dosing. For cycles 5 and beyond, for participants receiving nivolumab alone, who have never had a documented infusion reaction, pre and post-dosing vital signs may be obtained.

d. Hematology labs to include hemoglobin, hematocrit, red blood cell count, white blood cell count, platelets (direct platelet count), as well as total and differential CBC counts. These labs must be done and reviewed before ipilimumab and nivolumab infusion. These labs are required to be done throughout follow-up regardless if the participant goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed.

e. Serum chemistry laboratory analysis includes albumin, urea or BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, HCO3 (CO2; venous blood), calcium, phosphorous. These labs must be done 2 weeks before enrollment for eligibility purposes, and within 48 hours before drug infusion (See 8.2). These labs are required to be done throughout follow-up regardless if the participant goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 4 weeks prior to registration. Serum or urine pregnancy test must be done within 72 hours prior to Day 1 of cycles 1-5 and then every 12 weeks thereafter.

f. At screening, testing should be performed for HIV antibody (refer to eligibility criteria 3.1.2), hepatitis C antibody, and Hepatitis B antigen utilizing local standard informed consent procedures prior to this laboratory collection. Viral load for HIV and if positive for Hepatitis C/Hepatitis B should repeated prior to cycle 5 and every 12 weeks thereafter till participant off study. Refer to eligibility criteria at 3.1.12 and 3.1.13.

g. The following immunologic labs are to be done at baseline (within 4 weeks of starting the study drug) prior to cycle 5 and then every 12 weeks: rheumatoid factor, C-reactive protein and ANA, SPEP, quantitative immunoglobulins and FLC. These labs must be repeated prior to cycle 5 and then every 12 weeks after that until the participant goes off study.

h. Endocrine labs: To be done at the indicated visits. These include TSH, free T4, TPO, morning ACTH, morning cortisol if ACTH is

abnormal.

i. All AEs must be collected whether they occur on treatment or non-treatment weeks and must be submitted utilizing the corresponding AE Forms, covering all time periods specified on the forms. AE assessment must include assessment of immune-mediated AEs.

j. Scans will be performed at baseline, before cycle 5, and thereafter every 12 weeks. It is critical that all imaging studies or CT scans be performed in an identical way to the baseline scan with the same scanner, same scan direction, and consistent arm pointing. Confirmatory scans should also be obtained 4 weeks following initial documentation of objective response. If tumor flare is suspected, the participant must undergo a second scan within a month of the first scan and the case must be discussed with the principal investigator. If progression is documented at that time, participant must come off study. If a participant has a history of brain metastasis, a brain MRI at baseline is required. Participants with KS will be evaluated for response before odd numbered cycles (5, 7, 9, etc.). Scans will not be required for participants with KS unless clinically indicated.

   For participants with relapsed refractory HIV-cHL, F-FDG-PET /CT scans will be done at baseline, and weeks 17 and 25. At week 49, an F-FDG-PET/CT scan is required for patients who did not have two consecutive negative scans before this time point. If F-FDG-PET/CT scans are done without contrast, a full scan with and without contrast will be required unless there is an allergy to IV contrast. CT with and without contrast will follow the same schedule as stated above. For patients with bone marrow involvement at screening, a bone marrow biopsy was required to confirm complete remission.

k. Participants with ongoing toxicities should be seen more often as clinically indicated. AEs Assessment on the study will continue for all participants until 90 days after the last study drug administration. End of Study Assessment will be done within 6 weeks (±1 week) and at 16 weeks (± 1 week) of the last dose of study treatment.

l. For participants with KS, tumor assessments with photographic record must be performed within 7 days prior to initiating treatment. Tumor assessments and photographs should be performed as outlined in the AMC Kaposi's Sarcoma (KS) Tumor Assessment MOP. KS tumor assessment will occur every 4 weeks during study participation.

Biological Sample Submissions for Correlative Studies for participants on combination therapy solid tumor expansion cohort

Specimens are to be submitted as outlined in Section 8. All samples will be submitted to the AMC Biorepository unless noted otherwise. All blood tubes are to be filled completely.

	Visit -1	C1 Pre D1 Post D1 D8	C4 Pre D1 Post D1 D8	Pre C5 D1	C7 and Pre D1 Post D1 D8	C10 Pre D1 Post D1 D8	C15 D1	After Completion of Therapy/ Off Study[9]
MANDATORY								
Two 6 mL red top tubes	X			X				X
Twelve 8.5 mL ACD tubes[1,3] OR two 8.5 mL ACD tubes[1] Ship to AMC Biorepository	X						X	X
Five 8.5 mL ACD tubes[2, 3, 7] Ship to AMC Biorepository		X	X		X	X		
Four 4 micron thick unstained slides from initial biopsy[6,8]	X							
ADDITIONAL BIOPSIES DEPENDING ON TUMOR TYPE Submit from participants who answer *"Yes"* to *"I agree to participate in the laboratory research studies that are being done as part of this clinical trial."*								
Tumor Tissue Biopsy if participants have KS[4] OR Anal swab for participants with anal cancer[5]	X			X				X

1. If consenting to participate in the HIV latent reservoirs study and hematocrit ≥ 25. To be shipped unprocessed. If a participant has not consented to the HIV latent reservoir testing blood draw, two 8.5 yellow top tubes will be collected for herpesvirus load and latent virus studies (Appendix XIV and Appendix XVI).
2. To be processed on site for viable PBMC and plasma within 6 hours of collection. If processing cannot be completed on site, specimens should be shipped via priority overnight service.
3. Only to be completed for participants with hematocrit ≥ 25.

4. Two 2x2 mm biopsies in RNALater and formalin as described in Appendix XIV. The AMC Genomics Core Laboratory will provide kits for 2x2 punch biopsy collection and shipping samples.
5. Anal swabs as described in Appendix XVII.
6. Representative tumor tissue from the initial diagnostic biopsies or repeat diagnostic biopsy prior to initiating treatment and related pathology reports are to be submitted within one (1) month following registration or collection as outlined in Appendix XIV.
7. Collect within 2 days prior to day 1 of each cycle for pre-dose samples, and on day 1 for post-dose samples. The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days, and should be collected at least 12 hours apart. All samples on day 8 of a given cycle may be collected up to 3 days prior to day 8.
8. A minimum of four unstained sections (or three unstained sections plus one H&E), adhered to positively charged slides, and cut at a thickness of four microns are preferred for testing. Slides should be stored refrigerated if possible.
9. Samples are to be collected within 6 weeks (± 1 week) of the last dose of study treatment only.

APPENDIX II: COLLABORATIVE RESEARCH AGREEMENT

The agents, ipilimumab and nivolumab (hereinafter referred to as "Agent(s)"), supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between BMS (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:

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 investigational 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 participant or participant'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.

For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the participant 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 NIH, 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 investigational 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 investigational Agent.

Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively 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.

- 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.

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.

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.

APPENDIX III: PERFORMANCE STATUS SCALES

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

APPENDIX IV: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS

A. GENERAL

To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website: www.saftpak.com. The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

NOTE: Specimens MUST BE SHIPPED **Mondays** through **Thursday** as an OVERNIGHT PRIORITY shipment. Specimens are **NOT ACCEPTED ON SATURDAYS OR SUNDAYS** in the ACSR.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

Blood Specimens

Draw two 8.5 mL yellow top [acid citrate dextrose (ACD)] tubes from study participant. With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol # 095
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood, P=Plasma, S=Serum, or Tissue
- Specimen purpose: Donation

Specimen Shipment

- Seal the tops of the two 10 ml yellow tops with parafilm.
- Place the two sealed tubes into bubble wrap (provided in STP-210 kit).
- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and “self-seal.”
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX airbill on blank side of the shipper making sure that it is marked “FED-EX PRIORITY OVERNIGHT.”

- Mark “OTHER” in the airbill under “Packaging.” Please refer to the MOP and the AMC Operations website’s (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.Under airbill section “Special Handling” indicate “YES-SHIPPERS DECLARATION NOT REQUIRED.”
- Place “From/To” information onto areas provided on the shipper.

Blood specimens should be shipped by overnight express at room temperature to:

Jeff Bethony, PhD
AMC Biorepository
George Washington Medical Center
Ross Hall, Room 118
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056
Email: amc-bio@emmes.com

- Make certain that shipper is already either pre-labeled with ‘UN#3373’ stamp, or make a paper label with ‘UN#3373” and affix it to the shipper.
- Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in ml and affix to the shipper.
- Affix airbill to shipper so that the ‘UN’ and ‘VOLUME’ labels are visible.
- RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.
- Place the box in the FedEx pickup area at your site or call to request a package pickup.

Please Note: The shippers will be mailed back to each AMC site.

INSTRUCTIONS FOR BLOOD SPECIMENS COLLECTED ON FRIDAY OR SATURDAY

Preparation of Plasma and Mononuclear Cells

Refer to the ACSR’s SOP on Separation of Plasma and Mononuclear Cells on the AMC Operations web site for instructions on preparing plasma and PBMC aliquots. It is preferable that separation occurs as soon as possible. If necessary, whole blood in ACD (yellow top tubes) can be held at room temperature for no more than 24 hours.

Freeze the cell suspension in 0.5 ml aliquots in sterile NUNC vials by placing the NUNC tubes in a room temperature, alcohol saturated, control rate freezer container and store in the -80°C freezer overnight. Transfer the cell suspension into the liquid nitrogen temperature freezer for long term storage the next working day.

*****PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING. Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.**

PREPARATION OF TISSUE SAMPLES

Tissue specimens to be fresh frozen should be placed in OCT and then on dry ice immediately. The specimens may stay on dry ice until being transferred to a -80ºC freezer.

Tissue specimens for donation may be batched for shipping after storage in -80ºC freezer. *NOTE: Specimens can only be accepted Monday through **Friday**. Therefore, specimens can only be shipped **Sunday-Thursday** for delivery the next day. Shipping frozen tissue requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.

TISSUE specimens should be shipped by overnight express to:

Jeff Bethony, PhD
AMC Biorepository
George Washington Medical Center
Ross Hall, Room 118
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056
Email: amc-bio@emmes.com

C. RECORD OF SPECIMENS

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC^{SM} system. The GlobalTrace^{SM} shipment manifest must accompany all specimen shipments.

APPENDIX V: ACSR INFORMED CONSENT

Study Title for Study Participants:	Collecting Blood and Tissue Sample Donations for Research for HIV/AIDS-Related Cancers
Official Study Title:	Biospecimen Collection and Donation to the AIDS and Cancer Specimen Resource (ACSR)

What is the usual approach to donate blood and/or tissue to the ACSR?

You are being asked to donate blood and/or tissue for future research. You are being asked to donate your blood and/or tissue samples to the ACSR because you have HIV infection and are being considered for participation in an AIDS Malignancy Consortium (AMC) clinical trial. The AMC works with the ACSR to collect donated samples from persons with HIV infection for research studies. People who do not take part in an AMC clinical trial can also donate samples to the ACSR.

What are my other choices if I do not take part in this study?

It is your choice to donate or not donate your blood and/or tissue samples. You may still take part in the AMC clinical study if you choose not to donate blood or biopsy samples to the ACSR.

You may also choose to donate:

- Blood but not tissue, or
- Tissue but not blood.

What is the AIDS and Cancer Specimen Resource (ACSR)?

The ACSR is a biorepository (biobank) that collects human biological specimens (samples) from persons who have HIV or cancers related to HIV/AIDS. The ACSR stores the samples and some of the donor's medical information for use by researchers in future research studies. The National Cancer Institute (NCI) has set up the ACSR to assist researchers locate samples needed for their studies.

The ACSR has an independent research panel that approves researchers' requests to use the ACSR's stored samples for research studies. The ACSR only gives samples and medical information to researchers after their projects have been approved. Researchers may use the samples to study cancers and other diseases associated with HIV disease. This information may help us learn more about the causes of HIV-related diseases and cancers and to develop better ways to screen, diagnose, and treat them.

Why is this study being done?

The purpose of this study is to collect samples for the ACSR for future research studies. Researchers may study samples from the ACSR in combination with hundreds or thousands of other samples to explore how biologic or genetic factors may be related to HIV-related diseases and cancer. The information might help doctors in the future to identify who will or will not benefit from treatment. The samples may be used to learn more about how HIV-related diseases and cancers develop. The samples may also lead to new tests or discoveries. Finally, researchers may use the samples to study the genetic material from your cancer tissue and compare it to the material from your normal tissue (blood) to try to find the differences that exist. These studies could make it possible to identify many of the changes that are associated with diseases such as

cancers. It may also help us tailor treatments to a patient's unique genetic make-up and/or to the genetic markers of the tumors.

What extra tests and procedures will I have if I take part in this study?

1. If you agree to donate blood, the medical team will draw about 2 tablespoons of blood to give to the ACSR. This takes about 10 minutes.
2. If you agree to donate tissue, your leftover tissue biopsy material will be donated to and stored by the ACSR.
3. Some of your clinical information will be released to the ACSR and entered into their database. The information given to the ACSR will not include your name or any information that could personally identify you.

We will only give the ACSR tissue that is left over after making decisions about your treatment or diagnosis. The study doctor will not take any extra biopsies just for the ACSR.

We cannot tell you right now what future research these samples would be used for. Instead, we are asking that you give approval to give your samples for future testing without contacting you again. The results of whatever research is done on your samples will *not* be told to you or your doctor. The results of the tests will *not* be placed in your study records.

How long will ACSR keep my samples?

Your blood and/or tissue sample will be stored until it is used for research. The samples may be stored indefinitely.

What possible risks can I expect from taking part in this study?

- Blood Draw: The risks of drawing blood include temporary discomfort from the needle stick, bruising, and, rarely, infection.
- Confidentiality: The ACSR will receive study samples with code numbers. There will be no personal identifiers on the samples. Then the samples will be re-labeled with a barcode and stored for future testing. While the ACSR and researchers who study ACSR samples will have no information that could identify you, there is a risk that someone could use information from genetic studies to trace your samples back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. In some cases, this information could be used to make it harder for you to get or keep a job. There are laws against misuse of genetic information, but they may not give full protection. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

Let your study doctor know of any questions you have about these possible risks. You can ask the study doctor questions about side effects at any time.

What possible benefits can I expect from taking part in this study?

This study is unlikely to help you. This study may help us learn things that may help people in the future.

The information may help to identify those who are at increased risk and those who may benefit from targeted treatment and screening. In turn, these studies could help find ways to prevent or improve treatments for HIV-related diseases and AIDS-related cancers.

Can I stop taking part in this study?

Yes, you may withdraw your samples from the ACSR at any time. You may contact your AMC study coordinator if you would like to withdraw your samples. The coordinator can ask in writing that your sample be removed from research use and that any identifiable sample and information still in their possession be destroyed. However, if any research has already been done using some of your samples, the data will be kept and analyzed as part of those studies.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the _____ *(insert name of center)* Institutional Review Board at _____ *(insert telephone number). (Note to Local Investigator: Contact information for patient representatives or other individuals at a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can also be listed here.)*

What are the costs of taking part in this study?

There will be no cost to you for donating your samples to the ACSR. You will not be paid for taking part in this study.

What happens if I am injured or hurt because I took part in this study?

If you are injured or hurt as a result of taking part in this study and need medical treatment, please tell your study doctor. The AMC will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance, you would be responsible for any costs.

If you feel this injury was a result of medical error, you keep all your legal rights to seek payment for injury even though you are in a study.

Who will see my medical information?

Your privacy is very important to us and the researchers will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, the researchers will do their best to make sure that any information that is released will not identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private, unless required by law to provide information. Some of these organizations are:

- The AIDS Malignancy Consortium (AMC)
- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Office for Human Research Protections and the National Cancer Institute in the U.S.

To protect your privacy, the AMC does not keep identifying information that links study participants to specific samples. As a result, the AMC and ACSR will not be able to link the results from studies that use your samples back to you. Thus, information, including genetic information, that researchers may obtain in studies that use your samples may not be directly linked to you and will not be placed in your medical record. However, some clinical and basic information obtained confidentially from the AMC will be attached with these data. It is possible that findings may one day help, for example, people of the same race or sex as you. It also is possible that genetic factors might come to be associated with people who have HIV and cancer through these kinds of studies.

Where can I get more information?

You may visit the NCI Web site at **http://cancer.gov/** for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at 1-800-4-CANCER (1-800-422-6237).

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ *(insert name of study doctor[s])* at _____ *(insert telephone number).*

Please circle your answer to show whether or not you would like to take part in each option:

1. **I agree to donate my blood to the ACSR for future research that may be used to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.**

   YES NO

2. **I agree to donate my blood to the ACSR for future research that may include genetic testing to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.**

   YES NO

3. **I agree to donate some of my tissue biopsy material that is not required for my treatment or diagnosis to the ACSR for future research that may be used to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.**

   YES NO

4. **I agree to donate some of my tissue biopsy material to the ACSR for future research that may include genetic testing to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.**

   YES NO

My Signature Agreeing to Take Part in the Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the optional study.

Participant's signature_____

Date of signature_____

Signature of person(s) conducting the informed consent discussion_____

Date of signature_____

APPENDIX VI: AMC DATA AND SAFETY MONITORING PLAN

(Version 9.0 October 6, 2020)

Introduction

The AMC Data and Safety Monitoring Plan (DSMP) outlines the measures employed by the group to monitor the safety of participants and ensure the data validity and integrity for all clinical trials it conducts. This includes methods to: 1) monitor the progress of trials and the safety of participants; 2) comply with regulatory requirements for adverse event (AE) reporting; 3) processes for trial termination or temporary suspension and major modifications; and 4) plans for ensuring data accuracy and protocol compliance. As the AMC conducts protocols of varying research phase, region of conduct (which may include trials conducted in the U.S., international sites, or both), IND sponsor (AMC investigator, CTEP, or industry-sponsored) and clinical data entry system use, this plan addresses broad processes applying to the range of trial designs and requirements. Refer to the individual AMC protocol to identify the applicable study characteristics for the relevant requirements described in this plan.

Monitoring the Progress of Trials and the Safety of Participants

Routine and expedited AE reporting

All AMC protocols that collect safety data adhere to the *National Cancer Institute (NCI), Cancer Therapy Evaluation Program (CTEP) Guidelines: Adverse Event Reporting Requirements* (https://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm), as applicable to the clinical protocol. AEs are to be recorded in the source documents, assessed by a clinical investigator for the AE reporting criteria, and promptly reported in the clinical data entry system as required by each protocol. For AMC trials conducted under a CTEP IND and AMC trials conducted within the U.S., all AEs that meet the NCI's expedited reporting requirements are reported to the NCI via the CTEP Adverse Event Reporting System (CTEP-AERS) web application, either directly or through integration with Medidata Rave where this system is employed for AMC protocols. Use of this system ensures notification to the protocol chair and Investigational Drug Branch (IDB) at CTEP, as required for trials conducted under a CTEP IND, and a uniform expedited reporting and safety review process for AMC domestic trials. The system may also be programmed to include sponsor notification as required for trials with industry support. Alternate process for expedited AE reporting to the AMC protocol chairs and AMC Operations and Data Management Center (ODMC) within the clinical data entry system (AdvantageEDC or Advantage eClinical only) may be defined in the protocol for select trials (international studies and The ANCHOR Study).

All serious adverse events (SAEs) received by the AMC ODMC will be reviewed by the AMC medical monitor at the AMC ODMC for consideration of individual participant safety, safe trial conduct, data reporting quality for AE term selection, and appropriate application of the regulatory criteria for seriousness, expectedness, and relatedness to the investigational therapy. If alternate procedures are followed for SAE review, the process for adequate medical monitoring will be defined in the AMC protocol and the Transfer of Regulatory Obligations (TORO) with the sponsor. AMC medical monitor review includes review of the CTEP-AERS report before CTEP submission for IDB review (if applicable), or review of the SAE report in the data entry system for trials not using CTEP-AERS for expedited reporting. The IND sponsor or its designee will issue the determination as to whether the AE requires IND safety reporting

to FDA as a serious and unexpected suspected adverse drug reaction (SUSAR). For protocols not conducted under an IND, in the event of disagreement between the reporting physician and the AMC medical monitor regarding the relationship of the AE to the investigational agent(s) (i.e., determination of whether the attribution is unrelated or unlikely, or possible, probable, or definite), the AMC medical monitor will provide the final determination of the relationship. IND safety reporting to FDA is performed by CTEP for trials conducted under a CTEP IND; IND safety reporting is performed by the sponsor or sponsor's designee (AMC ODMC or other party defined in the study agreement or TORO) for IND studies sponsored by AMC investigators or industry sponsors.

Expedited reporting to the Institutional Review Board (IRB)

The requirements for IRB review will be identified in the protocol section on ethical and regulatory obligations. All AMC trials initiated before September 1, 2020 and all international sites for all AMC studies are subject to local IRB review; only U.S. sites are subject to the NCI requirement to use a single IRB for protocols initiated on or after September 1, 2020. For trials subject to local IRB review, the site principal investigator is responsible for ensuring that expedited AE reports for its trial participants and any unanticipated problems that affect the local institution only are submitted to the local IRB of the reporting institution, per the local IRB's requirements for such reporting. For studies reviewed by the single IRB, the protocol chair will render a determination as to whether a SAE or other problem constitutes a trial-wide unanticipated problem that requires reporting to that IRB, in accordance with its standards of procedure.

To comply with investigator notification requirements for IND studies under 21 CFR 312.32 and 312.55, IND safety reports from all trials the AMC conducts and reports from external sponsors investigating the same agents are made available to all investigators upon receipt from the sponsor or its designee, either via the password-protected section of the AMC Operations web site (AMC trials subject to local IRB review only) or the CTSU website (U.S. trials subject to single IRB review/CTEP IND agents). The site clinical investigator responsible for the applicable AMC protocol(s) is responsible for reviewing any IND safety reports received and documenting submission to the IRB of record (if required by local policy) within the timeline defined by the Clinical Trials Monitoring Branch (CTMB) audit guidelines.

Procedures for monitoring trial progress and pharmacovigilance

For trials using AdvantageEDC or Advantage eClinical for clinical data entry, the AMC ODMC provides on demand tabular listings of all reported AEs and SAEs on a participant level to the protocol chair and co-chair(s) for review via the password-protected section of the AMC Operations web site, www.AIDScancer.org. For trials using OPEN and Medidata Rave for clinical data collection, data listing will be made available using that system. Summary reports of AEs by frequency and relationship to the investigational agent(s) are provided to all AMC investigators and their staff It is the responsibility of each site to provide trial-specific AE listings to their respective IRB, if required by its policies. For blinded studies, the AE and SAE listings are reviewed and tabulated without treatment assignment.

Accrual summaries for each AMC trial are updated nightly on the password-protected section of the AMC web site. The progress of each AMC trial is reviewed regularly by the protocol chair and also by the appropriate Scientific Working Group (SWG) during scheduled conference calls (monthly SWG calls and as required, protocol-specific monitoring conference

calls). Summary accrual, summary AE, and individual SAE reports are provided to SWG leadership and protocol chairs to monitor participant safety during these monthly calls.

The AMC medical monitor reviews listings of all reported AEs on a quarterly basis for assuring compliance with the protocol requirements for AE reporting and the identification of any safety concerns (individual AE or increased frequency/severity of expected AEs) for the agents under investigation. Findings from these reviews are communicated to the protocol chairs and all AMC investigators, and posted to the AMC Operations web site.

Data and Safety Monitoring Board Review (DSMB) review

The AMC has formed an independent Data and Safety Monitoring Board (DSMB) for AMC trials and for the ANCHOR Study. As required by NCI policy, the AMC requires DSMB review for all phase III randomized trials. All other clinical trials that the AMC initiates will be reviewed by the AMC ODMC and AMC Statistical Center during protocol development to issue a recommendation as to whether the study requires DSMB oversight, which will require the approval of the AMC Executive Committee. This determination will be based on the phase of the study, experimental design, risk posed by the investigational approach, extent of data available on the safety of an investigational agent, risk posed by the natural course of the health condition under research, and the categories of vulnerable populations involved. The involvement of a DSMB in reviewing an AMC protocol will be identified in each clinical protocol as approved by CTEP and, as applicable, required by the IRB of record.

Regarding the composition of the AMC DSMB, voting members usually include physicians, statisticians, an ethicist, and a patient advocate. All voting members have no other affiliation to the AMC and are appointed by the AMC Executive Committee with the approval of the OHAM Director. Nonvoting members are the AMC group statistician, the protocol statistician, an AMC ODMC staff member, two representatives (normally a clinician or statistician) from CTEP, and the grant program directors from the NCI Office of HIV and AIDS Malignancy (OHAM).

The DSMB reviews all applicable AMC studies in accordance with the National Cancer Institute's Policy for Data and Safety Monitoring. Confidential reports of all trials under review are prepared by the AMC group statistician with support from the AMC ODMC. A written report containing the current status of each trial monitored, and when appropriate, any toxicity and outcome data, are sent to DSMB members by the AMC ODMC within the timelines specified by the DSMB charter. This report addresses specific toxicity issues and any other concerns about the conduct of the trial, as defined by the protocol plan for DSMB review. The report may contain information for the DSMB to render determinations for participant safety, early trial termination, results reporting, or continuing accrual or follow-up.

The results of each DSMB meeting are summarized in a formal report sent by the DSMB chair to the AMC group chair and AMC ODMC. The DSMB report contains recommendations on whether to close each study reviewed, whether to report the results, and whether to continue accrual or follow-up. A primary recommendation (e.g., continue with no change; recommended or required modification; stop) must be included in the document. The group chair or designee is then responsible for notifying the protocol chair and relevant SWG chair before the recommendations of the DSMB are carried out. In the unlikely event that the protocol chair does not concur with the DSMB, then the OHAM program directors and the NCI division director or designee must be informed of the reason for the disagreement. The protocol chair, relevant SWG chair, group chair, DSMB chair, and NCI division director or designee will be

responsible for reaching a mutually acceptable decision about the study. CTEP approval of a protocol amendment will be required prior to any implementation of a change to the study.

Following a DSMB meeting, the DSMB's recommendations are provided to all AMC investigators and staff. It is each site principal investigator's responsibility for conveying this information to its local IRB as relevant for its protocol participation. For trials reviewed by a single IRB, the AMC ODMC will support notification to the IRB as required per its procedures.

Cohort trial reviews not subject to DSMB review

For phase I dose escalation trials, dose escalation (or dose de-escalation) is based on the rules in the protocol and the protocol chair, AMC medical monitor, and protocol statistician determine whether these criteria have been met based on a review of all safety data for the protocol-defined evaluation period. If applicable for phase II trials, stopping the trial for toxicity or efficacy, or suspending enrollment pending observation of responses in a multi-stage phase II trial, is based on meeting criteria stated in the protocol, and the protocol chair, AMC medical monitor, and protocol statistician determine whether these criteria have been met.

Plans for Assuring Compliance with Requirements Regarding AE Reporting

The protocol chair, AMC group chair, and the AMC ODMC share responsibility in assuring that participating investigators comply with applicable regulatory and protocol requirements for AE reporting. The AMC site principal investigator certifies compliance with NCI and FDA requirements for trial conduct by signing the site subaward agreement for the grant and the AMC Adherence Statement for site membership; clinical investigators also certify compliance in completing the protocol signature page for each protocol active at the site, and Form FDA-1572 for CTEP investigator registration, and also for AMC IND studies sponsored by AMC investigators or industry sponsors. Protocol compliance with AE identification, assessment and reporting requirements is assessed by the AMC ODMC using several methods: 1) programmed system checks and messages to instruct the site to complete routine and/or expedited reporting when certain criteria are reported in the clinical data entry system; 2) programmed data reports provided to the protocol chairs that identify reports requiring expedited AE reporting; 3) remote review of data entry or data reports to ensure compliance with protocol and NCI AE reporting requirements; 4) AMC medical monitor review described in the section above; and, 5) routine site audits by reviewing the site's source documentation.

The clinical data entry systems used for AMC studies include the Oncology Patient Enrollment Network, OPEN for enrollment, and Medidata Rave for clinical data entry for enrolled participants; trials activated before September 1, 2020 or that involve only AMC international sites may be reported in AdvantageEDC/Advantage eClinical, a web-based data entry and enrollment system. These data entry systems are programmed to notify the site investigator, protocol chair, AMC medical monitor, and AMC ODMC via e-mail in the event that a site reports an AE that meets expedited reporting criteria to NCI and/or FDA. Additional reporting conditions may be programmed depending on the sponsor reporting requirements of a given protocol (e.g., adverse events of special interest [AESI]). If the site does not follow with an expedited report, the AMC ODMC contacts sites to request compliance with reporting requirements. Additionally, the protocol chair, AMC ODMC, and the AMC medical monitor review reported AEs on a routine basis to identify AEs reported by sites that require expedited reporting. The protocol chair, AMC SWG chairs, AMC group chair, and IND sponsors have

general oversight for assuring that routine and expedited adverse reporting requirements are met by the responsible parties.

For studies monitored by CTEP using the Data Mapping Utility (DMU), cumulative protocol- and participant-specific data will be submitted weekly to CTEP electronically via the DMU. For trials monitored by the NCI's Clinical Data Update System (CDUS), AE information is transmitted electronically to NCI on a quarterly basis. For trials monitored by NCI's Clinical Trials Monitoring Service (CTMS), AE information is transmitted electronically to NCI every two weeks.

Plans for Assuring that any Action Resulting in a Temporary or Permanent Suspension of an NCI-Funded Clinical Trial is Reported to the NCI Grant Program Director Responsible for the Grant

In the event that temporary or permanent suspension of a trial, or major modification to the protocol is under consideration, the protocol chair will convene the AMC ODMC, AMC Statistical Center, and SWG chair by conference call to discuss the options. Suspension actions will also be reviewed by the AMC Executive Committee for program oversight and direct communication of the action with the OHAM program directors. For phase III trials, closure decisions are typically rendered by the AMC DSMB; if the trial in question is under AMC DSMB oversight but rendered by the AMC investigators, the AMC DSMB will be notified of the suspension and the reason. For phase I and II trials, the protocol chair also has the option of asking the DSMB to review the study. The AMC ODMC will inform the CTEP Protocol Information Office (PIO), with copy to OHAM Directors, when studies are temporarily or permanently closed. In the event of major trial modification, CTEP must approve all protocol amendments prior to distributing to the AMC sites.

Plans for Assuring Data Accuracy and Protocol Compliance

All study data for AMC clinical trials are entered directly by AMC clinical site staff into the applicable clinical data entry system for the trial. During data entry, the system performs validation checks on many fields and performs consistency checks between select fields. Range checks are placed on each field to eliminate entry of out-of-range values. Edit check programs are run on the database on a set schedule to identify and resolve inconsistencies between forms or data collected at different points in time. Submitted data entry forms are reviewed for compliance with the protocol and data entry instructions according to the AMC ODMC's standards for data quality processes. AMC ODMC staff routinely interacts with site staff to resolve any data submission problems.

In accordance with NCI guidelines, the AMC ODMC conducts audits at the AMC sites to evaluate compliance with regulatory issues, and to review data for specific cases by checking source documents. These reports are sent to the site principal investigator and to the NCI. In the event that major violations are identified, sites are asked to provide a written corrective and preventative action plan to correct deficiencies. If needed, a repeat site audit is conducted. In the event that a site does not correct deficiencies in a pre-determined time frame, the AMC Executive Committee has the option to implement remedial action(s) for the site. Possible actions include, but are not limited to, suspending enrollment of new participants to AMC trials until deficiencies are corrected; recommending a decrease in funding to the site; and requiring specific training for site investigators or staff members.

APPENDIX VII: 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 VIII: PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS IN PATIENTS WITH AIDS

Infection	Preferred drug	Indications
Pneumocystis carinii pneumonia	Trimethoprim-sulfamethoxazole (double-strength tablet daily)	CD4 count <200 cells/microL; thrush; unexplained fever for more than two weeks; history of PCP
Toxoplasmosis	Trimethoprim-sulfamethoxazole (double-strength tablet daily)	CD4 count <100 cells/microL and Toxoplasma sero-positive
Mycobacterium avium complex	Azithromycin (1200 mg weekly)	CD4 count <50 cells/microL
Histoplasmosis	Itraconazole (200 mg daily)	CD4 count <100 cells/microL and lives in an endemic area

APPENDIX IX: 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 participants where there is clinical suspicion of deep soft tissue metastases (e.g., 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.

Brain MRI/CT

Brain scans (MRI or CT) are required at Screening. Brain scans should be conducted during the course of the study if the Screening scan was positive as per study protocol or as clinically indicated.

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 all subsequent response assessments. 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 RC (Section 9.1). 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.

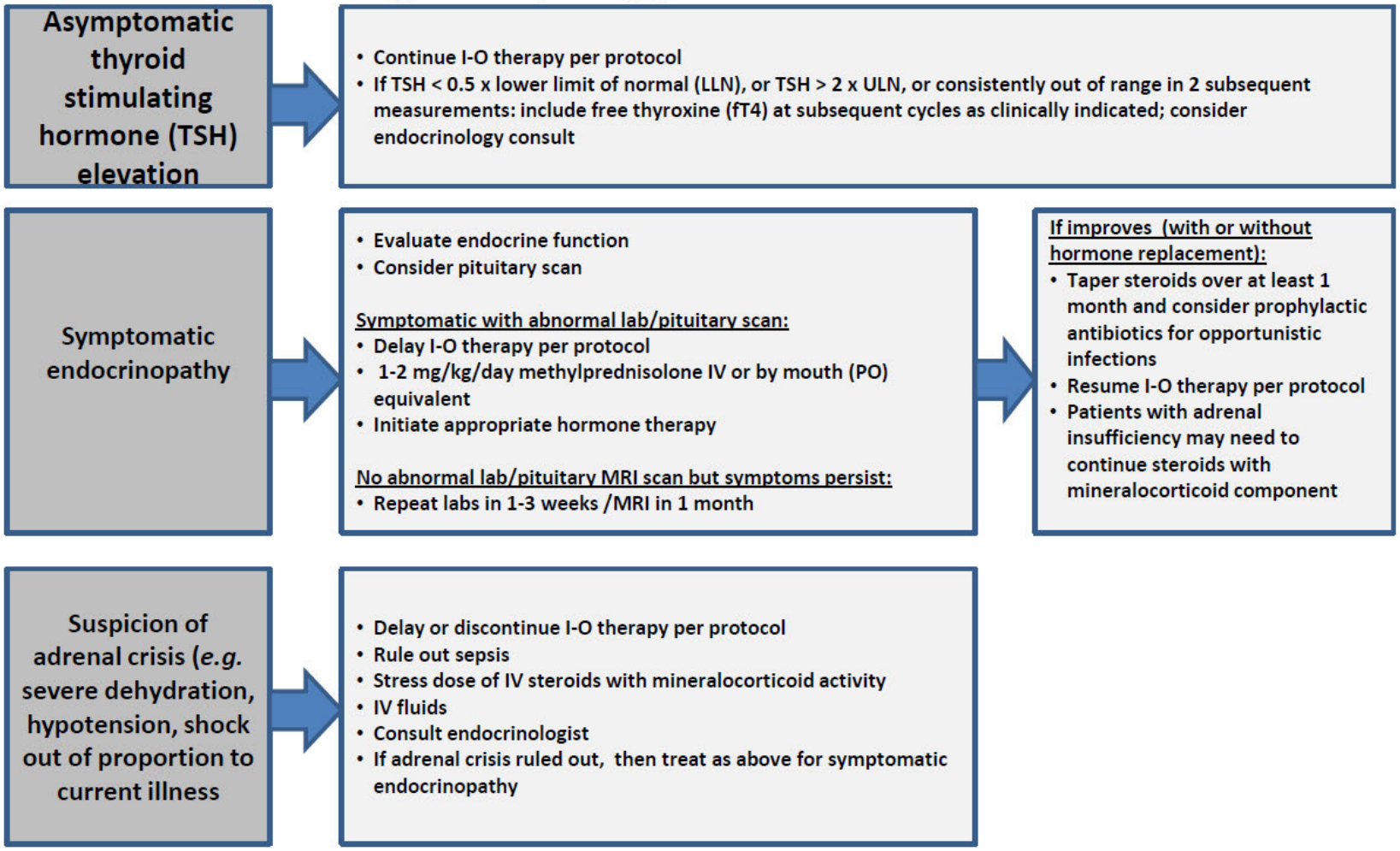
IV contrast should be used for all CT scans. If IV contrast is contraindicated, CT may be performed without contrast. Alternatively, MRI can be used to assess lesions in the participant. Participants who develop contrast allergy after study enrollment may be followed by MRI for subsequent tumor measurements. Sections should be contiguous, similarly sized and consistent from visit-to-visit. Section thickness must be based on institutional standards (e.g., 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 X: NIVOLUMAB: MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

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

Endocrinopathy Management Algorithm

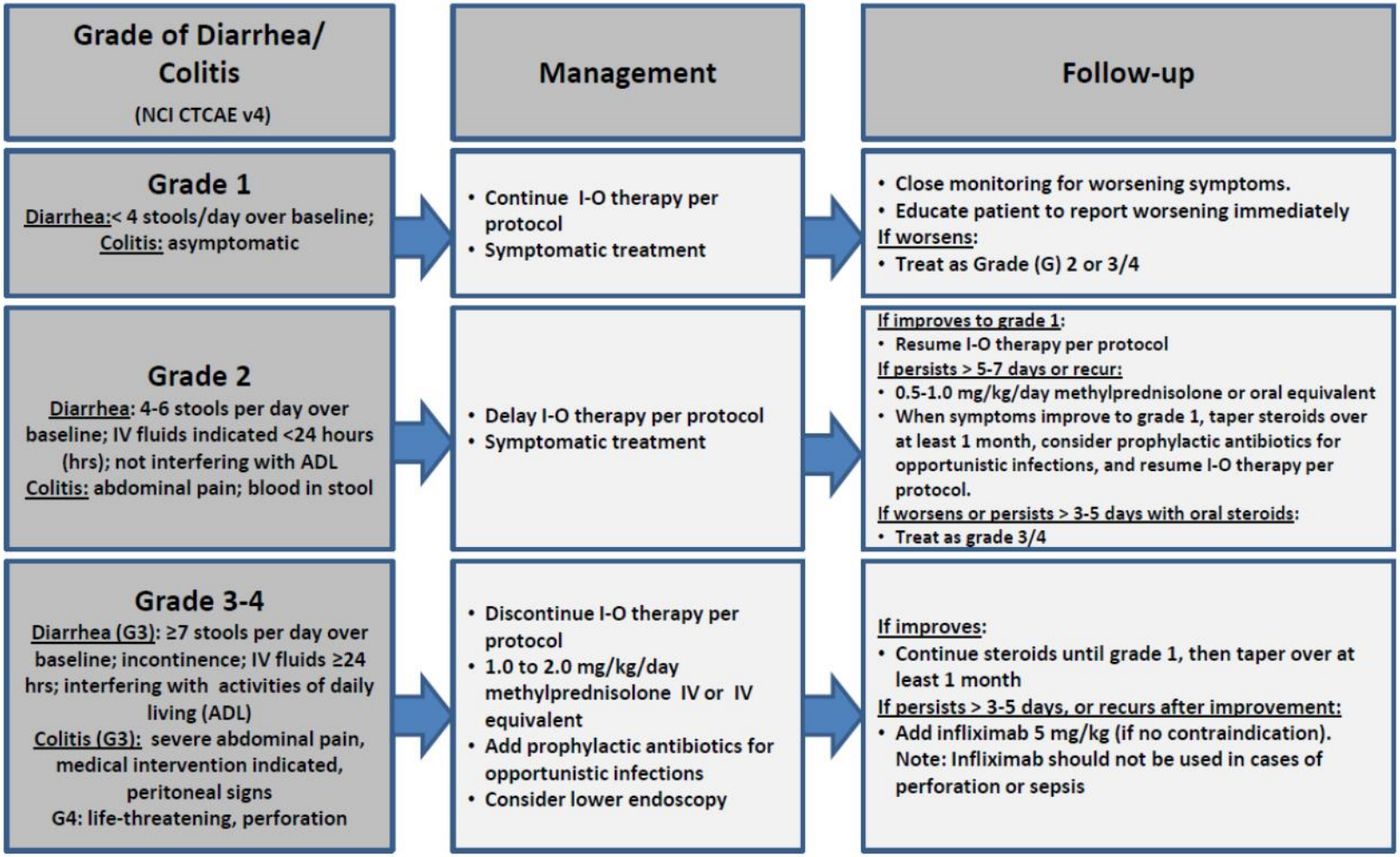
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy.
Consider visual field testing, endocrinology consultation, and imaging.



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

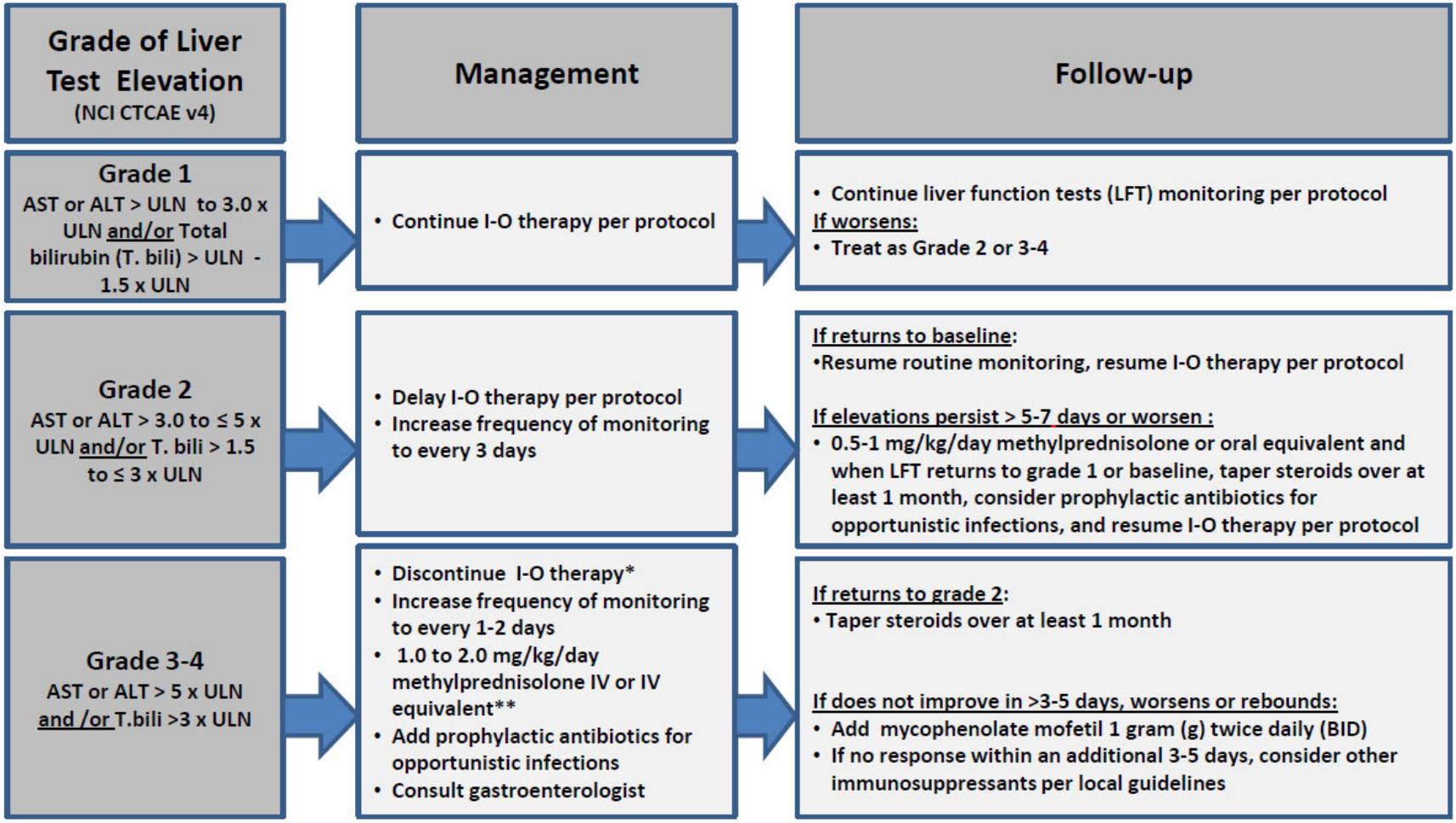
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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.



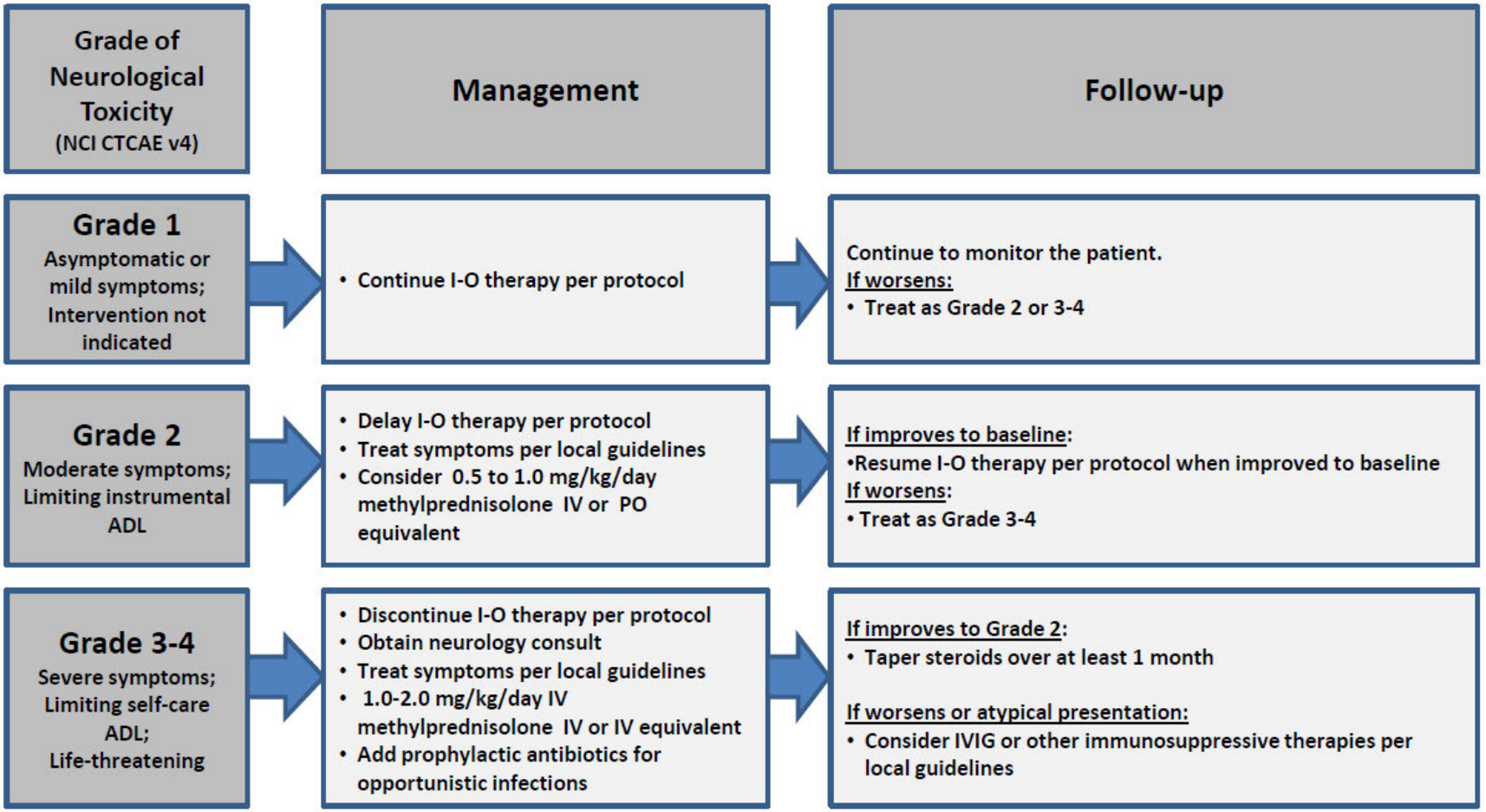
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

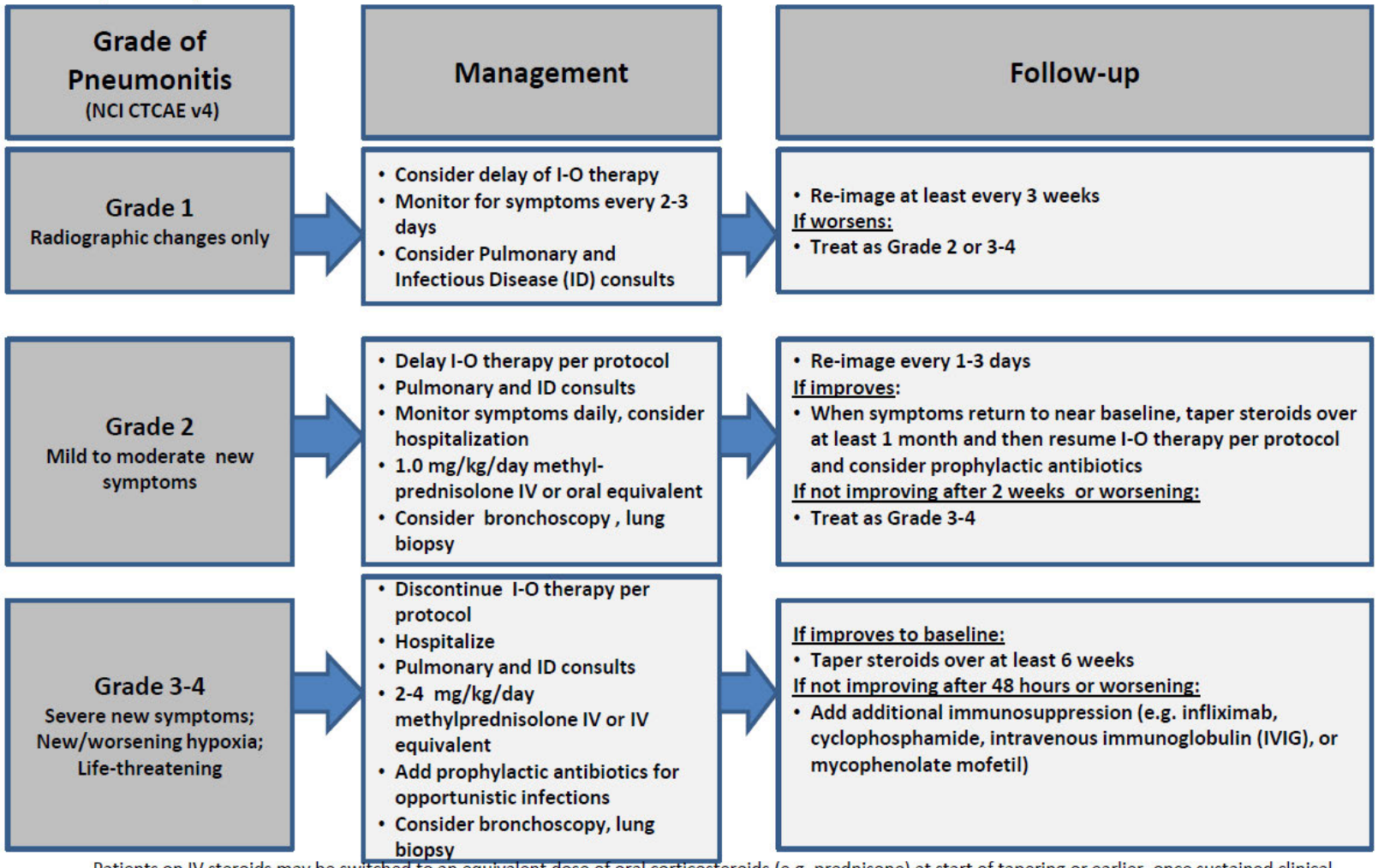
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

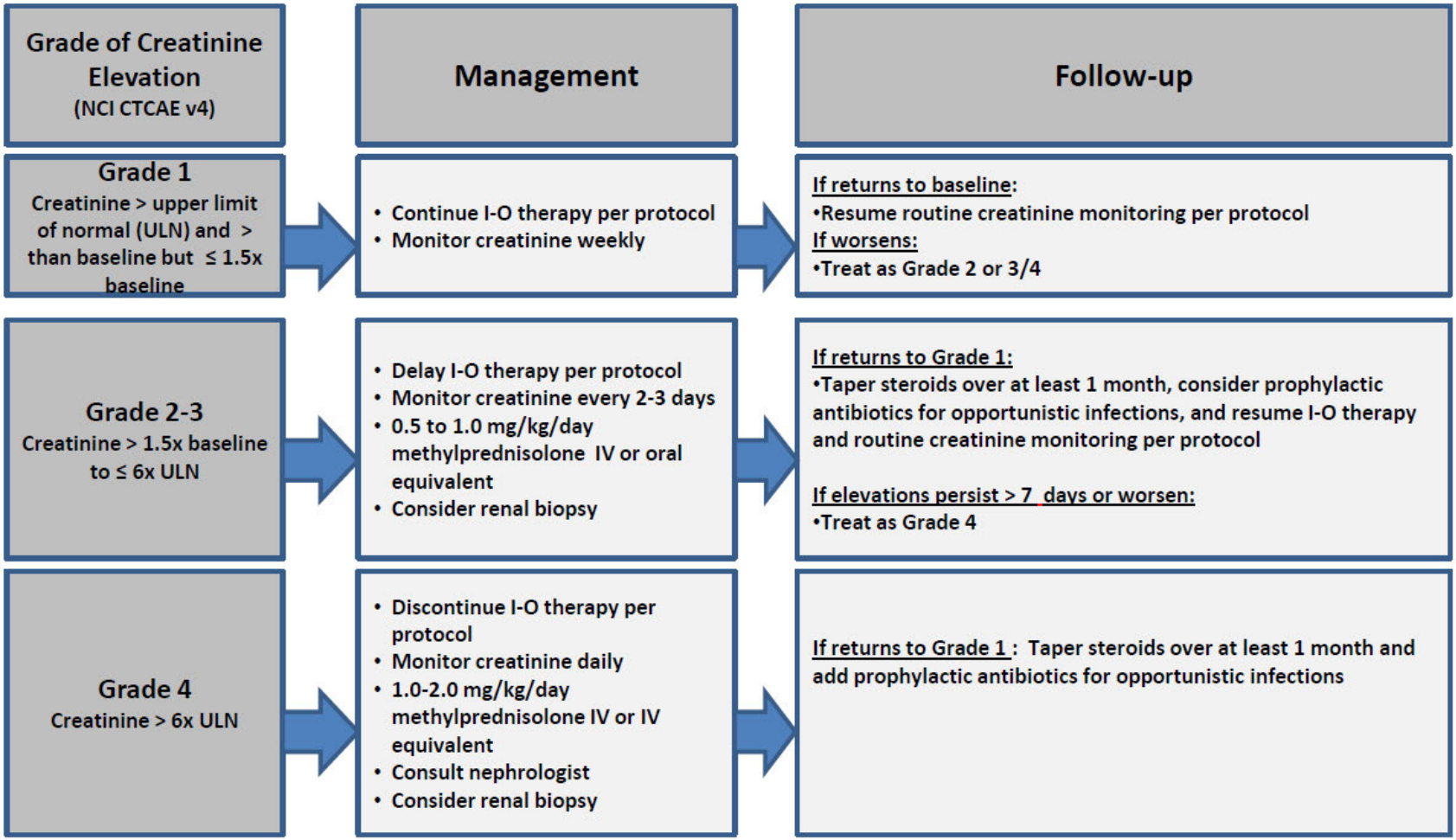
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Renal Adverse Event Management Algorithm

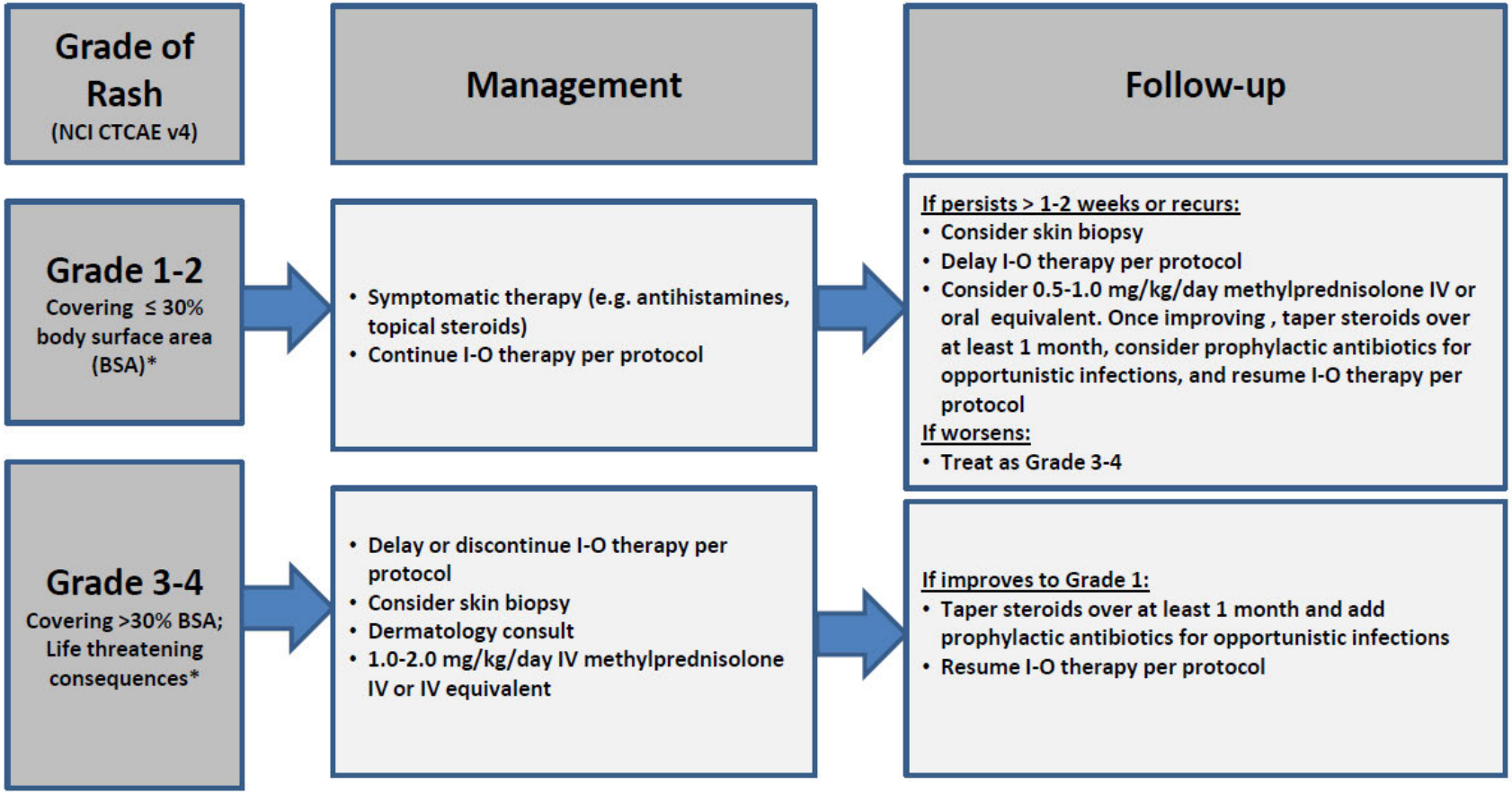
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Skin Adverse Event Management Algorithm

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



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

APPENDIX XI: DEFINITION OF AIDS INDICATOR CONDITIONS

- Aspergillosis, invasive *
- *Bartonella henselae* infection, disseminated (bacillary angiomatosis, peliosis hepatis)*
- Candidiasis of bronchi, trachea, or lungs *
- Candidiasis, esophageal *
- Cervical cancer, invasive *
- Coccidioidomycosis, disseminated or extrapulmonary *
- Cryptococcosis, extrapulmonary *
- Cryptosporidiosis, chronic intestinal (> 1 month's duration) *
- Cytomegalovirus disease, invasive *
- Cytomegalovirus retinitis *
- Encephalopathy, HIV-related *
- Herpes simplex: chronic ulcer(s) (> 1 month's duration), bronchitis, pneumonitis, or esophagitis *
- Histoplasmosis, disseminated or extrapulmonary *
- Isosporiasis, chronic intestinal (> 1 month's duration) *
- Kaposi's sarcoma (progression to visceral disease)
- Lymphoma, Burkitt's (or equivalent term) *
- Lymphoma, immunoblastic (or equivalent term) *
- Lymphoma, primary, of brain *
- Microsporidiosis, diarrhea > 1 month or disseminated *
- *Mycobacterium avium* complex or *M. kansasii*, disseminated or extrapulmonary *
- *Mycobacterium tuberculosis*, any site (pulmonary[1] or extrapulmonary) *
- *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary *
- Nocardiosis, pulmonary, brain or disseminated *
- *Pneumocystis jirovecii* pneumonia (new or recurrent diagnosis)
- Progressive multifocal leukoencephalopathy *
- *Salmonella* septicemia, recurrent *
- Toxoplasmosis of brain *
- Wasting syndrome due to HIV *
- New Diagnosis *

APPENDIX XII: PHOTOGRAPHIC RECORD OF KS LESIONS

Photographs will be taken to assist in documentation of the diagnosis of KS and for clinical monitoring purposes. The difficulty in standardizing these photographs is acknowledged.

In all participants, photographs will be needed of the five marker lesions. The five markers lesions must be labeled in the photographs #1-#5. The same lesions must be consistently labeled throughout the trial. For each lesion, two photos will be taken. The first photo will be a close-up of the lesion. A millimeter ruler should be included in the photograph to demonstrate the size of the lesion. The second photo will be a larger view of the photo that will show the lesion's location on the body.

All participants will also need photos of larger views of the back, chest, arms (front and back), legs (front and back), feet (including soles), whether involved with KS or not. In addition, photos should be taken of any other area with significant involvement at baseline (e.g., the face)

In participants with >50 cutaneous lesions, photographs will be taken of the three representative areas (each with ≥ 5 lesions), defined at study enrollment and used for clinical assessment of response.

Photographic documentation will be completed at each visit when the KS response category changes (as described in Section 9.2). For example, if a participant's KS response category changes from no response to PR, the site will take photos of this to document the category change. If there was no change in the KS response category, no photos are required

Photographs will be stored electronically under the participant ID number and back-up electronic storage will be kept.

APPENDIX XIII: KS STAGING CRITERIA

	GOOD RISK (0) (All of the following)	POOR RISK (1) (Any of the following)
Tumor (T)	• Confined to skin and/or lymph nodes and/or minimal oral disease2	• Tumor-associated edema or ulceration • Extensive oral KS • Gastrointestinal KS • KS in other non-nodal viscera
Immune system (I)	• CD4 cells > 200/μL	• CD4 cells < 200/μL
Systemic illness (S)	• No history of OI or thrush • No "B" symptoms3 • Performance status > 70 (Karnofsky)	• History of OI and/or thrush • "B" symptoms present • Performance status < 70 • Other HIV-related illness (e.g., neurological disease, lymphoma)

T_0 =tumor confined to skin, lymph nodes and/or minimal oral disease.

T_1 =any tumor falling under the "Poor Risk" criteria.

S_0 =no history of OI or thrush, no "B" symptoms, and Karnofsky Performance status $\geq$ 70.

S_1 =any "Poor Risk" systemic illness signs and symptoms.

NOTE: Staging criteria taken from: Krown SE, Metroka C, Wernz JC. Kaposi's sarcoma in the acquired immunodeficiency syndrome: A proposal for uniform evaluation, response, and staging criteria. J Clin Oncol 1989;7:1201-1207. This criteria was adopted by the ACTG Oncology Committee.

[2] Minimal oral disease is nonnodular KS confined to the palate.

[3] "B" symptoms are unexplained fever, night sweats, > 10% involuntary weight loss, or diarrhea persisting more than 2 weeks.

APPENDIX XIV: AMC BIOREPOSITORY SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS

Regimen: Required study specimens are included in Appendix I. Unless otherwise specified (HIV latent reservoir and viral outgrowth assays requiring twelve 8.5 ACD tubes, or two 8.5 ACD tubes where consent for larger draw was not obtained), all specimens will be shipped to and banked at the AMC Biorepository, which will serve as a tissue source site for processing samples for all AMC sites, for future testing at AMC core labs and outside labs. Tests will be conducted at the end of enrollment for each phase or within one year of acquisition of samples.

Assays procedures are described in the Network Core Laboratories' Manual of Operation(s) and available from the network core laboratories. Assays details may change as technology progresses.

Assays as proposed in exploratory objectives:

- Plasma for herpesvirus (HCMV, EBV, KSHV) viral load and PBMC for herpesvirus (HCMV, EBV, KSHV) latent virus will be provided to the AMC Virology Core Laboratory and the Lewin Laboratory at the University of Melbournefrom the material collected under Appendix XVI (10 million PBMC and plasma from all time points where 12 ACD tubes are collected (submitted by the site directly to the AMC Biorepository), or 2 ACD tubes where consent for larger blood draw was not obtained
- Four 4 micron unstained slides for PD-L1 testing (AMC Pathology Core Lab)
- For KS participants: Tissue (skin of KS) biopsies: RNALater for gene expression profiling (AMC Biomarker Core Laboratory)
- Tissue (skin of KS) biopsies: formalin for IHC (PD1, PDL-1, LANA, orf59, CD4, CD8, other immune cell populations)
- Serum for Luminex multiplex cytokine assay(s) (AMC Biomarker Core Laboratory) (See Appendix XV)
- For anal cancer participants: anal swabs for HPV type determination (AMC HPV Virology Core Laboratory, see Appendix XVII).

GENERAL

A. To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website: www.saftpak.com. The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

NOTE: Specimens MUST BE SHIPPED **Sunday** through **Thursday** as an OVERNIGHT PRIORITY shipment. Specimens are **NOT ACCEPTED ON SATURDAYS OR SUNDAYS** in the AMC Biorepository.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

Instructions for Peripheral Blood Specimens (Sunday – Thursday)

From study participant, draw:

Five 8.5 ml yellow top [ACD] tubes for HIV studies at: To be collected within two days before the Day 1 dose, post-dose Day 1, and Day 8 on the indicated cycles.

- For participants on dose levels 1 (including 240 mg every 2 weeks) 2, collect at the following cycles: Cycles 1, 4, 7, 10, 13, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 46.
- For participants on dose levels -1 and -2, collect at the following cycles: Cycles 1, 7, 10, 19, 25, 31, 37, and 43.
- For participants on the combination therapy solid tumor expansion cohort collect at the following cycles: Cycles 1, 4, 7, and 10.
- The pre-dose sample and the Day 1 Cycle 1 sample must be drawn on different calendar days, and should be collected at least 12 hours apart.
- With a black, water resistant, sharpie pen, label each specimen with the following information:
    - AMC Protocol # 095
    - AMC Participant ID#
    - Date and time of collection
    - Specimen type, i.e., WB=Whole Blood, P=Plasma, PBMCs=Peripheral blood mononuclear cells
    - Specimen purpose: HIV and Herpesvirus Viral Load Studies

Specimen Shipment

- Seal the tops of the tube(s) with parafilm.
- Place the sealed tube(s) into bubble wrap (provided in STP-210 kit).
- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and "self-seal."
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX airbill on blank side of the shipper making sure that it is marked "FED-EX PRIORITY OVERNIGHT."
- Mark "OTHER" in the airbill under "Packaging." Please refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.Under airbill section "Special Handling" indicate "YES-SHIPPERS DECLARATION NOT REQUIRED."
- Place "From/To" information onto areas provided on the shipper.

C. BLOOD SPECIMENS should be shipped by overnight express at room temperature to:

AMC Biorepository at GWU
Jeff Bethony, PhD
George Washington University Medical Center
Ross Hall, Room 118
2300 I Street NW
Washington, DC 20037
Tel: (202) 994-3422
Fax: (202) 994-5056
Email: amc-bio@emmes.com

- Make certain that shipper is already either pre-labeled with 'UN#3373' stamp, or make a paper label with 'UN#3373" and affix it to the shipper.
- Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in ml and affix to the shipper.
- Affix airbill to shipper so that the 'UN' and 'VOLUME' labels are visible.
- RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.
- Place the box in the FedEx pickup area at your site or call to request a package pickup.

Please Note: The shippers will be mailed back to each AMC site.

Specimens should be sent by 24-hour shipment at room temperature. If collecting on a Thursday through Saturday where 24-hour shipment is not possible, please see Section below for instructions regarding separation of PBMCs from blood. In this case separation will need to occur on site and within 6 hours of blood collection.

D. *INSTRUCTIONS FOR SEPARATION OF BLOOD FOR PBMCS (FOR RESEARCH) COLLECTED ON SUNDAY – THURSDAY AND PERIPHERAL BLOOD COLLECTED ON FRIDAY - SATURDAY*

From study participant, draw:

Five 8.5 ml yellow top (ACD) tubes for PBMCs (see Appendix XVI for PBMC separation directions) at the time points described in Appendix I.

With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol # 095
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., P=Plasma, PBMCs=Peripheral blood mononuclear cells
- Specimen purpose: HIV and herpesvirus assays

Specimen Shipment

- Seal the tops of the tube(s) with parafilm.
- Place the sealed tube(s) into bubble wrap (provided in STP-210 kit).

- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and "self-seal."
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX airbill on blank side of the shipper making sure that it is marked "FED-EX PRIORITY OVERNIGHT."
- Mark "OTHER" in the airbill under "Packaging." Please refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.Under airbill section "Special Handling" indicate "YES-SHIPPERS DECLARATION NOT REQUIRED."
- Place "From/To" information onto areas provided on the shipper.

Preparation of Peripheral Blood Mononuclear Cells

Refer to Appendix XVI for instructions.

*****PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING. Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.**

E. *PREPARATION OF TISSUE SAMPLES*

- From all participants, obtain four 4 micron unstained tissue slides from the diagnostic biopsy.
- From KS study participant, obtain:
    - Skin biopsy; formalin-fixed. Biopsy will be obtained at baseline/Day 1 of Cycle 1, Day 1 of Cycle 5, and at treatment discontinuation.
    - Skin biopsy; RNALater. Biopsy will be obtained at baseline/Day 1 of Cycle 1, Day 1 of Cycle 5, and at treatment discontinuation. RNALater biopsy must be kept at -20°C or lower, -80°C is preferred.
    - Tissue specimens (punch biopsies) should be placed (a) into no more than 5 mL buffered formalin and (b) 1 ml RNALater provided by the AMC Core Lab in batches to each site.

***NOTE**: Specimens can only be accepted **Monday through Friday**. Therefore, specimens can only be shipped **Sunday-Thursday** for delivery the next day. Samples in RNALater are to be shipped on dry ice, and tissue slides and samples in formalin are to be shipped in ambient temperature.

TISSUE specimens should be shipped to:

Jeff Bethony, PhD
AMC Biorepository
George Washington Medical Center
Ross Hall, Room 118
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056
Email: amc-bio@emmes.com

F. BILLING

Please refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.. It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.

G. RECORD OF SPECIMENS

This study will track specimens via GlobalTrace[SM], a component of the AMC AdvantageEDC[SM] system. The GlobalTrace[SM] shipment manifest must accompany all specimen shipments.

H. TECHNICAL QUESTIONS

Questions regarding these specimens may be directed to Dr. Jeff Bethony using the above contact information. Available hours: Monday-Friday (9AM-5PM EST)

APPENDIX XV: COLLECTION AND SHIPPING INSTRUCTIONS FOR PERIPHERAL BLOOD FOR IMMUNE RESPONSE (CYTOKINES AND SOLUBLE RECEPTORS)

1.0 OBJECTIVES

The aim of this proposed pilot study is to evaluate the effect of therapy with ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) on plasma levels of biomarkers of immune activation, at baseline/day 1 of cycle 1, day 1 of cycle 5, and treatment discontinuation, to identify the clinical correlates with regard to tumor response and disease free survival.

2.0 BACKGROUND

Ipilimumab and nivolumab, monoclonal antibody-based therapeutics for the treatment of cancer, work by enhancing T cell activation and effector functions. They do this by reversing the inhibition of T cell activation and effector functions due to ligation of CTLA-4 and PD-1. Therefore, measurement of biomarkers of T cell activation, including cytokines, soluble cytokine receptors, and biomarkers of immune activation, may provide insights into the biological effects of treatment with these agents, and may provide prognostic information regarding responses to treatment. These molecules are products of activated T cells, including TH1, TH2, TH17, T follicular helper, and regulatory T cells.

3.0 RATIONALE

Inhibition of T cell activation and effector functions has the potential to contribute to the growth of cancers in HIV infected persons, by allowing these cancers to evade host immune responses. Reversal of T cell inhibition mediated by CTLA-4 and/or PD-1 ligation by ipilimumab and nivolumab may lead to potent antitumor immune responses. Additionally, enhanced T cell immunity following treatment may provide more effective responses to HIV infection or to other viral infections. However, there is little information available on the effect of treatment with these agents on serum levels of cytokines and immune activation biomarkers, nor on the prognostic significance of such biomarkers.

The recent availability of multiplexed immunometric assays has made possible the simultaneous assessment of several of these immune activation and inflammation-associated factors, using small volumes (<500 μl) of serum or plasma.

The results of this study will provide information on immune activation/inflammation-associated biomarker plasma levels in HIV+ cancer participants treated with ipilimumab and nivolumab.

4.0 EVALUATIONS

Serum levels of cytokines and inflammation-associated molecules will be determined at the following study visits: pre-study (entry), and prior to Cycle 5 of therapy, and then at the off study or early discontinuation visit.

5.0 ANALYSES

The working hypothesis that will be tested is that participants who are treated with ipilimumab and nivolumab will display elevated levels of T cell-produced cytokines following treatment initiation, and that the level of T cell cytokine responses will diminish with time, following the conclusion of therapy.

Levels of cytokines and inflammation-associated biomarkers will be determined using two multiplexed (Luminex platform) panels. The first is a high-sensitivity cytokine panel that can detect human IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNFα, and IFNγ. The second is a soluble receptor/inflammatory cytokine panel: sCD14, sIL-6R, sIL-2R, sTNFR1, sTNFR2, CXCL13, IP10, and sCD30. Both of these panels are produced by R&D Systems, and we have used variations of these assays extensively in prior epidemiologic studies. The cytokine panel has yielded results that compare well to ELISA results for IL-6 and IL-10. The soluble receptor/inflammation panel yields detectable results for these biomarkers in the majority of persons tested.

2 ml of serum in 0.5 ml aliquots should be sent in batch at the end of the study to the Martinez-Maza laboratory.

6.0 SHIPPING INSTRUCTIONS AND SAMPLE PROCESSING

A. GENERAL

To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website www.saftpak.com. The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

NOTE: Specimens MUST BE SHIPPED Monday through Thursday as an OVERNIGHT PRIORITY shipment. Specimens are NOT ACCEPTED ON SATURDAY OR SUNDAY in the AMC BIOREPOSITORY.

Should the site collect specimens on Friday through Saturday, samples should be processed for serum according to the procedures in Section C below for serum separation. At least four 0.5 ml aliquots of serum are required. Aliquots should be frozen at -80 C and shipped on dry ice to the AMC Biorepository the following week.

B. SPECIMEN PREPARATION AND PACKAGING FOR SAME DAY SHIPMENT

Draw two 6 ml red top (uncoated) tubes from study participant. Seal the tops of the tubes with parafilm. Individually wrap the sealed tubes in bubble wrap (provided in STP-210 kit) and tape around the bubble wrap so that the tubes are secured and the tubes cannot fall out or break. Place an absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and "self-seal." Place poly-bag containing tubes into the white TYVEK bag and seal. Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.

Affix the FedEx airbill on blank side of the shipper making sure that it is marked "FedEx PRIORITY OVERNIGHT." Mark "OTHER" in the airbill under "Packaging." Under airbill section "Special Handling," indicate "YES-SHIPPERS DECLARATION NOT REQUIRED." Place "From/To" information onto areas provided on the shipper.

Blood specimens should be shipped by overnight express at room temperature to:

Jeff Bethony, PhD
AMC Biorepository
George Washington Medical Center
Ross Hall, Room 118
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056
Email: amc-bio@emmes.com

Make certain that shipper is already either pre-labeled with 'UN#3373' stamp. Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in ml and affix to the shipper. Affix airbill to shipper so that the 'UN' and 'VOLUME' labels are visible. RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS. Place the box in the FedEx pickup area at your site or call to request a package pickup.

C. SERUM SEPARATION SPECIMEN PREPARATION AND PACKAGING FOR DELAYED SHIPMENT

- For immediate processing, after blood is drawn, gently invert collection tubes five times and allow the blood to clot 60 minutes at room temperature, leaving the tube in an undisturbed upright position during the clotting process. Once clotted, the collection tube should not be allowed to sit at room temperature for more than 1 hour prior to centrifugation and separation of the serum from the clot.
- If the site would like to hold the sample for processing later the same day, store the sample at 4°C until ready to invert the tube and clot the sample.
- Centrifuge the collection tube for 10 minutes at 1000 x g. After separation, the serum should be clear and free from all blood cells. Using a clean serological pipette tip for each sample, dispense the serum into a minimum of four 0.5 ml aliquots in cryovials
- Discard the red top tube and residual blood fraction in accordance with local procedures for medical waste.
- Label each serum aliquot tube with an AMC GlobalTrace specimen label. Specify the specimen type as "Serum" when entering each sample into GlobalTrace. With a black, water resistant, sharpie pen, label each specimen with the following information:
    - AMC Protocol # 095
    - AMC Participant ID#
    - Date and time of collection
    - Specimen type, i.e., S=Serum
    - Specimen purpose: Cytokine assays

- Separated sera aliquots must be stored frozen until ready for shipment to the AMC Biorepository on next operating day (no longer than 72 hours after collection).

Once sera aliquots are frozen at -80°C, sera must be shipped on dry ice to avoid thawing during shipment. Specimens should be placed on dry ice immediately. The specimens must stay on dry ice until being transferred to a -80ºC freezer.

Specimens may be batched for shipping after storage in -80ºC freezer. *NOTE: Specimens can only be accepted Monday through **Friday**. Therefore, specimens can only be shipped **Sunday-Thursday** for delivery the next day. Shipping frozen specimens requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.

Billing

Please refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.

Contact for Site Questions (9AM-5PM Pacific Time)

Lab Manager: Larry Magpantay (lmagpantay@mednet.ucla.edu)

Lab phone: 310-206-6846

Oto Martinez-Maza's office phone: 310-825-2542

7.0 AMC BIOREPOSITORY INSTRUCTIONS ONLY

The AMC Biorepository will separate the whole blood samples, aliquot the serum into as many 0.5 ml aliquots until the sample is used as per AMC Biorepository SOPs, and store at -80C. Specimens should be sent in batch at the end of the study to:

Larry Magpantay
BSRB 173
UCLA AIDS Institute
615 Charles Young Drive
Los Angeles, CA 90095-7363
Phone: 310-206-6846
Fax: 310-206-5387
Email: lmagpantay@mednet.ucla.edu

APPENDIX XVI: HIV LATENT RESERVOIR AND VIRAL OUTGROWTH ASSAY SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS

Blood samples for HIV latent reservoir testing should only be collected for participants with hematocrit ≥ 25.

Latent Reservoir Quantification

To ship blood for latent reservoir quantitation, place twelve (or two) 8.5 mL yellow top (ACD) tubes into a canister of a STP-100 SAF-T-PAK shipper (VWR# 11217-163) wrapping each tube in bubble wrap and using the absorbent paper at the bottom of the canister. Each sample tube should be labeled using a Sharpie pen with the following information:

- Protocol #: AMC-095
- 9 digit Participant #
- Date and time of collection
- Specimen type: "Whole Blood"
- Specimen purpose: "HIV latent reservoir"

Specimen Shipment. Specimens are accepted TUESDAY through FRIDAY. All specimens should be shipped to:

AMC Biorepository at GWU
Jeff Bethony, PhD
George Washington University Medical Center
Ross Hall, Room 118
2300 I Street NW
Washington, DC 20037
Tel: (202) 994-3422
Fax: (202) 994-5056

- Email: amc-bio@emmes.comPlease refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.It is the responsibility of the Primary Investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens act in conformance with International Air Transport Association (IATA) regulations (IATA Packing Instruction 650) relating to the handling and shipping of hazardous goods. **(IATA Packing Instruction 650 is located on the AMC web site.)**

- Use a federally approved shipper for biological substance shipment (Category B). Label the shipper with the "Infectious substance" diamond-shaped label. On one side, in black marker write "Biological Substance, Category B, UN 3373", your name or name of responsible person, date of collection, and phone number of the person responsible for the package. Package and label the shipment in accordance with the instructions provided for that specific shipper.

- A *Shipper's Declaration for Dangerous Goods* is **not** required. If shipping separated specimens on dry ice, the following information must be shown in sequence on the airway bill in the "Nature and Quality of Goods" box: Dry Ice, 9, UN1845, number of boxes being shipped, net weight of dry ice per box.

Please Note: The shipper will be mailed back to the AMC site.

The STP-100 SAF-T-PAK shipper (VWR Cat #11217-163) is a complete shipment kit. To reuse the shipper, there is a refurbishment kit with sufficient replacement materials (STP102) (VWR Cat # 11217-166) for 15 mailings.

INSTRUCTIONS BELOW FOR BLOODS COLLECTED ON FRIDAY:

In the event that blood samples are drawn on Friday, the samples must be processed into plasma and PBMC immediately to maintain their viability for analysis, frozen over the weekend, and shipped to the lab on Monday. The HIV latent reservoir assay will be conducted using frozen PBMC and the single copy HIV viral load assay will be conducted using frozen plasma specimens.

Preparation of Plasma and PBMCs

Blood is to be processed on site within 6 hours of collection.

Materials

- Lymphocyte Separation Medium (LSM Solution, Ficoll-Hypaque - sterile)
- 15 ml conical centrifuge tubes (sterile)
- PBS (sterile)
- 1 ml, 5 ml and 10 ml serologic pipettes (sterile)
- 1.5 ml NUNC tubes
- Alcohol saturated, control rate freezer container
- DMSO freezing media
- 50% Cryoprotective Medium, Cambrex (catalog no.:12-132A)
- 50% Heat Inactivated Fetal Bovine Serum

Plasma Separation and Freezing Procedures

1. The 10 ml tubes of whole blood in ACD should be rotated gently two or three times before being centrifuged. Do not transfer before centrifugation.
2. Separate the cells by centrifugation at 500 g for 10 minutes.
3. Remove 0.5 ml aliquots of plasma and put into separate 1.5 ml NUNC tubes and transfer to liquid nitrogen storage.

PBMC Separation and Freezing Procedures

1. The cells and plasma remaining from the previous step are transferred into a 15 ml conical tube or 50 ml centrifuge tube depending on volume.
2. Sterile PBS should be added to the suspended whole blood cells in an equal volume and pipetted up and down to mix (1:1).
3. The whole blood-PBS mixture should be carefully overlaid onto 4-5 ml of room temperature LSM or Ficoll-Hypaque solution in a sterile 15 ml conical centrifuge tube. A sharp interface should exist between the LSM and the whole blood mixture. (If the layer of LSM gets mixed with the blood-PBS, the tube should be gently rotated to mix the blood, PBS, and LSM, and transfer to a 50 ml sterile conical tube. An equal volume of PBS is added, and the cells are separated at 600 g for 15 minutes. After removal of LSM-PBS supernatant, return to Step 2).

4. Centrifuge the 15 ml conical tube for 30 minutes at 900 g at room temperature. The mononuclear leukocytes (principally lymphocytes and monocytes) will band at plasma/LSM interface.
5. The fluffy white layer just below the plasma layer should be aspirated off and transferred to an appropriately labeled 15 ml sterile conical centrifuge tube. Be careful to remove only the interface and a minimum amount of the LSM or Ficoll-Hypaque.
6. Add three volumes of PBS to the cell suspension and or enough to fill conical and mix by pipetting up and down.
7. Centrifuge at 500 g for 10 minutes.
8. Aspirate off and discard supernatant, taking care not to disturb pellet.
9. Resuspend in 12 ml of PBS. Take 10 □l of suspension for cell counting (dilute accordingly whether using a hemocytometer or automated cell counter). Centrifuge again for 10 minutes at 500g to wash cells.
10. Using a 1 ml pipette, the *DMSO freezing mixture should be added dropwise to the cell pellet suspension. Gently finger-tap between drops to resuspend cells. If the cell pellet is small, only 0.5 ml of freezing media is added (and only one aliquot of cells is frozen). If the cell pellet is large, up to 2 ml of freezing media can be added in a drop wise fashion. (Cell densities of 1-10 million PBMC/ml are best for cryopreservation. If a hemocytometer is available, the optimal concentration is 5 x 10^6 PBMC/ml).

    ***Important: Do not put the DMSO containing media on the cell button all at once.**

    Freeze the cell suspension in 0.5 ml aliquots in sterile NUNC vials by placing the NUNC tubes in a room temperature, alcohol saturated, control rate freezer container and store in the -80°C freezer overnight. Transfer the cell suspension into the liquid nitrogen temperature freezer for long term storage the next working day.

RECORD OF SPECIMENS

This study will track specimens via GlobalTrace℠, a component of the AMC AdvantageEDC℠ system. The GlobalTrace℠ shipment manifest must accompany all specimen shipments.

TECHNICAL QUESTIONS

AMC Biorepository at GWU
Jeff Bethony, PhD
George Washington University Medical Center
Ross Hall, Room 118
2300 I Street NW
Washington, DC 20037
Tel: (202) 994-3422
Fax: (202) 994-5056
Email: amc-bio@emmes.com

APPENDIX XVII: PROCEDURE FOR INTRA-ANAL HPV DNA PCR SAMPLE COLLECTION, PACKAGING, AND SHIPPING

All HPV DNA PCR specimens will be collected at the local institution and shipped to the AMC Biorepository. Specimens will be shipped to the Joel Palefsky Laboratory at the end of the study for testing.

PROCEDURE FOR OBTAINING INTRA-ANAL HPV DNA PCR SAMPLE

The subject should undress from the waist down, and either bend over the exam table or lay on his side in the fetal position. The examiner should use one hand to spread the buttocks and expose the anoderm. A Dacron or polyester (not cotton, and not scored) swab moistened in sterile saline will then be inserted as far as is comfortable into the anus, a minimum of 2-3 inches. Do not use the swab provided with the Digene kit as it is scored to break easily into the collection tube and can break in the anus. If there is difficulty inserting the swab, the subject should also retract their buttocks. With pressure on the distal end of the swab, rotate it gently and slowly in a circular fashion as it is withdrawn over 15 to 30 seconds. Do not retract the buttocks when the swab is close to the verge to ensure that it is sampled as well.

Immediately immerse the swab into a tube containing 1 mL of Specimen Transport Medium (Digene Female Swab Specimen Collection Kit, Qiagen Catalog #5123-1220, 1-800-426-8157), agitating vigorously over 10 to 15 seconds to disperse the cells.

The swab should be placed in the collection vial and cut with sterile or single-use scissors to allow the swab to fit in the vial. The cap should then be replaced securely.

Note: Specimens will be processed for HPV DNA PCR, **not** Digene HCII.

SAMPLE LABELING

Each sample must be labeled with the following information using AMC GlobalTrace labels. Each label is preprinted with a 9-digit specimen ID number:

- **Nine (9) Digit Subject ID:** 095-XXX-XXX
- **Specimen Type:** "Anal Swab"
- **Specimen Purpose:** "HPV DNA PCR"
- **Date of Sample Collection:** MM/DD/YYYY

STORAGE

Anal specimens obtained for HPV DNA testing should be stored at -80° C until they are ready to be shipped. All specimens will be batch shipped quarterly on **dry ice** by overnight delivery to the AMC Biorepository.

If the site does not have a -80° C freezer, the site must store the specimens in refrigeration if possible (2-8° C) and ship the specimens to the AMC Biorepository within two weeks of collection.

SHIPPING

Please note that the shipment of these samples requires certified training in IATA regulations. All shipping materials can be ordered from SAF-T-PAK at www.saftpak.com.

1. Please use the Saf-T-Pak STP 320 shipper (each tube must be sealed with moisture-resistant tape or parafilm under current IATA rules). Place securely fastened tubes in the clear biohazard bag with the absorbent strip. Seal bag.
2. Place clear biohazard bag into the white TYVEK bag and seal bag.
3. Place white TYVEK bag into inner box.
4. Place the inner box into the STP 320 insulated box and place 5 lbs of dry ice in the insulated box around each side of the inner box. For sites shipping refrigerated specimens, use four frozen cold packs instead of dry ice, each placed in the space between the inner box and the inner wall of the insulated box.
5. Place the lid on the insulated box. Place the GlobalTrace shipping manifest on top of the lid.
6. Seal the box and place your FedEx airbill on the outside.
7. Please refer to the MOP and the AMC Operations website's (www.AIDSCancer.org) Shipping tab for more information and the FedEx account details.It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.Include a return FedEx Express or Ground Airbill charged to the AMC FedEx account for the return of shippers.
8. Specimens should be shipped by overnight express to:

    Jeff Bethony, PhD
    AMC Biorepository
    George Washington Medical Center
    Ross Hall, Room 118
    2300 I Street, NW
    Washington, DC 20037
    Tel: (202) 994-2945
    Fax: (202) 994-5056
    Email: amc-bio@emmes.com

***PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING. Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package. STP-320 will come pre-printed with: Biological Substance Category B Marking, Exempt Human/Animal Specimen Marking, UN3373 Marking, Orientation Arrows, Class 9 Label and UN1845 Dry Ice Marking

RECORD OF SPECIMENS

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC^{SM} system. The GlobalTrace^{SM} shipment manifest must accompany all specimen shipments.

AMC BIOREPOSITORY INSTRUCTIONS

Specimens will be frozen at -80C and stored until the end of the trial. Following receipt of the last specimens, the Biorepository will ship all specimens on dry ice to the Joel Palefsky Laboratory.

Joel Palefsky Laboratory
C/o Maria Da Costa
University of California, San Francisco
513 Parnassus Ave., Room S-420
San Francisco, CA 94143
Tel: 415-476-8885
Email: Maria.DaCosta@ucsf.edu

Specimens must only be shipped on Mondays and Tuesdays and not during the week of a federal holiday.

TECHNICAL QUESTIONS

Questions regarding these specimens may be directed to Maria Da Costa using the above contact information for the Palefsky Laboratory.