SUMMARY OF CHANGES

For Protocol Amendment #12 to: NRG-GY003

NCI Protocol #: NRG-GY003
Local Protocol #: NRG-GY003

NCI Version Date: February 28, 2020

#	Section	Change
1.	Title Pages	• NCI Version Date is now February 28, 2020. • Includes Amendments #1-12. • Dr. Dmitriy Zamarin is now the Study Chair. • Dr. Khleif's contact information was updated. • Dr Powell's email was updated.
	ICD	The only change that was made to the ICD was the NCI Version Date.

NRG ONCOLOGY

NRG-GY003
(ClinicalTrials.gov NCT #02498600)

Includes Amendments #1-12

Phase II Randomized Trial of Nivolumab with or without Ipilimumab in Patients with Persistent or Recurrent Epithelial Ovarian, Primary Peritoneal or Fallopian Tube Cancer

This trial is part of the National Clinical Trials Network (NCTN) program, which is sponsored by the National Cancer Institute (NCI).

Lead Organization: NRG / NRG Oncology
This study is limited to NRG Oncology participation

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NCI-Supplied Agents: BMS-936558 (Nivolumab, MDX-1106) NSC #748726
Ipilimumab (BMS-734016; MDX-010 Transfectoma-derived) NSC #732442

IND Sponsor: Division of Cancer Treatment and Diagnosis, NCI **(02/22/2016)**

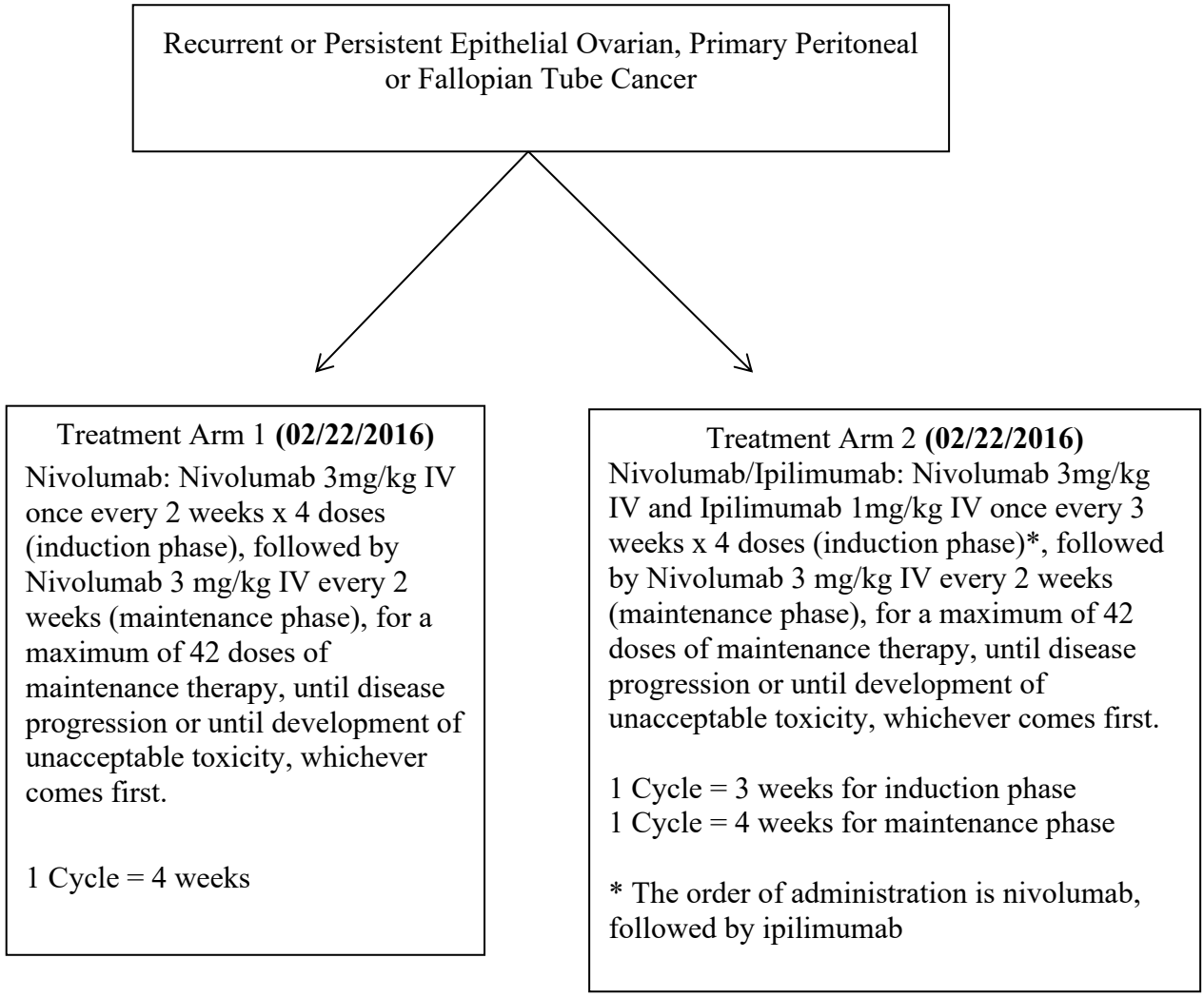
Participating Sites

☒ U.S.
☐ Canada
☐ Approved International Member Sites

Document History

	Version/Update Date	
Amendment 12	**February 28, 2020**	
Amendment 11	06/10/2019	
Amendment 10	**02/28/2019**	
Amendment 9	**12/21/2018**	
Amendment 8	**09/07/2018**	
Amendment 7	**07/30/2018**	**Broadcast Date**
Closure	**07/14/2017**	**08/28/2017**
Amendment 6	**07/14/2017**	**07/31/2017**
Re-activation and Amendment 5	**04/28/2017**	**05/22/2017**
Amendment 4	**01/19/2017**	**02/13/2017**
Amendment 3	**02/23/2016**	**03/21/2016**
Amendment 2	**02/02/2016**	Amendments #1 and 2 Broadcast together: **02/22/2016**
Amendment 1	**01/25/2016**	
Temporary Closure	**05/21/2015**	**10/07/2015**
Activation	**05/21/2015**	**06/29/2015**

NRG-GY003
SCHEMA



Tumor measurements scheduled according to time period since beginning protocol therapy: every 8 weeks (+/- 7 days) from cycle 1, day 1 (regardless of delays and/or changes in treatment schedule) for the first 8 months; then every 12 weeks (+/- 7 days) thereafter until disease progression is confirmed; also repeat at any other time if clinically indicated based on symptoms or physical signs suggestive of new or progressive disease. See Section 4 for more details.

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To estimate the proportion of patients who have objective tumor response (complete or partial) by modified RECIST 1.1 in patients with persistent or recurrent epithelial ovarian, fallopian tube, primary peritoneal cancers, treated with nivolumab or the combination of nivolumab and ipilimumab and to assess the difference in ORR between patients treated with nivolumab versus those treated with the combination of nivolumab and ipilimumab.

1.2 Secondary Objectives (09/07/2018)

1.2.1 To estimate the PFS hazard ratio for patients treated with nivolumab versus those treated with the combination of nivolumab and ipilimumab. To estimate and compare the duration of OS for patients treated with nivolumab or the combination of nivolumab and ipilimumab. PFS by modified RECIST 1.1 will also be examined.

1.2.2 To determine the frequency and severity of adverse events associated with treatment with nivolumab or the combination of nivolumab and ipilimumab as assessed by Common Terminology Criteria for Adverse Events (CTCAE).

1.2.3 To determine whether cellular and molecular laboratory parameters in pre-treatment tissue and peripheral blood specimens predict overall survival (OS), tumor response by modified RECIST 1.1, and progression-free survival (PFS): **(09/07/2018)**

(1) PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes (TILs) measured by quantitative immunohistochemistry (IHC);
(2) Natural anti-tumor immunity in tumor cells and TILs measured using IHC and T cell repertoire analyses;
(3) Tumor "immunogenicity" as determined by the neo-epitope landscape using next-generation whole exome sequencing (NGS);
(4) Anti-tumor immune response in peripheral blood, including serologic responses and analysis of T cell receptor (TCR) repertoires by deep sequencing; and
(5) Shift in quantitative peripheral blood parameters after the first 6 and 12 weeks of therapy.

2. BACKGROUND

2.1 Rationale for Immunotherapy

Most epithelial cancers of ovarian, fallopian tube and primary peritoneal origin are genetically unstable, aggressive neoplasms accounting for the majority of deaths related to gynecologic malignancies.[1] Unfortunately, despite front-line therapy including surgical resection and combination platinum-taxane chemotherapy, the vast majority of patients will recur and ultimately die from disease. Thus, there is a great unmet need for the development of complementary and augmentative therapeutic approaches. In particular, strategies reversing tumor promoting processes involving the interaction between tumor-infiltrating T-cells and the host microenvironment may be worthwhile, especially in genetically unstable tumors.

There is abundant evidence that native host anti-tumor cell mediated immune mechanisms play a role in controlling malignant progression of epithelial ovarian cancer, the majority of which exhibit high grade serous morphology. Investigators at the University of Pennsylvania first discovered the impact of anti-tumor immune response on overall survival (OS) in patients with newly diagnosed advanced epithelial ovarian cancer, where the presence of CD3+ T-cells infiltrating tumor islets (intra-tumor T-cells) was found to be a predictor of prolonged OS.[2] The five-year OS rate was 38 percent among patients whose tumors contained CD3+ tumor infiltrating lymphocytes (TILs) versus 4.5 percent among patients whose tumors contained no CD3+ TILs within tumor islets. Furthermore, in a Cox proportional hazards regression model, the prognostic significance of TIL was independent of residual disease after initial surgical cytoreduction, tumor grade, cell type, previous receipt of taxane therapy, and age at diagnosis. Finally, the presence of TILs was associated with increased tumor expression of interferon gamma (IFN-γ) and other lymphocyte-attracting cytokines. Sato et al. independently showed that the accumulation of TILs in ovarian cancers was associated with improved progression-free survival (PFS) and OS.[3] The accumulation of CD8+ TILs, but not CD4+ TILs, was shown to predict improved OS, suggesting a role for CD8+ T-cells in tumor control. TILs isolated from ovarian cancers can recognize autologous tumor and known tumor antigens *in vitro*,[4-8] can exhibit tumor-specific cytolytic activity *ex vivo*[9,10] and are oligoclonal in composition.[11,12] We demonstrated that tumor-specific T-cell precursors are also detected in the peripheral blood of approximately 50% of patients with advanced ovarian carcinoma.[13] Furthermore, we have shown that TILs within epithelial ovarian cancers exhibit tumor-rejecting features *in vivo* and that ovarian cancers can be immunogenic (see below). Thus, naturally arising T lymphocytes with anti-tumor potential pre-exist in patients with epithelial ovarian cancer and underscore the potential for immunotherapy to affect the natural course of Mullerian duct carcinomas in order to improve patient outcome.

Until recently, the immuno-biology of spontaneous tumor-reactive T-cells in ovarian cancer was not well-defined, largely due to the difficulty in identification and interrogation of responses. Recently, we elucidated CD137 (4-1BB), an activation induced TNF receptor family member, as a biomarker for bona fide naturally-occurring tumor-reactive T-cells in epithelial ovarian cancers.[14] CD137+ TILs, but

not CD137- TILs, demonstrated HLA-dependent IFN-γ secretion in response to autologous tumor stimulation and were capable of controlling epithelial ovarian cancer outgrowth *in vivo*. These results have enabled rapid identification of tumor-reactive T-cells in this disease. We have now validated the existence of CD137+ TILs in ovarian cancer samples via analytical immunohistochemistry (IHC), allowing for the quantification of tumor-reactive TILs in biopsied tumor specimens. Taken together, we have demonstrated the potency of TILs in the control of epithelial ovarian cancer progression and have revealed an important role for CD137 in the immuno-biology of ovarian cancer, wherein this marker is preferentially expressed on the tumor-reactive TIL subset, rationalizing the use of immunotherapy to bolster the accumulation of CD137+ TIL.

2.2 Evasion of Natural Cell-Mediated Immunity

On the other hand, solid tumors may successfully evade immune attack by blocking effector T-cell trafficking into tumor islets and inhibiting effector T-cell activation. There are two important parallel mechanisms of tumor induced cell mediated immunologic tolerance in ovarian cancer which may operate in a complementary fashion and represent candidate therapeutic targets to reverse spontaneous T-cell mediated anti-tumor immunity.

First, in general, activation of T-cells requires binding of the T-cell receptor (TCR) to antigen in association with major histocompatibility (MHC) molecules, plus co-stimulation by antigen-presenting cells (APCs).[15] Such co-stimulation involves binding of CD86 and CD80 on APCs to CD28 on T-cells, and absence of co-stimulation through CD28 is associated with anergy. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a negative regulatory homologue of CD28 found on activated T-cells and, by competing with CD28 for CD86 and CD80 binding functions as a checkpoint to prevent auto-immunity. CTLA-4 is also expressed ubiquitously on a naturally occurring subpopulation of $CD4^+$ regulatory T-cells (Treg) constitutively expressing FOXP3 and CD25 (the IL-2 receptor-α chain) and thought to inhibit autoimmune pathology, mediate transplantation tolerance, impede anti-tumor immunity and prevent the expansion of other T-cells *in vivo*.[16] Treg cells require activation via their T-cell receptor (TCR) to become suppressive, and once activated inhibit the proliferation of effector T-cells in an antigen-unrestricted manner. Cancer cells may secrete chemokines such as CCL22 that specifically recruit Treg cells. The paradigm of Treg-mediated immunosuppression in human cancer was originally defined in epithelial ovarian cancer.[17] In a detailed analysis of Treg cells in 104 ovarian carcinoma cases, it was shown that human tumor-infiltrating Treg cells suppressed tumor-specific T-cell immunity and contributed to growth of human tumors *in vivo*. At all stages of disease, increased accumulation of Treg cells in tumor was associated with poor OS. In addition, a high CD8+/Treg ratio has been associated with favorable prognosis in epithelial ovarian cancer,[3] suggesting that an abundance of CD8 effector cells may overcome Treg-mediated suppression. We therefore hypothesize that those immune therapies that enhance CD8+ T-cell accumulation and activity while reducing the population of Treg cells will demonstrate anti-tumor activity. Experimental depletion of Treg cells in tumor-

bearing mice has been shown to improve immune-mediated tumor clearance, indicating that strong anti-tumor immunity may require breaking Treg-mediated tolerance to tumor antigens.[18,19] van Elsas et al showed that treatment with anti-CTLA-4 antibody promoted tumor rejection and T-cell activation in a mouse model of melanoma.[20] Experimental work in animal models using this approach ultimately led to clinical development of ipilimumab (see full details below in protocol section 2.5), a CTLA-4 neutralizing humanized monoclonal antibody FDA approved for the treatment of metastatic melanoma[21] based on a phase III trial demonstrating significant prolongation of OS.[22] Though the application of CTLA-4 targeted therapy with Ipilimumab represents a rational approach in the treatment of advanced Mullerian duct adenocarcinomas (particularly high-grade serous cancers), clinical trial experience to date for this disease entity has been limited to two patients with relapsed epithelial ovarian cancer in the initial phase I study.[23]

A second mechanism involves signaling through the negative regulatory co-stimulatory T-cell receptor, human programmed death-1 (PD-1, CD279).[24,25] PD-1 is highly expressed on activated T-cells. The principal ligands for PD-1 are PD-L1 (B7-H1, CD274),[25,26] expressed on tumor cells, antigen-presenting cells and dendritic cells and PD-L2 (B7-DC, CD273),[27,28] commonly expressed on endothelial cells. Ligand binding leads to de-activation of T-cells. PD-L1 is expressed in some normal tissues and thought to maintain host T-cell-mediated immunologic tolerance. Of note, expression of PD-L1 by a variety of solid tumors has been implicated in evasion of anti-tumor immunity. PD-L1 expressed on tumor cells from epithelial ovarian cancers has been shown to inhibit anti-cancer immunity.[26] A study by Hamanishi et al. suggested that expression of both PD-L1 and PD-L2 in human ovarian cancers is associated with poor OS and that expression of PD-L1 was inversely correlated with the presence of CD8+ (effector) T-cells in tumor islets.[29] The same group also showed in a pre-clinical model that PD-L1 bearing tumors inhibited tumor-directed cytotoxic T-cell activity. Maine et al. showed that PD-L1 was over-expressed on monocytes within ascites of patients with ovarian cancer; they demonstrated functional links between PD-L1 expression on monocytes and tumor cells.[30] Furthermore, blocking the interaction of PD-L1 with PD-1 on T-cells has resulted in tumor regression in a mouse epithelial ovarian cancer model.[31] We recently showed that nearly all human tumor-reactive TILs in epithelial ovarian cancer express high levels of PD-1,[14] requiring signaling through its ligands (most notably PD-1) to suppress T-cell function. Thus, disruption of PD-1/PD-L1 interaction is a rational strategy for immunotherapy in patients with advanced epithelial ovarian cancer. Nivolumab (see full details below in protocol section 2.4) is a humanized monoclonal antibody binding to PD-1 and blocking the interaction between PD-1 and its ligands, thus inhibiting PD-1 signal transduction in activated T-cells. A phase I dose-finding trial involving 296 patients with multiple non-gynecologic tumors demonstrated response rates of 18% (14 of 76), 28% (26 of 94) and 27% (9 of 33) among patients with non-small-cell lung cancer, melanoma and renal-cell cancer, respectively.[32] Overall, two-thirds of responses were durable for at least one year. Importantly, response in 42 patients with pretreatment tumor specimens was associated with tumor expression of PD-L1 by IHC analysis, with no

responses in 17 patients bearing PD-L1 (-) tumors and 9 (36%) responses in 25 patients with PD-L1 (+) tumors. Further rationale for targeting PD-1/PD-L1 pathway in gynecologic malignancies was demonstrated in a phase I study of anti-PD-L1 antibody in patients with advanced cancer, which included 17 patients with ovarian cancer.[33] Of the ovarian cancer cohort, 22% of the patients had evidence of objective response or stable disease, lasting at least 24 weeks.[33]

2.3 Rationale for Combined CTLA-4 and PD-1 Targeting

The non-redundant nature of CTLA-4 and PD-1 mediated T-cell inhibition suggests the potential for additive or synergistic anti-tumor activity with dual therapeutic targeting. To determine whether concurrent therapy with nivolumab and ipilimumab may have applicability for human epithelial ovarian cancer, we performed molecular profiling of IFN-γ+ CD137+ tumor-reactive TILs from fresh tumor biopsies, which revealed that the tumor-reactive fraction of TILs in epithelial ovarian cancers express both surface PD-1 and CTLA-4 **(Figure 1)**. To model concurrent therapy for epithelial ovarian cancer, we showed that blockade of both PD-1 and CTLA-4 resulted in reversal of CD8+ TIL dysfunction and led to tumor rejection in two thirds of treated mice.[34] Double blockade was associated with increased proliferation of antigen-specific effector CD8+ and CD4+ T-cells, antigen-specific cytokine release, inhibition of suppressive functions of Treg cells, and up-regulation of key signaling molecules critical for T-cell function. In summary, our results suggest that clinical activity from concurrent nivolumab and ipilimumab can act in a complementary fashion to provide significant clinical activity in epithelial ovarian cancer and related malignancies.

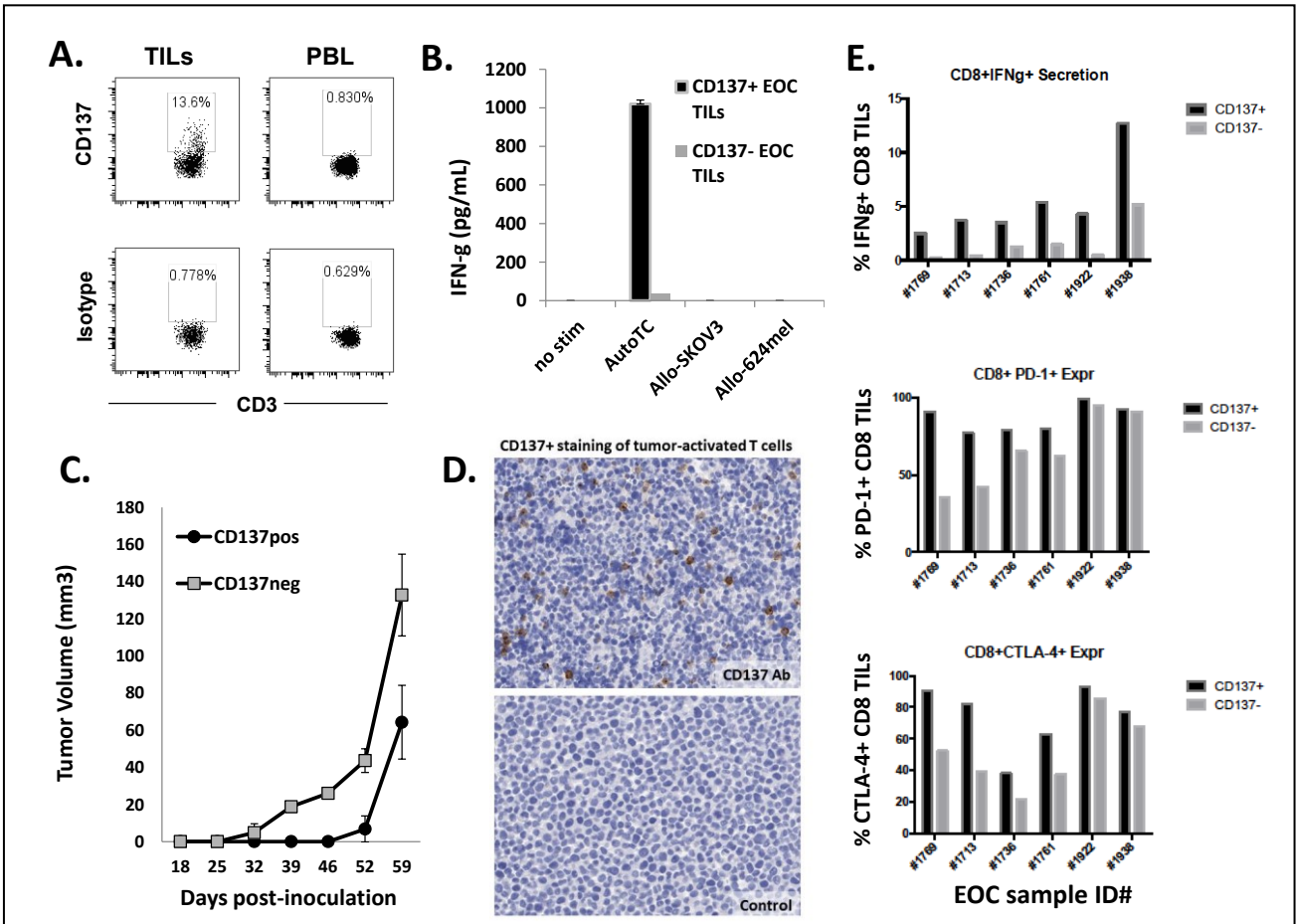


Figure 1. Tumor-reactive T-cells in advanced ovarian cancer express PD-1 and CTLA-4. (A) A subset of epithelial ovarian cancer CD3+ TILs express activation-induced CD137 on their surface which is detectable by flow cytometry. (B) Enriched CD137+ TILs are the bona fide tumor-reactive TIL subset, and react against autologous tumor cells and produce IFN-γ in overnight co-culture. All other TILs (CD137-) do not. (C) Tumor-specific CD137+ TILs control human ovarian cancer progression in an immuno-deficient mouse model; CD137 TILs do not. (D) Tumor-activated T-cells in a field of tumor cells are detectable by IHC using anti-CD137 antibody. CD3, CD8, CD4, FOXP3 and PD-L1

Wolchok et al. recently reported the results of a dose-finding trial in advanced melanoma which included 53 patients receiving concurrent Nivolumab and Ipilimumab.[35] The objective response rate was 40%. At maximum doses associated with an acceptable level of adverse events, the objective response rate was 53%, and all 17 of these patients experienced tumor reduction of at least 80% by modified World Health Organization (WHO) criteria. Callahan et al. are currently conducting an open label phase I/II trial of Nivolumab as monotherapy or combined with Ipilimumab in advanced or metastatic solid tumors.[36]

Recently, efficacy and safety results of a phase I clinical trial of Nivolumab in women with platinum-resistant ovarian cancer was presented.[37] Nivolumab was administered every 2 weeks to patients with advanced or relapsed, platinum–

resistant ovarian cancer. Eighteen patients were treated at two dose levels, 1 (10 patients) or 3 (8 patients) mg/kg until disease progression or dose limiting toxicity. CT imaging was performed every 8 weeks. The majority of women had serous tumors (14, serous; 2 endometrioid; 2 clear cell), and 11/18 had received at least 4 prior chemotherapy regimens. Seven of 18 experienced grade 3-4 adverse events possibly related to treatment (rash (n=1), hypothyroidism (n=2), fever (n=1), arrhythmia (n=1), arthralgia (n=1), lymphocytopenia (n=1); the distribution did not appear to be dose dependent in this dose range. In addition, there were two serious adverse events thought possibly to be treatment related. One patient at the 1 mg/kg dose level developed grade 3 disorientation and fever, and another at the 3 mg/kg dose level developed grade 3 fever in association with deep vein thrombosis. Three of 18 (17%) had a confirmed objective response (2 CR [serous, clear cell], 1 PR [serous]) with the two complete responses seen at the 3 mg/kg dose. An additional 5 (28%) patients demonstrated stable disease. These results provide preliminary evidence of single agent activity in patients with highly pre-treated, platinum-resistant ovarian cancer.

2.4 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 .[38] 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).[38]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 23-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta (TGF-beta).

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

PD-1 knockout mice develop strain-specific lupus-like glomerulonephritis (C57BL/6) and cardiomyopathy (BALB/c). In transplantable tumor models that expressed PD-1 and LAG-3 on tumor-infiltrating CD4+ and CD8+ T cells dual anti-LAG-3/anti-PD-1 antibody treatment cured most mice of established tumors that were largely resistant to single antibody treatment.[39] Despite minimal immuno-pathologic 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,[40] renal,[41-43] esophageal, [44]gastric,[45] ovarian,[46] pancreatic,[47] lung,[48] and other cancers.[38]

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

2.4.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated.[38] 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 Kd = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

2.4.2 Clinical Development of Nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC, hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications (Investigator Brochure, 2013). In addition, two investigator-sponsored trials (ISTs) of nivolumab in combination with a peptide vaccine in melanoma are being conducted in the adjuvant setting and advanced disease.

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

2.4.2.1 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve from time zero to infinity (AUC0-∞), with low to moderate inter-subject variability observed at each dose level.[38] Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

2.4.2.2 Efficacy

In a phase 1 (1, 3, and 10 mg/kg nivolumab doses) dose-escalation study the 3 mg/kg dose was chosen for expanded cohorts. Among 236 patients, objective responses (ORs) (complete or partial responses [CR or PR]) were seen in NSCLC, melanoma, and RCC. ORs were observed at all doses.[49] 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.[50] In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year.[51]

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered IV every 3 weeks for 4 doses followed by nivolumab alone every 3

weeks for 4 doses (concurrent regimen).[35] The combined treatment was subsequently administered every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent regimen (53 patients), 53% of patients had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced-regimen (33 patients), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC patients, 43 patients were treated with nivolumab + PT-doublet.[52] No dose-limiting toxicities (DLTs) were reported and total/confirmed ORRs were 43/33%, 40/33%, and 31/31% in nivolumab/gemcitabine/cisplatin, nivolumab/pemetrexed/cisplatin, and nivolumab/carboplatin/paclitaxel arms, respectively.

2.4.2.3 Toxicology

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

2.4.2.4 Pharmacodynamics/Biomarkers

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

2.5 Ipilimumab

Ipilimumab (MDX-010, MDX-CTLA4, BMS-734016) is being developed by CTEP as an anticancer agent in collaboration with Bristol-Myers-Squibb (BMS). On March 25, 2011, the FDA approved ipilimumab injection (YERVOY, BMS) for the treatment of unresectable or metastatic melanoma. Ipilimumab is a human $IgG_1\kappa$ monoclonal antibody (mAb); it is specific for human cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, CD152) expressed on activated T cells. Ipilimumab is now produced and formulated from transfected Chinese hamster ovary (CHO) cells.

CTLA-4 is a negative regulator of T-cell responses following T-cell stimulation.[53,54]CTLA-4 knockout mice suffer from a fatal lymphoproliferative disorder, supporting the idea that CTLA-4 functions as a negative regulator of T-cell responses *in vivo*.[55-57] Disrupting CTLA-4 interaction with its ligands B7-1 (CD80) and B7-2 (CD86), which are expressed on antigen-presenting cells (APCs), with ipilimumab, augments immune responses.[58] *In vivo* blockade of CTLA-4, utilizing anti-CTLA-4 mAb, induced regression of established tumors and enhanced antitumor immune responses in several murine tumor models. Blockade of CTLA-4-mediated signals is effective in inducing rejection of immunogenic cancers in mice. Moreover, when anti-CTLA-4 mAb is used in conjunction with granulocyte macrophage-colony stimulating factor (GM-CSF)-secreting tumor vaccines, poorly immunogenic cancers in mice are rejected. These findings suggest that CTLA-4 blockade, alone or in combination with antigenic stimulation and other immune modulating agents, can induce a potent antitumor response.

2.5.1 Pharmacology of Ipilimumab

In vitro studies were performed with ipilimumab to demonstrate that it is specific for CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2, does not show any cross-reactivity with human B7.1 or B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys. Blockade of CTLA-4/B7 interactions enhanced T-cell responses to CD3 / CD28, peptide antigens, or super-antigens in mice.[59-62] CTLA-4 knockout mice appear to have spontaneously activated T cells evident at approximately 1 week after birth, followed by rampant lymphoproliferation and lymphadenopathy. These mice die at approximately 3 weeks of age, either as a result of polyclonal T-cell expansion and tissue destruction or as a result of toxic shock resulting from lymphokine production. Genetically engineered mice heterozygous for CTLA-4 (CTLA-4+/-), appeared healthy and gave birth to healthy CTLA-4+/- heterozygous offspring. Mated CTLA-4+/- heterozygous mice also produced offspring deficient in CTLA-4 (homozygous negative, CTLA-4-/-). Since thymocyte differentiation and selection proceed normally in CTLA-4-deficient mice, the rampant T-cell expansion that occurs in the mice indicates that CTLA-4 plays a critical role in down-regulating post-thymic T-cell responses in the periphery following stimulation of naïve, memory, and effector T cells.[62]

2.5.2 Pharmacokinetics

The pharmacokinetics (PK) of ipilimumab was studied in 499 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg administered once every 3 weeks (q3w) for four doses. Peak concentration (Cmax), trough concentration (Cmin), and area under the curve (AUC) of ipilimumab were found to be dose proportional within the dose range examined. Upon repeated dosing of ipilimumab administered q3w, ipilimumab clearance was found to be time-invariant, and minimal systemic accumulation was observed as evident by an accumulation index of 1.5-fold or less. Ipilimumab steady-state concentration was reached by the third dose. The following mean (percent coefficient of variation) parameters were generated through population PK analysis: terminal half-life of 14.7 days (30.1%); systemic clearance (CL) of 15.3 mL/h (38.5%); and volume of distribution at steady-state (Vss) of 7.21 L (10.5%). The mean (±SD) ipilimumab Cmin achieved at steady-state with the 3-mg/kg regimen was 21.8 mcg/mL (±11.2).

Specific Populations: Cross-study analyses were performed on data from patients with a variety of conditions, including 420 patients with melanoma who received single or multiple infusions of ipilimumab at doses of 0.3, 3, or 10 mg/kg. The effects of various covariates on ipilimumab PK were assessed in population PK analyses.

Ipilimumab CL increased with increasing body weight; however, no dose adjustment of ipilimumab is required for body weight after administration on a mg/kg basis.

The following factors had no clinically meaningful effect on the CL of ipilimumab: age (range 26 to 86 years), gender, concomitant use of budesonide, performance status, HLA-A2*0201 status, positive anti-ipilimumab antibody status, prior use of systemic anticancer therapy, or baseline lactate dehydrogenase (LDH) levels. The effect of race was not examined as there were insufficient numbers of patients in non-Caucasian ethnic groups.

Renal Impairment: Creatinine clearance at baseline did not have a clinically important effect on ipilimumab PK in patients with calculated creatinine clearance values of 29 mL/min or greater.

Hepatic Impairment: Baseline AST, total bilirubin, and ALT levels did not have a clinically important effect on ipilimumab PK in patients with various degrees of hepatic impairment.

2.5.3 Clinical Pharmacodynamics

In clinical studies, ipilimumab increased absolute lymphocytes counts (ALC) in peripheral blood.[58] However, CD4+/CD8+ ratio did not appear to be affected. Across three phase 2 studies in 463 subjects with advanced melanoma, ipilimumab increased ALC in a dose-dependent manner, with the largest increase observed at 10 mg/kg dose. ALC continued to increase over time during the induction treatment at least until week 12 at the 3 mg/kg and 10 mg/kg dose, but not at the 0.3 mg/kg dose.

The slope of ALC increase also suggested the 10 mg/kg dose is more biologically active than the 3.0 mg/kg or 0.3 mg/kg dose.

2.5.4 Mechanism of Action

The proposed mechanism of action for ipilimumab is T-cell potentiating through interference of the interaction of CTLA-4 with B7 (CD80 or CD86) molecules on APCs, with subsequent blockade of the inhibitory function of CTLA-4 (Investigator Brochure, 2011). Ipilimumab impacts tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. These immunologic responses may take time to develop and so tumor responses may be delayed and tumor progression may occur during the initial period followed by responses. In some cases, tumor response based on tumor infiltration with immune cells may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be taken for tumor progression on radiological evaluations. Delayed responses following increasing tumor size or appearance of new lesions have been seen in approximately 10-20% of patients with metastatic melanoma. For patients who are not experiencing rapid clinical deterioration, allowing sufficient time to observe responses including disease stabilization or confirmation of progression is recommended; as discussed in the section "Overall Risk/Benefit Assessment" may allow better assessment of clinical activity and avoid unnecessarily initiating additional therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations in patients with melanoma to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

2.5.5 Nonclinical Toxicology

Please note relevant toxicity for single-agent ipilimumab has been almost completely derived from clinical studies.

In a study using cynomolgus macaques, anti-melanocyte responses were observed in animals given up to four doses of 10 mg/kg ipilimumab after receiving a melanoma cell vaccine.[63] Depigmentation has been observed in other nonclinical immunotherapy studies that involve treatment with melanoma peptides.[64-69] The symptoms in animals appear to resemble vitiligo observed in clinical immunotherapy trials of melanoma patients and may be an unavoidable consequence of treatment.[70]

Additional repeat-dose toxicity studies conducted using cynomolgus macaques demonstrated that the IV administration of ≤30 mg/kg every 3 days for three doses, 10 mg/kg weekly for 1 month, 1 mg/kg weekly for 10 weeks, or 10 mg/kg monthly for 6 months was generally well tolerated, without significant clinical, immuno-toxicological, or histo-pathological findings.[58] However, when ipilimumab was administered in combination with another immuno-modulatory antibody (BMS-663513, a fully human anti-CD137 mAb) and simian immunodeficiency virus (SIV)

DNA, two immune-related adverse events (irAEs) were observed: severe colitis requiring euthanasia in one monkey and reversible dermatitis/rash in the inguinal area and peripheral lymphadenopathy in another monkey.

Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB).[58] Non-clinical toxicity assessments included *in vitro* cynomolgus monkeys alone and in the presence of vaccines. Low to moderate ADCC activity was noted at concentrations up to 50 mcg/mL. These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC or CDCC. No mortality or signs of toxicity were observed in three independent 14-day intravenous (IV) toxicology studies in cynomolgus monkeys at multiple doses up to 30 mg/kg/dose. Furthermore, ipilimumab was evaluated in sub-chronic and chronic toxicology studies in cynomolgus monkeys with and without Hepatitis B (HepB) Vaccine and Melanoma Vaccine. Ipilimumab was well tolerated alone or in combination in all studies. There were no significant changes in clinical signs, body weight values, clinical pathology values or T-cell activation markers. In addition, there were no significant histopathology changes in the stomach or colon.

2.5.6 Clinical Development of Ipilimumab

Company-Sponsored Studies

BMS and Medarex (acquired by BMS in September 2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing more than 4000 subjects in several cancer types in 33 completed and ongoing studies.[58] The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as single agent and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapy.

Phase 3 programs are ongoing in melanoma and prostate cancer (Investigator Brochure, 2011). In the phase 3 combination study of ipilimumab with glycoprotein 100 (gp100) peptide vaccine (melanoma study MDX010-20), ipilimumab was administered at a dose of 3 mg/kg of body weight, with or without gp100 q3w for up to four treatments.[22] The median overall survival (OS) in the ipilimumab plus gp100 group was 10.0 months (95% confidence interval [CI], 8.5 to 11.5 months) compared with 6.4 months (95% CI, 5.5 to 8.7 months) in the gp100-alone group (hazard ratio [HR] for death, 0.68; $p<0.001$). Grade 3 or 4 immune related events (irAEs) occurred in 10 to 15% of patients treated with ipilimumab and in 3% of patients treated with gp100 alone.

A second phase 3 study in melanoma was reported for ipilimumab in combination with dacarbazine versus dacarbazine alone in previously untreated advanced melanoma. CA184024 evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m^2 q3w, with either ipilimumab 10 mg or placebo for cycles 1-4 and as maintenance

after completion of chemotherapy. Ipilimumab AEs were consistent with previous studies and predominantly affected skin, GI tract, liver, and the endocrine system. Events were managed with established guidelines and were generally responsive to dose interruption/discontinuation, corticosteroids and/or other immunosuppressive drugs.

There are two ongoing studies of ipilimumab as adjuvant monotherapy for high-risk Stage III melanoma (CA184029 and ECOG 1609).

Two phase 3 studies are ongoing in subjects with castration-resistant prostate cancer who have received prior chemotherapy (CA184043, ipilimumab in combination with radiation therapy) and those who are chemotherapy-naïve (CA184095, ipilimumab monotherapy).

An ongoing large phase 2 study (CA184041) is investigating the addition of ipilimumab to carboplatin and paclitaxel using two different schedules (concurrent and phased) in subjects with non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC).[58] The results demonstrate an improved immune-related progression-free survival (irPFS) for the subjects who received combination therapy with paclitaxel/carboplatin/ipilimumab compared with paclitaxel/carboplatin alone. The improvement in irPFS with the combination therapy met the pre-specified protocol criteria for significance for both the concurrent and phased schedules (p=0.0935 and 0.0258, respectively). Using modified World Health Organization (mWHO) criteria (a key secondary endpoint), PFS was significantly improved with the phased schedule but not the concurrent schedule (p=0.0240 and 0.2502, respectively).

2.5.7 Other Clinical Studies with Ipilimumab

Renal cell carcinoma (RCC)

Yang and colleagues presented data on a phase 2 study of ipilimumab conducted in patients with metastatic RCC.[71] Sequential cohorts received either 3 mg/kg followed by 1 mg/kg or all doses at 3 mg/kg q3w. One of 21 patients receiving the lower dose had a PR. Five of 40 patients at the higher dose had PRs (95% CI, cohort response rate 4 to 27%) and responses were seen in patients who had previously not responded to IL-2. Thirty-three percent of patients experienced grade 3 or 4 irAEs. There was a highly significant association between autoimmune events and tumor regression (response rate = 30% with AE, 0% without AE). The authors concluded that CTLA-4 blockade with ipilimumab induced cancer regression in some patients with metastatic clear cell renal cancer, even if they had not responded to other immunotherapies.

Melanoma (ipilimumab plus bevacizumab)

At the 2011 ASCO meeting Hodi and colleagues presented results on 21 evaluable patients (22 patients enrolled) with unresectable stage III or stage IV melanoma treated with the combination of 10 mg/kg ipilimumab and 7.5 mg/kg bevacizumab on a phase 1 study.[72] AEs included giant cell arteritis (1), hypophysitis (3), thyroiditis (4), grade 3-4 hepatitis (2), bilateral uveitis (2), and grade 2 colitis (2); 5

patients required systemic steroids and stopped treatment. All toxicities were resolved. Eight PRs and 6 SDs were observed. All responses were durable (>6 months). Post-treatment biopsies in 12 patients revealed activated vessel endothelium with extensive T-cell trafficking non-productive central angiogenesis, and peripheral blood monitoring revealed a marked increase in CD4/CCR7/CD45RO central memory cells in the majority of patients, not seen with ipilimumab alone. The authors concluded that the combination of ipilimumab with bevacizumab can be safely administered with clinical activity and correlatives suggesting synergistic effects.

Bladder cancer

Carthon and colleagues reported immuno-modulatory effects following a brief exposure of anti-CTLA-4 in patients with urothelial carcinoma of the bladder requiring surgery (BMS study CA184027).[73] 12 patients were enrolled (six patients received 3 mg/kg/dose of ipilimumab and another six patients received 10 mg/kg/dose for two doses prior to surgery). The treatment was found to be tolerable in the cohort of patients with 11 of 12 patients receiving both doses of antibody. Grade 1-2 diarrhea and rash were the most common drug-related AEs. The only noted grade 3 irAEs were ischemic papillopathy and diarrhea, which were both responsive to treatment with steroids.

Liakou and colleagues found that CD4 T cells from peripheral blood and tumor tissues of all bladder cancer patients treated with anti-CTLA-4 antibody had markedly increased expression of inducible co-stimulator (ICOS).[74] These $CD4^{+}ICOS^{hi}$ T cells produced interferon-gamma (IFN-γ) and could recognize the tumor antigen NY-ESO-1. Increase in $CD4^{+}ICOS^{hi}$ cells led to an increase in the ratio of effector to regulatory T cells. The authors indicated that these immunologic changes were reported in both tumor tissues and peripheral blood as a result of treatment with anti-CTLA-4 antibody, and they may be used to guide dosing and scheduling of this agent to improve clinical responses. A sustained increased frequency of $CD4^{+}ICOS^{hi}$ T cells may serve as a biomarker of anti-CTLA-4 activity and/or of clinical benefit for patients who are being treated with this novel agent.[73]

Pancreatic cancer

Royal and colleagues presented the results on 27 patients (metastatic disease: 20 and locally advanced: 7).[75] Three subjects experienced ≥ grade 3 irAEs (colitis:1, encephalitis:1, hypophysitis:1). One subject experienced a delayed response after initial progressive disease. In this subject, new metastases after 2 doses of ipilimumab established progressive disease. However, continued administration of the agent per protocol resulted in significant delayed regression of the primary lesion and 20 hepatic metastases with normalization of tumor markers and clinically significant improvement of performance status. The investigators concluded that single agent ipilimumab at 3.0 mg/kg/dose was ineffective for the treatment of advanced pancreatic cancer. However, a significant delayed response in one subject of this trial suggests that immunotherapeutic approaches to pancreatic cancer deserve further exploration.

2.5.8 CTEP-Sponsored Studies

The DCTD, NCI, has sponsored nine studies with ipilimumab including one pilot study (NCI #5744, lymphoma), three phase 1 studies (5708 [ovarian], 6082 [solid tumors], and 7458 [solid tumors]), one phase 1/2 study (6359 [non-Hodgkin's lymphoma]) with single agent ipilimumab, two phase 1 combination studies in prostate cancer with GM-CSF (6032) and with prostate-specific antigen (PSA)-TRICOM vaccine (7207), one phase 2 combination study of ipilimumab with GM-CSF (E1608, melanoma) and one phase 3 study (E1609) of adjuvant ipilimumab therapy versus high-dose interferon alpha-2b in patients with resected high-risk melanoma.

Results from 11 patients (colon, n=3; non-Hodgkin's lymphoma, n=4; prostate, n=4) who received ipilimumab on study 5744 included tumor regression in 2 patients with lymphoma; 1 of whom (follicular lymphoma patient) had a partial response (PR) of 14-month duration.[76] Ipilimumab was well tolerated with predominantly grade 1/2 toxicities. One drug-related grade 3 AE was observed. Tregs, as detected by expression of $CD4^{+}CD25^{+}CD62L^{+}$, declined at early time points but rebounded to levels at or above baseline values at the time of the next infusion. The investigators concluded that ipilimumab treatment depressed Treg numbers at early time points in the treatment cycle but was not accompanied by an increase in vaccine-specific CD8+ T-cell responses in these patients previously treated with a variety of investigational anticancer vaccines.

Hodi and colleagues reported preliminary results on 20 patients (11 metastatic melanoma patients and 9 metastatic ovarian carcinoma patients) on study 5708.[77] None of the 11 patients from the metastatic melanoma cohort manifested grade 3 or 4 inflammatory toxicities; however, all subjects revealed mild inflammatory pathologies associated with low-level constitutional symptoms. The most common toxicity (10/11 subjects) was a grade 1-2 reticular and erythematous rash on the trunk and/or extremities that arose between 3 days and 3 weeks after antibody administration and then gradually resolved without specific intervention. Biopsies of involved skin revealed low-grade interface dermatitis, minor to moderate mononuclear infiltrates surrounding the superficial dermal vasculature, and increased mucin deposition in the papillary and reticular dermis. These pathologic features resembled those observed in mild cutaneous forms of systemic lupus erythematosus. Three PRs (range, 21-34+ months) and five events of stable disease (SD) (range, 4-25 months) were observed. One PR and three SDs (2, 4, and 6+ months) were observed in the ovarian carcinoma group. The investigators concluded that selective targeting of antitumor regulatory T cells (Treg) may constitute a complementary strategy for combination of ipilimumab and GM-CSF-based antigen tumor cell vaccine therapy.

Results from 29 patients with malignancies that were recurrent or progressive after allogeneic hematopoietic cell transplantation (allo-HCT) demonstrated that drug was well tolerated at single doses up to 3 mg/kg.[78] Four patients experienced organ-specific irAEs of reversible grade 3 arthritis, grade 2 hyperthyroidism, dyspnea, and

grade 4 pneumonitis. Three patients had objective responses: one PR lasting for 2 months, and two durable complete responses (CRs). Two additional patients with Hodgkin's disease who had evidence of rapid disease progression prior to ipilimumab treatment achieved SD for 3 and 6 months, following infusion at the 3 mg/kg dose level. Median OS was 24.7 months. At a 3.0 mg/kg dose, active serum concentrations of ipilimumab were maintained for >30 days following a single infusion. Zhou and colleagues reported immuno-phenotypes of peripheral blood T cells, including T-cell reconstitution, activation, and Treg expression, in 29 patients before and after a single-dose infusion of ipilimumab.[79] CTLA-4 blockade by a single infusion of ipilimumab increased $CD4^+$ and $CD4^+HLA\text{-}DR^+$ T lymphocyte counts and intracellular CTLA-4 expression at the highest dose level (3.0 mg/kg). There was no significant change in Treg cell numbers after ipilimumab infusion. These data demonstrate that significant changes in T-cell populations occur on exposure to a single dose of ipilimumab.

Harzstark and colleagues reported results on 36 patients[80] with hormone refractory metastatic prostate cancer.[81] Of six patients treated with ipilimumab at a dose of 3 mg/kg, three patients had confirmed PSA declines of ≥50%, with a time to progression (TTP) of 22, 26, and 103 weeks. One of these patients had a PR in hepatic metastases. Grade 3 IrAEs consisted of rash in five patients, pan-hypopituitarism in one patient, temporal arteritis in one patient, and diarrhea in three patients. Non-irAEs included grade 3 and 4 cerebrovascular events (one patient each), grade 3 angina (one patient), grade 3 atrial fibrillation (one patient), grade 3 fatigue (four patients), and grade 5 pulmonary embolism (one patient). One patient treated at 10 mg/kg had a PSA decline of ≥50% with a TTP of 39 weeks. Higher doses of treatment with MDX-010 + GM-CSF induced the expansion of activated circulating $CD25^+$, $CD69^+$, and $CD8^+$ T cells more frequently than was seen in patients who received the same doses of either MDX-010 or GM-CSF alone.[82] The sera screening with protein arrays showed that the treatment can induce antibody responses to the testicular antigen NY-ESO-1.

Patients with metastatic prostate cancer were treated with ProstVac vaccine and ipilimumab before chemotherapy. The median OS for all patients on study was 31.8 months with a 74% survival probability at 24 months.[83] The median Halabi predicted OS for all patients was 18.5 months. There was no significant difference in OS at different dose levels of antibody (range 1-10 mg). A unique effect of the vaccines on the rate of tumor growth may be a novel method to evaluate the anti-tumor effects of the vaccine.[84] The authors suggested that the addition of immune checkpoint inhibition may augment the clinical benefit of vaccines.

Ansell and colleagues reported data on 18 treated patients with NHL.[85] Two clinical responses were observed: one patient with diffuse large B-cell lymphoma (BCL) had an ongoing CR (>31 months), and one with follicular lymphoma had a PR lasting 19 months. In 5 of 16 cases tested, T-cell proliferation to recall antigens was >2 fold increased after ipilimumab therapy. The investigators have found that blockade of CTLA-4 signaling with the use of ipilimumab is well tolerated at the

doses used. Ipilimumab has antitumor activity in patients with BCL, resulting in durable responses in a minority of patients. Ipilimumab at 3 mg/kg monthly for 4 months can be given safely and is the dose recommended for future combination studies.

2.5.9 Clinical Safety

Safety Experience

The most common treatment-related AEs (those considered possibly, probably, or definitely related to study drug by the investigator) associated with the use of ipilimumab were immune related irAEs.[58] The irAEs primarily involved the gastrointestinal (GI) tract (*e.g.*, diarrhea and colitis) and skin (*e.g.*, pruritus and rash), and less frequently, the liver, endocrine glands (including the thyroid, pituitary, and adrenal glands) and nervous system. IrAEs were generally managed with either symptomatic therapy (grade 1-2 events), systemic corticosteroids (grade 3-4 events), or other immunosuppressive drugs (*e.g.*, infliximab, mycophenolate mofetil) for steroid-unresponsive GI or hepatic irAEs, as appropriate. Management of irAEs was usually paired with omission of dosing for mild or moderate events and permanent discontinuation for severe irAEs. Ipilimumab can result in severe and fatal immune-mediated reactions due to T-cell activation and proliferation. Fatalities due to GI perforation, hepatic failure, toxic epidermal necrolysis, and Guillain-Barre syndrome have been reported in clinical trials of ipilimumab.

Clinical trials are conducted under widely varying conditions so that extrapolation to novel settings and combinations regarding rates and severity of events may be unreliable. Given the expected rate of toxicity which may require stopping study drug but may also be related to a therapeutic immunologic response alternative DLT criteria are discussed in section 6.

Min and colleagues reported three patients who received ipilimumab alone or combined with bevacizumab therapy and developed thyroiditis, and the first report of euthyroid Graves' ophthalmopathy.[86] They recommend that all patients on ipilimumab alone or combined with bevacizumab therapy have baseline thyroid function tests and careful monitoring for new onset of thyroid disease, particularly during the first 3 months of treatment. See specific events in section 5.

Safety Profile of Ipilimumab at a Dose of 10 mg/kg (Phase 2 data)

The safety profile of ipilimumab as monotherapy over multiple doses at a dose of 10 mg/kg in 325 subjects was determined from 4 completed melanoma studies. Overall, the incidence of grade 3/4 AEs attributable to study drug was 31%. The target organ system, the incidence, and the severity of the most commonly observed irAEs vary among studies and with drug combinations. Typically, the severity but not necessarily the overall incidence increases with dose. Additional information on specific events is provided in section 7.1 and the IB:

Summary of irAE Safety Data for 10 mg/kg in Melanoma

	Total	Low-grade (Grade 1 - 2) (%)	High-grade (Grade 3 - 4) (%)	Median Time to Resolution of Grade 2 - 4 irAEs (weeks)
All irAEs	72.3	46.2	25.2	-
Skin (*e.g.*, rash, pruritus)	52.0	49.2	2.8	6.14
GI (*e.g.*, colitis, diarrhea)	37.2	24.9	12.3	2.29
Liver (*e.g.*, LFT elevations)	8.0	0.9	6.8	4.0
Endocrine (*e.g.*, hypophysitis, hypothyroid)	6.2	3.7	2.5	20.1

Pregnancy

Preliminary results are available in cynomolgus monkeys. Pregnant monkeys received ipilimumab every 21 days from the onset of organogenesis in the first trimester through delivery, at dose levels either 2.6 or 7.2 times higher than the clinical dose of 3 mg/kg of ipilimumab (by AUC). No treatment-related adverse effects on reproduction were detected during the first two trimesters of pregnancy. Beginning in the third trimester, the ipilimumab groups experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower birth weight), and higher incidences of infant mortality in a dose-related manner compared to controls. Based on animal data, ipilimumab may cause fetal harm. The use of ipilimumab during human pregnancy has not been formally studied in clinical trials. There have been 7 known pregnancies during ipilimumab treatment: in 3 female subjects and in the partners of 4 male study subjects. Two (2) of the 3 female pregnancies ended with elected terminations. The third female subject had a history of seizures and delivered the baby at 36 weeks gestation. The baby had respiratory complications that resolved by birth week 16. Three (3) of the 4 partners of male study subjects had full term, normal babies. The fourth baby had small ureters, which are expected to grow as the baby matures. Although these outcomes do not indicate that stillbirths or other severe abnormalities will occur, pregnancy should be avoided during treatment with ipilimumab.

Immunogenicity

In clinical studies, 1.1% of 1024 evaluable patients tested positive for binding antibodies against ipilimumab in an electro-chemiluminescent (ECL) based assay. This assay has substantial limitations in detecting anti-ipilimumab antibodies in the presence of ipilimumab. Infusion-related or peri-infusional reactions consistent with

hypersensitivity or anaphylaxis were not reported in these 11 patients nor were neutralizing antibodies against ipilimumab detected. Because trough levels of ipilimumab interfere with the ECL assay results, a subset analysis was performed in the dose cohort with the lowest trough levels. In this analysis, 6.9% of 58 evaluable patients, who were treated with 0.3 mg/kg dose, tested positive for binding antibodies against ipilimumab. These results are highly dependent on methodology, and comparison of incidence of antibodies to ipilimumab with the incidences of antibodies to other products may be misleading.

2.5.10 Study Results and Clinical Efficacy

The clinical efficacy of ipilimumab as a single agent at a dose of 3 mg/kg administered q3w for 4 doses has been established in MDX010-20 (a randomized, controlled study in second line, locally advanced/metastatic melanoma), which led to approval of ipilimumab by the FDA. In study CA184024, the addition of 10 mg/kg ipilimumab to dacarbazine led to a prolongation of overall survival in patients with previously untreated melanoma.

In melanoma studies, disease stabilization in subjects receiving ipilimumab is characteristic of anti-tumor activity. Stable disease, sometimes of long duration, or slow steady decline of tumor lesion size over long periods of time, has been observed. Some subjects demonstrate initial tumor volume increase before response, possibly due to T-cell infiltration as shown by biopsies or to the time required for immunologic activation. Consequently, an initial determination of progressive disease and consequently PFS may not capture all patterns response and may underestimate the clinical activity of ipilimumab. Please see section "Considerations for Using Immune-Related Tumor Assessment Criteria (irRC)."

MDX010-20 (Phase 3, 3 mg/kg, previously treated melanoma)

MDX010-20, a randomized (3:1:1), double-blind, double-dummy study included 676 randomized subjects with unresectable or metastatic melanoma previously treated with one or more of the following: aldesleukin, dacarbazine, temozolomide, fotemustine, or carboplatin. Of these 676 subjects, 403 were randomized to receive ipilimumab at 3 mg/kg in combination with an investigational peptide vaccine with incomplete Freund's adjuvant (gp100), 137 were randomized to receive ipilimumab at 3 mg/kg, and 136 were randomized to receive gp100 alone. The study enrolled only subjects with HLA A2*0201 genotype; this HLA genotype facilitates the immune presentation of the investigational peptide vaccine. The study excluded subjects with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation.

The OS results are shown in the table below.

MDX010-20 Overall Survival Results

	Ipilimumab n = 137	**Ipilimumab + gp100 n = 403**	**gp100 n = 136**
Hazard Ratio (vs gp100) (95% CI)	0.66 (0.51, 0.87)	0.68 (0.55, 0.85)	
p-value	p = 0.0026[a]	p = 0.0004	
Hazard Ratio (vs ipilimumab) (95% CI)		1.04 (0.83, 1.30)	
Median (months) (95% CI)	10 (8.0, 13.8)	10 (8.5, 11.5)	6 (5.5, 8.7)

[a] Not adjusted for multiple comparisons.

CA184024 (Phase 3, previously untreated melanoma, 10 mg/kg)
CA184024 evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m^2 q3w, with either ipilimumab 10 mg/kg or placebo cycles 1-4, and as maintenance after completion of chemotherapy.

Patients on the ipilimumab arm received a median of 3 ipilimumab induction doses, versus 4 placebo induction doses on the placebo arm. A total of 17.4% and 21.1% of patients continued to receive maintenance ipilimumab or placebo, for a median of 4 and 2 doses, respectively. The number of patients who received all 8 dacarbazine doses was 12.2% in the ipilimumab arm, and 21.5% in the placebo arm.

The study met its primary end-point of prolonging overall survival in patients treated with ipilimumab (HR 0.72 [95% CI, 0.59 – 0.87], median OS 11.2 vs 9.1 months, p = 0.0009).

One, two, and three year survival rates were 47.3%, 28.5%, and 20.8% in the ipilimumab arm, and 36.3%, 17.9%, and 12.2% in the placebo arm.

PFS, a secondary end-point, was also prolonged by the addition of ipilimumab, HR 0.76 (95% CI, 0.63 - 0.93). The median PFS was 2.8 months in the ipilimumab vs 2.6 months in the placebo arm, p = 0.006.

Best overall response rate (BORR) was increased from 10.3% in the placebo arm to 15.2% in the ipilimumab arm. More importantly, duration of response was more than twice as long in the ipilimumab arm (19.3 months) than in the placebo arm (8.1 months).

10 mg/kg Dosing with Ipilimumab

Several additional trials studied the efficacy and safety of 10 mg/kg dosing, and additional information gained from these trials is listed below:
A dose of 10 mg/kg may be necessary to ensure blockade of the CTLA-4 pathway; *in vitro,* a concentration of 20 mcg/mL of ipilimumab was the minimal concentration able to fully abrogate the binding of CTLA-4 to B7.1 and B7.2. With a dose of 3 mg/kg q3w, 30% achieved a trough concentration of ipilimumab greater than 20 μg/mL, compared to 95% of subjects treated at 10 mg/kg q3w.

In addition, in all ipilimumab trials examined to date, mean Absolute Lymphocyte Count (ALC) increased after ipilimumab treatment throughout the 12-week induction-dosing period, in a dose-dependent manner. In an analysis of ipilimumab at 0.3, 3, or 10 mg/kg in melanoma studies CA184007, CA184008, and CA184022 combined, the rate of change in ALC after ipilimumab treatment was significantly associated with dose ($p = 0.0003$), with the largest rate at 10 mg/kg ipilimumab. Moreover, the rate of change in ALC over the first half of the induction-dosing period was significantly associated with clinical activity in these studies ($p = 0.009$), where clinical activity was defined as CR, PR, or prolonged SD (*i.e.*, SD lasting at least 6 months from first dose). Although these analyses alone could not determine whether the rate of change in ALC was specifically associated with clinical activity in response to ipilimumab treatment, as opposed to being generally prognostic, these results do suggest a potential benefit to higher rates of ALC increase after ipilimumab treatment. Among the 3 doses evaluated, 10 mg/kg ipilimumab led to the greatest such rates.

In the 3 primary studies conducted in advanced melanoma (CA184007, CA184008, and CA184022), subjects treated with 10 mg/kg single agent ipilimumab had the highest response, disease control rates, median OS as well as 1-year and 2-year survival rates compared to lower doses. The CA184022 data are summarized in the table below.

Summary of Phase 2 Response Data in Melanoma (CA184022 see IB)

	10 mg/kg (n = 72)	**3 mg/kg (n = 72)**	**0.3 mg/kg (n = 73)**
BORR (mWHO) – % (95% CI)	11.1 (4.9 - 20.7)	4.2 (0.9 - 11.7)	0 (0.0 - 4.9)
DCR (mWHO) – % (95% CI)	29.2 (19.0 - 41.1)	26.4 (16.7 - 38.1)	13.7 (6.8 - 23.8)
Survival rate at 1 year - % %, 95% CI	48.64 (36.84, 60.36)	39.32 (27.97, 50.87)	39.58 (28.20, 51.19)
Survival rate at 2 year - % %, 95% CI	29.81 (19.13, 41.14)	24.20 (14.42, 34.75)	18.43 (9.62, 28.22)
Overall median survival 95%CI (months)	11.43 (6.90, 16.10)	8.74 (6.87, 12.12)	8.57 (7.69, 12.71)

2.5.11 Dose, Schedule, and Regimen

While optimal doses and schedules for ipilimumab have not yet been determined, in proposed proof of principle studies demonstration of efficacy at 10 mg/kg would allow future studies to explore biologic and clinical efficacy at lower doses with reduced toxicity. For most studies in new combinations or settings, a short phase 1 component at 3 mg would be appropriate with a 5 or 6 mg/kg dose added as an additional cohort if needed.

A recommended dose of 10 mg/kg is proposed by the manufacturer for most studies of ipilimumab. In melanoma, a similar survival benefit was demonstrated in phase 3 trials at the 3 mg/kg and at 10 mg/kg with DTIC. However, the incidence of grade $\geq$3 toxicity was 15 and 25% respectively.

Based on Phase 2 studies, response rates of ipilimumab appear to be dose dependent up to 10 mg/kg.

Exposure-response analyses [C_{min}ss Analysis of PK data from patients treated with ipilimumab at 0.3 mg/kg (N=47), 3 mg/kg (N=60) and 10 mg/kg (N=311)], showed that the target C_{min}ss target threshold of 20 mcg/ml was exceeded in 0%, 30% and 95% subjects respectively. The slope of change in absolute lymphocyte count

(ALC) correlated with clinical benefit and T-cell activation markers such as HLA-class II expression may also be dose dependent. Responses have not been compared systematically in randomized phase 2 or phase 3 studies in patients with tumor types other than melanoma.

Regarding schedule, the typical schedule for advanced melanoma at present is once q3w for four doses followed by a maintenance phase of four doses every 12 weeks. Of interest, ipilimumab was evaluated in NSCLC and SCLC using a dose of 10 mg/kg given concomitantly or following initial paclitaxel/cisplatin. When used in the phased schedule, 10 mg significantly improved irPFS and mWHO defined responses but not PFS determined by Response Evaluation Criteria in Solid Tumors (RECIST). There also was a trend for an improvement in OS in both indications. Doses less than 10 mg/kg have not been evaluated in either NSCLC or SCLC.

Studies comparing doses in non-melanoma and combinations have not been widely done. There are also no clear data that peak levels, Cmin, AUC, exposure and number of doses given, or the occurrence of autoimmune events, predict responses in individual patients. We note that the incidence of specific events such as hypophysitis may vary from study to study and with different combinations of agents. The severity and possibly time to onset but not necessarily the frequency of events increases with dose. In addition, there are rare but serious events such as toxic epidermal necrolysis (TEN) for which a dose relationship has not been established. Case report forms should include data on the prior treatment, timing, number of doses, duration of event, response to treatment, and complications to allow comparisons among studies.

2.5.12 Considerations in Using Immune-Related Tumor Assessment Criteria (irRC)

New endpoint definitions for trials of immunologic agents have been proposed based on novel patterns of clinical activity in malignant melanoma.[87,88] These alternative definitions allow time for immunologically mediated effectors to develop that may result in late tumor responses even after initial progression by RECIST. Also, in some patients, tumors necrosis and inflammation may increase tumor size radiographically prior to response. Changing the definitions of OR and PD may alter (increase) the number patients achieving responses and the duration of PFS.

On a protocol by protocol basis, investigators and study sponsors have considered allowing study treatment to continue during initial progression up to the 12-16 week assessment to allow time for responses to be observed, if the patient is clinically stable, there is no deterioration in PS, and there is no need for immediate additional treatment. While maintaining standard definitions of progression and response, this system has allowed new lesions and some progression beyond 20% increases in tumor measurements during the initial treatment period to allow time for responses to develop (these delayed tumor responses may be seen in 10-20% of melanoma patients who initially progress during the initial treatment cycles and evaluation). There has been little to no experience with response patterns with combination therapy or in diseases other than melanoma. It is suggested that standard response

definitions be used as the primary endpoint in these studies.

For the purpose of this trial, section 14.1 specifies modifications to RECIST 1.1 which take into account the possibility of delayed response following evidence of increased tumor size by imaging at first re-assessment 8 weeks after initiation of treatment.

2.5.13 Overall Risk/Benefit Assessment

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab affects tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in subjects who are not experiencing rapid clinical deterioration, confirmation of progression is recommended, at the investigator's discretion, to better understand the prognosis as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response, and in some instances is associated with slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses. Thus, the immune-based mechanism of action of ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its improvement in OS.

The immune-based mechanism of action is also reflected in the safety profile. The most common drug-related AEs are immune-mediated, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressive drugs. As previously discussed, the immune-mediated adverse reactions primarily involve the GI tract, skin, liver, endocrine glands, and nervous system.

The early diagnosis of immune-mediated adverse reactions is important to initiate therapy and minimize complications. Immune-mediated adverse reactions are generally manageable using symptomatic or immunosuppressive therapy as recommended through detailed diagnosis and management guidelines, as described fully in the current IB. The management guidelines for general immune-mediated adverse reactions and ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy are provided in the appendices of the current IB.

2.6 Translational Science Background

2.6.1 PD-L1 Expression (Integrated Biomarker)
The expression of PD-L1 has been previously demonstrated to be associated with improved clinical benefit from therapies targeting the PD-1/PD-L1 pathway[89]. The utility of PD-L1 expression as a predictive biomarker, however, has been debated as it is highly dynamic and can be up-regulated in response to immune activating factors. In this trial, pre-treatment formalin-fixed, paraffin-embedded (FFPE) tumor tissue will be evaluated by immunohistochemistry (IHC) for expression of PD-L1 as a predictive biomarker.

2.6.2 Analysis of Tumor Infiltrating Lymphocytes (TILs; Exploratory Biomarker)
Immune infiltration of tumors has been demonstrated in multiple studies to be both prognostic and predictive of response to immunotherapies, with tumors exhibiting inflamed phenotypes being most responsive. Assessment of the immune subsets in the tumors will help to establish predictive markers on the basis of pre-treatment phenotype as well to determine whether clinical efficacy correlates with the degree of infiltration of specific immune cell subsets. In addition, both PD-L1 and indoleamine-2,3-dioxygenase (IDO) have been demonstrated to be up-regulated in tumors in response to immune infiltration and may serve as negative feedback mechanisms[90]. Other immune checkpoints such as LAG-3 and TIM-3 may serve as additional immune inhibitory mechanisms, which may dampen the overall immune response. As such, pre-treatment FFPE will be evaluated for the presence of TILs and immune markers including CD3, CD4, CD8, FoxP3, CD16, CD68, CD137, OX40, GITR, CD40, IDO, LAG3, TIM3, B7-H3, B7-H4, PD-1, CTLA-4, and VISTA.

2.6.3 Neo-antigen Landscape (Exploratory Biomarker)
A recent study by the group at Memorial Sloan Kettering Cancer Center (MSKCC) used next-generation whole exome sequencing (NGS) to characterize neo-antigen landscape in patients with malignant melanoma and defined particular neo-epitope signatures that were highly predictive of clinical benefit from anti-CTLA-4 therapy in patients with malignant melanoma (Snyder et al, ASCO Annual Meeting 2014). To determine whether specific tumor genetic determinants, including neo-epitope signatures influence the response to either treatment, DNA isolated from archived tumor samples will be processed for whole exome analysis to assess for specific driver mutations and for potential neo-antigens using appropriate bioinformatics algorithms developed at MSKCC by Dr. Alexandra Snyder.

2.6.4 T Cell Receptor (TCR) Repertoires (Exploratory Biomarker)
Since cancer-testis (CT) antigens may not be present in all patients and may not be the immuno-dominant antigens that will determine tumor sensitivity to the immune system, the analysis of overall changes in TCR repertoires during therapy may help to determine whether certain dominant T cell clones emerge or persist in response to therapy. Recently, Cha et al, reported that maintenance of specific T cell clonotypes in blood of patients with advanced melanoma treated with ipilimumab was

associated with improved survival[91]. Similar TCR repertoire studies were performed in tumors at MSKCC in the setting of a clinical trial in patients with early stage breast cancer treated with neoadjuvant cryoablation of breast lesion and ipilimumab, where combination therapy demonstrated most significant increase in intra-tumoral T cell clones[92]. Thus, in this trial DNA will be used for TCR repertoire analysis and deep sequencing of TCR CDR3 regions (Adaptive Biotechnologies and Dr. Dmitriy Zamarin, respectively).

2.6.5 Tumor-Associated Antigen (TAA) Serologic Responses (Exploratory Biomarker) Expression of CT antigens in a large percentage of gynecologic malignancies has been reported in multiple studies. Monitoring for early responses to CT antigens and TAA such as NY-ESO1, MAGE 4, SOX2, and p53 may help identify the patients that are more likely to derive benefit from therapy. As such, pre- and on-treatment serum will used for assessment of TAA serologic responses. **(05/22/2017)**

2.7 Inclusion of Women and Minorities

NRG Oncology and NRG participating institutions will not exclude potential subjects from participating in this or any study solely on the basis of ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire ovarian, primary peritoneal and fallopian tube cancer population treated by participating institutions.

3. PATIENT SELECTION, ELIGIBILITY, AND INELIGIBILTY CRITERIA

Note: Per NCI guidelines, exceptions to inclusion and exclusion criteria are not permitted. For questions concerning eligibility, please contact the NRG Statistics and Data Management Center-Pittsburgh Office: 412-624-2666. **(02/13/2017)**

3.1 Patient Selection Guidelines

Although the guidelines provided below are not inclusion/exclusion criteria, investigators should consider these factors when selecting patients for this trial. Investigators also should consider all other relevant factors (medical and non-medical), as well as the risks and benefits of the study therapy, when deciding if a patient is an appropriate candidate for this trial.

3.1.1 Patients must have the psychological ability and general health that permits completion of the study requirements and required follow up.

3.1.2 Women of childbearing potential should be willing and able to use medically acceptable forms of contraception during the trial and for 23 weeks after the last dose of drug.

3.1.3 Submission of tumor tissue is required for all patients. Investigators should check with their site Pathology department regarding release of bio-specimens before approaching patients about participation in the trial. (See details of bio-specimen submissions in Section 11.)

3.2 Eligibility Criteria

A patient cannot be considered eligible for this study unless ALL of the following conditions are met.

3.2.1 Patients must have recurrent or persistent epithelial ovarian, fallopian tube, or primary peritoneal cancer with documented disease progression (disease not amendable to curative therapy). Histologic confirmation of the original primary tumor is required via the pathology report. NOTE: Patients with mucinous histology are NOT eligible. Patients with carcinosarcoma histology are NOT eligible. **(02/22/2016)**

3.2.2 All patients must have measurable disease as defined by RECIST 1.1. Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded). Each lesion must be ≥ 10 mm when measured by CT, MRI or caliper measurement by clinical exam; or ≥ 20 mm when measured by chest x-ray. Lymph nodes must be ≥ 15 mm in short axis when measured by CT or MRI.

3.2.3 Patients must have at least one "target" lesion" to be used to assess response on this protocol as defined by RECIST 1.1. Tumors within a previously irradiated field will be designated as "non-target" lesions unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

3.2.4 Appropriate for study entry based on the following diagnostic workup:

- History/physical examination within 28 days prior to registration;
- Imaging of target lesion(s) within 28 days prior to registration; **(02/22/2016)**
- Further protocol-specific assessments:
  - Recovery from effects of recent surgery, radiotherapy or chemotherapy
  - Free of active infection requiring antibiotics (with the exception of uncomplicated UTI)
  - Any hormonal therapy directed at the malignant tumor must be discontinued at least one week prior to registration
  - Any other prior therapy directed at the malignant tumor including chemotherapy, targeted agents, biologic agents, immunologic agents, and any investigational agents, must be discontinued at least 4 weeks prior to registration (6 weeks for nitrosoureas or mitomycin C).
  - Any prior radiation therapy must be completed at least 4 weeks prior to registration
  - At least 4 weeks must have elapsed since major surgery

3.2.5 Prior Therapy (1–3 Priors allowed as detailed below) (02/22/2016)
Patients are allowed to have received up to three prior cytotoxic regimens for treatment of their epithelial ovarian, fallopian tube, or primary peritoneal cancer.

They must have had one prior platinum-based chemotherapeutic regimen for management of primary disease, possibly including intra-peritoneal therapy, consolidation, biologic/targeted (non-cytotoxic) agents or extended therapy (maintenance/consolidation) administered after surgical or non-surgical assessment.

Patients are allowed to have received, but are not required to have received, one or two cytotoxic regimens for management of recurrent or persistent disease. (For the purposes of this study PARP inhibitors given for recurrent or progressive disease will be considered cytotoxic. PARP inhibitors given as maintenance therapy in continuation with management of primary disease will not be considered as a separate cytotoxic regimen.) If two cytotoxic regimens had been received for management of recurrent or persistent disease, one of these regimens would have had to contain either a platinum or a taxane agent. **(02/22/2016)**

3.2.6 Age ≥ 18; because no dosing or adverse event data are currently available on the use of nivolumab in patients <18 years of age, children are excluded from this study.

3.2.7 The trial is open to females only.

3.2.8 Performance Status of 0, 1 or 2 (see Appendix II) within 28 days prior to registration.

3.2.9 Adequate hematologic function within 14 days prior to registration defined as follows:

- ANC ≥ 1,500/ul
- Platelets ≥ 100,000/ul

3.2.10 Adequate renal function within 14 days prior to registration defined as follows:

- Creatinine ≤ 1.5 x institutional/laboratory upper limit of normal (ULN)

3.2.11 Adequate hepatic function within 14 days prior to registration defined as follows:

- Bilirubin ≤ 1.5 x ULN
- ALT and AST ≤ 3 x ULN
- Albumin ≥ 2.8 g/dL

For patients with Gilbert's Syndrome, Bilirubin ≤ 3.0 mg/dL is acceptable. **(02/22/2016)**

3.2.12 Adequate thyroid function within 28 days prior to registration defined as serum TSH in normal range.

3.2.13 The patient or a legally authorized representative must provide study-specific informed consent prior to study entry.

3.2.14 **Platinum-Free Interval (PFI)** - Patients must have progressed < 12 months after completion of their last platinum-based chemotherapy. The date (platinum free interval) should be calculated from the last administered dose of platinum therapy to documentation of progression.

3.2.15 Adequate oxygen saturation via pulse oximeter within 28 days prior to registration (i.e., patient can NOT have CTCAE hypoxia grade 2 or greater).

3.2.16 LVEF ≥ 50% (measured within 28 days of study entry). **(02/13/2017)**

3.3 **Ineligibility Criteria (02/22/2016)**

Patients with one or more of the following conditions are NOT eligible for this study.

3.3.1 Patients who have had prior therapy with nivolumab or 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 check point pathways.

3.3.2 History of severe hypersensitivity reaction to any monoclonal antibody.

3.3.3 Patients with a history of other invasive malignancies, with the exception of non-melanoma skin cancer **and other specific malignancies as noted in Section 3.34** are excluded if there is any evidence of other malignancy being present within the last three years (2 years for breast cancer, see Section 3.3.4). Patients are also excluded if their previous cancer treatment contraindicates this protocol therapy. **(12/21/2018)**

3.3.4 Patients who have received prior chemotherapy for any abdominal or pelvic tumor OTHER THAN for the treatment of ovarian, fallopian tube, or primary peritoneal cancer within the last three years are excluded. Patients may have received prior adjuvant chemotherapy and radiotherapy for localized breast cancer, provided that it was completed more than 2 years prior to registration, the patient remains free of recurrent or metastatic disease and hormonal therapy has been discontinued. Patients who have received prior radiotherapy to any portion of the abdominal cavity or pelvis or thoracic cavity within the last three years are excluded. Prior radiation for localized cancer of the head and neck or skin is permitted, provided that it was completed more than three years prior to registration, and the patient remains free of recurrent or metastatic disease.

3.3.5 Patients with uncontrolled illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure and unstable angina pectoris.

3.3.6 Patients with history of organ transplant.

3.3.7 Patients who are pregnant or nursing. The effects of nivolumab and ipilimumab on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) 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 23 weeks after the last dose of investigational drug. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IV/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab or nivolumab + ipilimumab. Women must not be breastfeeding. **(02/22/2016)**

Women who are not of childbearing potential (i.e., who are postmenopausal or surgically sterile) do not require contraception.

Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy and/or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 month 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 greater than 40mIU/mL.

If, following initiation of the investigational product(s), it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (*e.g.*, dose tapering if necessary for subject safety). The investigator must report this event and any outcomes by amendment through CTEP-AERS (see section 7.1).

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (*e.g.*, X-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report and follow-up on information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks. **(02/22/2016)**

3.3.8 History or evidence upon physical examination of CNS disease, including primary brain tumor, seizures which are not controlled with non-enzyme inducing anticonvulsants, any brain metastases and/or epidural disease, or history of cerebrovascular accident (CVA, stroke), transient ischemic attack (TIA) or subarachnoid hemorrhage within six months prior to the first date of study treatment.

3.3.9 In order for patients with known history of testing positive for human

immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS) to be eligible, they must be on a stable highly active antiretroviral therapy (HAART) regimen, have CD4 counts > 350, with no detectable viral load on quantitative PCR.

Patients with treated hepatitis virus infections (Hepatitis B or Hepatitis C) are eligible if they have been definitively treated for 6 months, have no detectable viral load on quantitative PCR, and LFTs meet eligibility requirements.

3.3.10 Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids, should be excluded. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease, Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease. Patient with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjogren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

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

3.3.12 Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses ≤10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). 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. **(02/22/2016)**

3.3.13 Any of the following within 2 months of registration: active peptic ulcer disease, diverticulitis, cholecystitis, symptomatic cholangitis or appendicitis, malabsorption syndrome. Any of the following within 6 months of registration: Intra-abdominal abscess, gastrointestinal obstruction requiring parenteral hydration and/or nutrition, gastrointestinal perforation. Note: complete resolution of an intra-abdominal abscess must be confirmed prior to registration even if the abscess occurred more than 6 months prior to registration. **(02/22/2016)**

3.3.14 No planned concomitant, non-protocol directed anti-cancer therapy.

3.3.15 Grade ≥2 peripheral neuropathy. **(02/22/2016)**

4. REQUIREMENTS FOR STUDY ENTRY, TREATMENT, AND FOLLOW-UP

4.1 PRE-TREATMENT ASSESSMENTS (02/22/2016)

Assessments	Prior to Registration (calendar days)	Prior to Treatment (calendar days) (Cycle 1, Day 1)
History and Physical	≤ 28 days	≤ 28 days
Vital Signs (Blood Pressure, Heart Rate, Temperature, and **Pulse Oximeter** Oxygen Saturation)	≤ 28 days	≤ 28 days
Echocardiogram **(02/13/2017)**	≤ 28 days	≤ 28 days
Performance Status	≤ 28 days	≤ 28 days
Toxicity Assessment	≤ 14 days	≤ 14 days
Concurrent Medications	≤ 14 days	≤ 14 days
CBC/Differential/Platelets	≤ 14 days	≤ 14 days
Electrolytes, including, BUN, creatinine, Ca, phosphorus, magnesium and glucose	≤ 14 days	≤ 14 days
Bilirubin, ALT, AST, Alkaline Phosphatase, Albumin	≤ 14 days	≤ 14 days
Hepatitis panel, including hepatitis B surface antigen and hepatitis C Antibody	≤ 28 days	≤ 28 days
Pregnancy Test (if childbearing potential exists)	≤ 28 days	≤ 24 hours[1]
TSH	≤ 28 days	≤ 28 days
Amylase/Lipase	≤ 14 days	≤ 14 days
CA-125		≤ 28 days
Radiographic Tumor Measurement[2]	≤ 28 days	≤ 28 days

[1] The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of hCG.
[2] Radiographic tumor measurements should be obtained via imaging of the chest, abdomen and pelvis. See RECIST 1.1 for allowable imaging modalities used to assess disease at baseline and subsequent assessments. Contrast CT is the preferred modality.

Note: See section 11.2 for mandatory and optional research specimens calendar

4.2 ASSESSMENTS DURING TREATMENT (02/22/2016)

Assessments	**Induction Phase,[1] Prior to Each Infusion (All Infusions after Cycle 1, Day 1)**	**Maintenance Phase,[2] Prior to Each Infusion**	**Maintenance Phase,[2] Prior to Every other Infusion (i.e., prior to maintenance infusion 1, 3, 5, ect)**	**Timed (Treatment Cycle Independent)**
History and Physical	≤ 1 day of treatment	≤ 1 day of treatment		
Vital Signs (Blood Pressure, Heart Rate, Temperature and **Pulse Oximeter** Oxygen Saturation)	Day of treatment	Day of treatment		
Performance Status	≤ 1 day of treatment	≤ 1 day of treatment		
Toxicity Assessment (including clinical assessment for irAEs)[3]	≤ 1 day of treatment	≤ 1 day of treatment		
Concurrent Medications	≤ 1 day of treatment	≤ 1 day of treatment		
CBC/Differential/Platelets	≤ 3 days of treatment	≤ 3 days of treatment		
Electrolytes, including, BUN, creatinine, Ca, phosphorus, magnesium and glucose	≤ 3 days of treatment	≤ 3 days of treatment		
Bilirubin, ALT, AST, Alkaline Phosphatase, Albumin	≤ 3 days of treatment	≤ 3 days of treatment		
Amylase/Lipase	≤ 3 days of treatment	≤ 3 days of treatment		
TSH	≤ 7 days of treatment		≤ 7 days of treatment	
CA-125			≤ 7 days of treatment	
Radiographic tumor measurement				X[4]
Echocardiogram[5] **(02/13/2017)**				

Note: See section 11.2 for mandatory and optional research specimens calendar

[1]Induction phase consists of first 4 infusions, administered either every 2 weeks (nivolumab arm) or every 3 weeks (nivolumab plus ipilimumab arm).
[2]Maintenance phase consists of every 2 week infusions of nivolumab only, following induction phase.
[3]Report all adverse events that occur within 30 days of last protocol treatment on the Toxicity form for the last cycle of therapy administered. For reporting of delayed toxicity, see Section 7.
[4]Every 8 weeks (+/- 7 days) from cycle 1, day 1 (regardless of delays and/or changes in treatment schedule) for the first 8 months; then every 12 weeks (+/- 7 days) thereafter. Radiographic tumor measurements are obtained until disease progression is confirmed; at the investigator's discretion, they can be repeated any other time if clinically indicated based on symptoms or physical signs suggestive of new or progressive disease. A tool is provided to calculate dates of re-imaging. Utilize **same** imaging modality of abdomen, pelvis and chest (see footnote 2 under Pre-Treatment Assessments) as for pre-cycle 1 baseline assessment. Chest imaging is also indicated to monitor for evidence of immune pneumonitis. See section 14.1 for guidance concerning continuation of treatment in cases of radiologic progression at the first 8 week (+/- 7 days) CT. **(02/22/2016)**
[5]Indicated for symptoms or physical signs of congestive heart failure or cardiomyopathy. **(02/13/2017)**

4.3 ASSESSMENTS IN FOLLOW UP

Assessments	Timed
Vital Status	1
Toxicity Assessment	2
Radiographic tumor measurement	3

[1]Every 3 months for 2 years and then every 6 months for 3 years. Follow-up Forms are collected for the 5-year follow-up period or until study termination.
[2]Patients will be followed for 100 days or 16 weeks (based on 5 half lives) from end of treatment on Follow Up Adverse Event Reporting Form. Patients who discontinue treatment for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. For reporting of delayed toxicity, see Section 7.
[3]In the case that protocol directed therapy is discontinued for reasons other than disease progression, follow radiographic tumor measurement schedule as defined under Assessments During Treatment (until disease progression documented by RECIST 1.1 or until patient initiates a subsequent cancer therapy).

5. TREATMENT PLAN/REGIMEN DESCRIPTION

The treatment plan consists of an induction phase in which treatment is administered every 2 weeks (nivolumab arm) or every 3 weeks (nivolumab plus ipilimumab arm) for 4 total doses, followed by a maintenance phase in which treatment with nivolumab only is administered every 2 weeks. Treatment is administered for a maximum of 42 doses of maintenance therapy, until disease progression or until development of unacceptable toxicity, whichever comes first.

5.1 Chemotherapy/Hormonal Therapy/Other Agent-Based Therapy (02/22/2016)

5.1.1 Protocol Directed Therapy

During maintenance, a patient will be permitted to have an infusion delayed up to 7 days (without this being considered to be a protocol violation) for major life events (e.g., serious illness in a family member, major holiday, vacation which is unable to be re-scheduled). Documentation to justify this decision should be provided.

During both induction and maintenance, it will be acceptable for individual infusions to be delivered within a 24-hour window before and after the protocol-defined date. If the treatment due date is a Friday, and the patient cannot be treated on that Friday, then the window for treatment would include the Thursday (1 day earlier than due) through the Monday (day 3 past due).

5.1.2 Nivolumab Regimen

Nivolumab 3 mg/kg IV every 2 weeks x 4 for induction, followed by nivolumab 3 mg/kg IV every 2 weeks for a maximum of 42 doses for maintenance.

5.1.3 Nivolumab plus Ipilimumab Regimen

Nivolumab 3 mg/kg IV plus ipilimumab 1 mg/kg, every 3 weeks x 4 for induction, followed by nivolumab 3 mg/kg IV every 2 weeks for a maximum of 42 doses for maintenance.

The order of administration is nivolumab, followed by ipilimumab. **(02/22/2016)**

5.1.4 Details of Nivolumab Administration **(02/28/2019)**

Patients may not be dosed less than 12 days from the previous dose of drug.
The dosing calculations should be based on the actual body weight. If the patient's weight on the day of dosing differs by >10% from the weight used to calculate the original dose, the dose must be recalculated. All doses should be rounded to the nearest milligram or the treating institutional guidelines. There will be no dose modifications allowed.

Nivolumab is to be administered over approximately 30 minutes as an IV infusion, using a volumetric pump with a 0.2-1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be below 0.35 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

5.1.5 Details of Ipilimumab Administration

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

Ipilimumab is to be administered over approximately 90-minutes as an IV infusion, using a volumetric pump with a PVC IV infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (0.2/1.2 micron) at the protocol-specified dose. The drug can be given undiluted or diluted with 0.9% normal saline for delivery with the total drug concentration of the solution between 1 mg/mL and 4 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

5.2 Radiation Therapy
Not Applicable.

5.3 Surgery
Not Applicable.

5.4 Device
Not Applicable.

5.5 Imaging
Not Applicable.

5.6 Integral Assay/Biomarker
Not applicable.

5.7 Intervention Not Otherwise Categorized
Not applicable.

5.8 General Concomitant Medication and Supportive Care Guidelines

5.8.1 Permitted Supportive/Ancillary Care and Concomitant Medications

- All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol and documented on each site's source documents as concomitant medication. Follow guidelines in section 6.0 and Appendix III regarding medication and supportive guidelines related to immune adverse effects. Please consult Study Chair or Study Co-Chair with questions.
- Analgesics
- Antibiotics
- Anticonvulsants
- Antiemetics
- Anticoagulants
- Antihistamines (as per Appendix III)
- Corticosteroids +/- mineralocorticoid component (as per Appendix III)
- Hydration
- Immunosuppressive agents – other (as per Appendix III)
- Infliximab (as per Appendix III)
- Mycophenolate mofetil (as per Appendix III)

- Nutritional supplementation

5.8.2 Prohibited Therapies

- Chronic systemic corticosteroids or other immunosuppressive agents for conditions other than for hypersensitivity or immune adverse effects associated with nivolumab or ipilimumab as specified in the protocol.
- Patients in this study may use standard vaccines. 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 nivolumab/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 nivolumab/ipilimumab treatments.

5.9 Duration of Therapy (Discontinuation Criteria) (02/22/2016)

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

- Disease progression (NOTE: the protocol specifies continuation of treatment in cases of radiologic progression at the first 8 week (+/- 7 days) CT if all of the criteria in section 14.1 are satisfied)
- Any clinical adverse event, laboratory abnormality, or on study illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued study drug dosing.
- Specified unacceptable adverse event(s) defined below and in section 6.1:
    - Any Grade 2 or greater drug-related uveitis, 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.
    - Grade 3 drug related laboratory abnormalities do not require treatment discontinuation except Grade 3 drug-related thrombocytopenia > 7 days or any grade thrombocytopenia associated with bleeding.
    - Any Grade 4 drug laboratory only abnormality with no clinical consequence may be managed medically, and treatment may be continued at investigator discretion. Examples are:
        - Grade 4 lymphopenia
        - Isolated Grade 4 electrolyte imbalance/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.
        - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis (see Section 6.1).
- Any patients who require additional immune suppressive treatment beyond

steroids.
- Patient decides to withdraw from study treatment.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

The Study Chair or Co-Chair must be consulted in any case where protocol therapy has not been discontinued but where protocol therapy dosing has been delayed > 6 weeks in order to determine whether or not protocol therapy may be resumed or must be discontinued.

Patients in the combination nivolumab and ipilimumab cohort who experience an unacceptable adverse event(s) (defined in section 5.9 and 6.1) during combination nivolumab and ipilimumab induction can, following resolution of toxicity to ≤ grade 1 and permission from the Study Chair or Co-Chair, continue on nivolumab alone to complete induction followed by maintenance nivolumab. In these cases, the schedule for the induction phase with nivolumab should resume as per the Treatment 1 - Nivolumab Regimen in section 5.1.2. In addition, the same management guidelines should be followed and any patient with any further unacceptable adverse event(s) while receiving nivolumab alone should be taken off study treatment permanently.

Tumor assessments should continue if protocol therapy is discontinued for reasons other than disease progression (see protocol section 4).

6. TREATMENT MODIFICATIONS/MANAGEMENT (02/22/2016)

No dose reductions or escalations for nivolumab or ipilimumab are permitted or specified. In the case of adverse effects, please see guidelines below, Appendix III and Section 5.9 for treatment delay/discontinuation and for management.

If treatment delay is required, patients should be reassessed weekly. Doses should be considered delayed not missed. See Section 5.9 for description of maximally allowed delay.

NOTE: In several places there are differences between protocol directed drug treatment delay and discontinuation rules and Appendix III, identified with (^). In these cases please follow the protocol specific guidelines in this section (as opposed to Appendix III).

The most common adverse effects of nivolumab and ipilimumab are related to their mechanisms of action and are auto-immune in nature. Management in cases of adverse effects in general depends on organ system involved, grade and duration.

6.1 Immune-Related Adverse Events (irAEs) General Definition, Monitoring, and Management (02/22/2016)

For the purposes of this protocol, an immune-related adverse reaction irAE is defined as an adverse reaction of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an event an irAEs. Serologic, immunologic, and histologic (biopsy) data should be used to support the diagnosis of an immune-related toxicity. Suspected immune-related adverse reactions must be documented on an AE or SAE form.

Overall, immune-related AEs commonly start within 3 to 10 weeks from initiation of therapy and are in most cases successfully managed by delaying doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as mentioned below. Immune-related AEs generally resolved within days to weeks in the majority of subjects.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic immune-mediated adverse reactions (e.g., systemic lupus erythematosus-like diseases) or organ-specific immune-mediated adverse reaction (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an immune-mediated adverse reaction is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary.

It is unknown if systemic corticosteroid therapy has an attenuating effect on nivolumab or ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from ipilimumab. If utilized, corticosteroid therapy should be individualized for each patient. For example, prior experience suggests that colitis manifested as ≥ grade 3 diarrhea requires corticosteroid treatment. If an irAE is documented, in general, delay protocol therapy and initiate corticosteroids earlier to obtain resolution with the possibility for resuming protocol therapy rather than waiting for higher grade events.

Below are dose delay tables for both nivolumab and ipilimumab (depending on assigned treatment regimen) related to adverse events. Patients may be dose-delayed for evaluation and restarted depending on results.

Adverse effects algorithms in Appendix III provide schematic representations of the guidelines in this section. These guidelines/algorithms should be followed barring specific clinical circumstances in which the treating physician indicates variations or alternative treatment is needed. In these cases, the investigator must contact the Study Chair or Study Co-Chair. If a patient experiences several adverse events where there are conflicting recommendations between the guidelines in this section and the appropriate algorithms, the investigator should follow the protocol specific guidelines in this section.

Investigators should as a rule evaluate suspected adverse effects early, and with any suspicion, erring on the side of caution by withholding drug and instituting appropriate treatment as indicated in the management tables and following event specific guidelines.

Immune-mediated Endocrinopathy. Agents such as ipilimumab and nivolumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thyroiditis 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. Patients 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 patient's symptoms should be excluded. Based on the available data with known outcome, most of the subjects symptomatically improved with hormone replacement therapy. Long-term hormone replacement therapy with hydrocortisone and thyroid hormone will typically be required for subjects developing hypophysitis/hypopituitarism after treatment with ipilimumab. Some patients have regained partial function following steroid treatment.

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. **Headache is often the first symptoms of hypophysitis.** Patients 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 endocrinopathy should be considered immune-mediated and drug withheld pending evaluation. Patients may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

In this study, TSH will be performed at baseline prior to initial treatment (see section 4). TSH levels, clinical chemistries and clinical assessment is to be performed using the schedule in section 4, with further evaluation as clinically indicated. Monitoring TSH may allow early detection of pituitary dysfunction and hypophysitis. Clinical monitoring of symptoms may be equally or more sensitive as an initial presentation. In a limited number of patients, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland.

Please see table below and Appendix III related to delays in protocol therapy and management related to endocrine related adverse effects.

Endocrine Thyroiditis Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Asymptomatic, TSH out of normal range and no evidence of hypophysitis. Continue protocol therapy per protocol. If a) TSH < 0.5 x lower limit of normal (LLN), b) TSH > 2 x upper limit of normal (ULN), or c) TSH consistently otherwise out of range in 2 subsequent measurements: Include free thyroxine (fT4) at subsequent cycles and consider endocrinology consultation**.**
Grade 2	Symptomatic. Hold protocol therapy pending evaluation and management as per Appendix III - Endocrinopathy Management Algorithm. Resume protocol therapy if patient on a stable replacement hormone regimen with toxicity resolved to ≤ grade 1 and having completed steroid taper (or tapered to <10 mg/kg prednisone equivalent) for patient treated with steroids. .
Grade 3	Hold protocol therapy pending evaluation and management as per Appendix III - Endocrinopathy Management Algorithm. Resume protocol therapy if patient on a stable replacement hormone regimen with toxicity resolved to ≤ grade 1 and having completed steroid taper (or tapered to <10 mg/kg prednisone equivalent) for patient treated with steroids.
Grade 4	Discontinue protocol therapy.
Notes: Prior to starting corticosteroids or hormone replacement for any reason, appropriate pituitary functional testing including 8 AM cortisol, 8 AM ACTH, TSH and T4 must be obtained. Isolated thyroid deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored. Central hypothyroidism should be considered hypophysitis (not thyroiditis). Symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI is considered a grade 3 event.	
Recommended management: See Endocrinopathy Management Algorithm, Appendix III	

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 in particular. Patients 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 patients 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 patients, 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 patients with colitis and may be an independent entity or may co-exist with ipilimumab induced inflammatory colitis.

See Appendix III (GI Adverse Event Management Algorithm).

Permanently discontinue ipilimumab in patients with severe enterocolitis (see table below on Management/Protocol Therapy Modifications for Diarrhea/Enterocolitis and Appendix III), and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of methylprednisolone IV or IV 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 of ipilimumab has resulted in recurrence or worsening symptoms of enterocolitis in some patients receiving ipilimumab. Patients have been treated with anti-TNF agents (e.g., infliximab) 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.

Diarrhea/ Enterocolitis	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy at investigator discretion. Treat symptoms. Monitor closely for worsening symptoms. Educate patient to report worsening symptoms immediately.
Grade 2	Delay protocol therapy. Treat symptoms. If improves to ≤ Grade 1, resume protocol therapy at investigator discretion. Consider steroid treatment as clinically indicated if symptoms fail to improve or worsen after evaluation and symptomatic treatment.
Grade 3	Discontinue protocol therapy
Grade 4	Discontinue protocol therapy.
Notes: See GI Adverse Event Management Algorithm in Appendix III for NCI CTCAE v4.03 definitions of diarrhea and colitis by grade and for management of diarrhea/colitis. Evaluate for additional causes including *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.	

Other GI - Nausea/Vomiting	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
≤ Grade 1	Continue protocol therapy and monitor. Symptomatic Treatment.
Grade 2	Hold protocol therapy pending evaluation for gastritis, duodenitis and other immune adverse events or other causes. Resume protocol therapy after resolution to ≤ Grade 1.
Grade 3	Hold protocol therapy pending evaluation until ≤ Grade 1. Then resume protocol therapy. If symptoms do not resolve within 7 days with symptomatic treatment, discontinue protocol therapy
Grade 4	Discontinue protocol therapy
Patients with grade 2 or 3 nausea or vomiting should be evaluated for upper GI inflammation and other immune related events. Recommended management: antiemetics.	

Immune-mediated Hepatitis and Pancreatitis. Hepatic immune-related AEs are 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 protocol directed therapy infusion 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 patients who had immune-related hepatotoxicity have shown evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Monitor levels of hepatic transaminases, bilirubin, and lipase and assess patients for signs and symptoms of hepatotoxicity/pancreatitis before each protocol directed therapy infusion. In patients with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution. Please see guidelines below in tables related to hepatic transaminase/bilirubin levels and pancreatitis/lipase levels. Please also see Appendix III.

Hepatic transaminase, bilirubin	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy at investigator discretion. Continue liver function tests (LFT) monitoring per section 4.2.
Grade 2	Hold protocol therapy. Increase frequency of laboratory monitoring to every 3 days. Consider steroid treatment as clinically indicated if symptoms fail to improve or worsen after evaluation and symptomatic treatment. If resolves to ≤ Grade 1 or baseline, resume protocol therapy.
Grade 3	Discontinue protocol therapy. Manage as per Appendix III – Hepatic Adverse Event Management Algorithm.
Grade 4	Discontinue protocol therapy. Manage as per Appendix III – Hepatic Adverse Event Management Algorithm.
Notes: See Hepatic Adverse Event Management Algorithm in Appendix III for NCI CTCAE v. 4.03 hepatic adverse event grading and management and Opdivo package insert. Continued treatment (nivolumab or nivolumab + ipilimumab) during active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug (nivolumab or nivolumab + ipilimumab) to evaluate LFT changes as indicated above and in Appendix III and early treatment are mandatory. 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 in Appendix III	

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy and monitor. Asymptomatic lipase/amylase elevation, continue protocol therapy and monitor patient clinically.
Grade 2	For amylase/lipase elevations only, continue protocol therapy and monitor patient clinically. For radiographic findings consistent with pancreatitis, hold protocol therapy until resolved.
Grade 3	Discontinue protocol therapy for development of Grade 3 pancreatitis or for new or worsening DM. Evaluate for co-existing hepatitis/cholecystitis. For amylase/lipase elevations only, continue protocol therapy and monitor patient clinically.
Grade 4	Discontinue protocol therapy for development of Grade 4 pancreatitis or for new or worsening DM. For amylase/lipase elevations only, continue protocol therapy and monitor patient clinically. Consider imaging if no improvement or worsening.

Immune-related Neurological Events. Fatal Guillain-Barre syndrome has been reported in clinical trials of ipilimumab. Patients may present with muscle weakness and myasthenia gravis, cranial nerve palsy (n VII Bell's palsy), and aseptic meningitis and 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.

Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Please see guidelines below in table related to neurologic adverse effects. Please also see Appendix III.

Withhold protocol therapy dosing in patients with any evidence of neuropathy pending evaluation. Permanently discontinue protocol therapy in patients 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 methylprednisolone IV or IV equivalent for severe neuropathies.

Neurologic events	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Hold protocol therapy pending evaluation and observation^. Then resume protocol therapy.
Grade 2	Hold protocol therapy pending evaluation and observation until ≤ Grade 1^. Discontinue protocol therapy if treatment with steroids is required^. Otherwise resume protocol therapy.
Grade 3	Discontinue protocol therapy
Grade 4	Discontinue protocol therapy
Discontinue protocol therapy for any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, or myasthenia gravis.	
Recommended management: See Neurologic Adverse Event Management Algorithm in Appendix III	

Pneumonitis	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Hold protocol therapy pending evaluation including pulmonary or ID consultation and resolution to baseline, including baseline pO2^. Then resume protocol therapy.
Grade 2	Hold protocol therapy pending evaluation including pulmonary or ID consultation and resolution to baseline, including baseline pO2. Then resume protocol therapy if lymphocytic pneumonitis is excluded; otherwise discontinue protocol therapy. Consider steroid therapy as clinically indicated, see Opdivo package insert for further information. Discontinue protocol therapy if steroids required^.
Grade 3	Discontinue protocol therapy
Grade 4	Discontinue protocol therapy
Notes: Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids, see Opdivo package insert for further information. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Consider recommending seasonal influenza killed vaccine for all patients.	
Recommended management: See Pulmonary Adverse Event Management Algorithm in Appendix III	

Renal Adverse Events	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy and monitor creatinine weekly
Grade 2	Delay protocol therapy, monitor creatinine every 2 – 3 days. Administer corticosteroids and manage as directed by the Renal Adverse Event Management Algorithm in Appendix III.
Grade 3	Delay protocol therapy, monitor creatinine every 2 – 3 days. Administer corticosteroids and manage as directed by the Renal Adverse Event Management Algorithm in Appendix III.
Grade 4	Discontinue protocol therapy. Manage as directed by the Renal Adverse Event Management Algorithm in Appendix III.
Recommended management: See Renal Adverse Event Management Algorithm in Appendix III.	

Immune-mediated Dermatitis. Skin immune-related Aes presented mostly frequently as a rash and/or pruritus. Some subjects reported vitiligo associated with ipilimumab administration. Fatal toxic epidermal necrolysis has been reported in clinical trials of ipilimumab.

Monitor patients 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.

Please see guidelines for management/protocol therapy modifications in the table below and Appendix III. **(02/22/2016)**

Permanently discontinue protocol therapy in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, 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 methylprednisolone IV or IV equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month.

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy. Symptomatic therapy (e.g. antihistamines, topical steroids).
Grade 2	Continue protocol therapy at investigator discretion. Symptomatic therapy (e.g. antihistamines, topical steroids).
Grade 3	Hold protocol therapy until ≤ Grade 1. Obtain Dermatology consultation, with consideration of skin biopsy.
Grade 4	Discontinue protocol therapy.
Notes: **Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphigoid.** Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note that skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: See Skin Adverse Event Management Algorithm in Appendix III	

FATIGUE	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Continue protocol therapy
Grade 2	Continue protocol therapy
Grade 3	Hold protocol therapy. Resume protocol therapy if ≤ Grade 2 within 7 days.
Grade 4	Discontinue protocol therapy
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, hepatic, and/or muscle (CPK) inflammation	

Fever	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
Grade 1	Hold protocol therapy until < grade 1. Then resume protocol therapy
Grade 2	Hold protocol therapy until < grade 1. Then resume protocol therapy
Grade 3	Hold protocol therapy until < grade 1. Then resume protocol therapy
Grade 4	Discontinue protocol therapy
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever.	
See section 6.3 regarding Infusion Reactions	

Other Immune-mediated Adverse Reactions (e.g. ocular, joint, myocardial, pericardial) not addressed above have been observed with agents such as ipilimumab and nivolumab.

See the table below regarding delay in protocol therapy related to other adverse events. Please refer to Section 5.9 prior to utilizing this table; in some cases specific rules in section 5.9 override guidance in this table.

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 methylprednisolone IV or IV equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis.

ALL OTHER EVENTS	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipilimumab**
≤ Grade 1	Continue protocol therapy at investigator discretion.
Grade 2	Consider corticosteroids as clinically indicated. Hold until ≤ grade 1 OR baseline, then resume protocol therapy.
Grade 3	Discontinue protocol therapy
Grade 4	Discontinue protocol therapy

Any patient started on corticosteroids initially who is subsequently determined not to require steroids treatment for an autoimmune adverse event may resume therapy at the discretion of the investigator. In such a case, steroids should be slowly tapered. **(02/22/2016)**

Cardiac *	**Management/Next Dose for BMS-936558 (Nivolumab) + Ipilimumab Cardiac Toxicities (02/13/2017)**
≤ 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.

6.2 Criteria to Discontinue Protocol Therapy

Refer to specific guidelines in sections 5.9 and 6.1.
Tumor assessments should continue if protocol therapy is discontinued for reasons other than disease progression (see protocol section 4).

6.3 Treatment of Nivolumab or Ipilimumab-Related Infusion Reactions (02/22/2016)

Since nivolumab and ipilimumab contain only human immunoglobulin protein sequences, they 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 an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE version 4.0 guidelines.

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

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

Remain at bedside and monitor subject until recovery from symptoms. Infusion rate may be slowed. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely.

The following prophylactic pre-medications 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.

For Grade 2 infusion reaction: (Therapy or infusion interruption indicated but responds promptly to symptomatic treatment [*e.g.*, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, IV fluids];prophylactic medications indicated for ≤ 24 hours).

Stop the nivolumab/ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor patient until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. If symptoms recur, then no further

nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the patient until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and paracetamol 325 to 1000 mg acetaminophen should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 infusion reaction: Grade 3: (Prolonged [*e*.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae); or **Grade 4:** (Life threatening consequences; urgent intervention indicated).

Immediately discontinue infusion of nivolumab/ipilimumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Patient should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab/ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor patient until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (*e.g*., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (*e.g*., oral antihistamine, or corticosteroids).

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

6.4 TREATMENT OF IPILIMUMAB/NIVOLUMAB-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 nivolumab/ipilimumab or to an infectious etiology. If a patient 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 nivolumab/ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient 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 patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further nivolumab/ipilimumab.

7. ADVERSE EVENTS REPORTING REQUIREMENTS

7.1 Protocol Agents

Investigational Agents

The investigational agents administered in NRG-GY003 are:

Nivolumab: IND #125336; IND Sponsor: DCTD, NCI

Ipilimumab: IND Sponsor: DCTD, NCI

For Nivolumab and Ipilimumab, determination of whether an adverse event meets expedited reporting criteria, see the reporting table in section 7.4.2 of the protocol.

7.2 Adverse Events and Serious Adverse Events

7.2.1 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 https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. **(07/30/2018)**

7.2.2 Definition of an Adverse Event (AE)

Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of unrelated, unlikely, possible, probable, or definite). (International Conference on Harmonisation [ICH], E2A, E6).

For multi-modality trials, adverse event reporting encompasses all aspects of protocol treatment including radiation therapy, surgery, device, and drug.

Due to the risk of intrauterine exposure of a fetus to potentially teratogenic agents, the pregnancy of a study participant must be reported via CTEP-AERS in an expedited manner.

7.3 Comprehensive Adverse Events and Potential Risks (CAEPR) List for CTEP Study Agents

7.3.1 Comprehensive Adverse Events and Potential Risks list (CAEPR) for BMS-936558 (Nivolumab, MDX-1106, NSC 748726) (03/21/2016) (02/13/2017) (07/30/2018)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the

comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2069 patients.* Below is the CAEPR for BMS-936558 (Nivolumab, MDX-1106).

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

Version 2.3, June 18, 2018[1]

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (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 2)***
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
		Pericarditis	
ENDOCRINE DISORDERS			
	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]	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Gastritis	
		Mucositis oral	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		***Injection site reaction (Gr 2)***
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)[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)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)[3]	
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-Barre syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<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]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)[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 disorders - Other (bullous pemphigoid)	
	Skin and subcutaneous 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]BMS-936558 (Nivolumab, MDX-1106) being a member of class of agents involved in the inhibition of "immune checkpoints", may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[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 BMS-936558 (Nivolumab, MDX-1106). These complications may occur despite intervening therapy between receiving BMS-936558 (Nivolumab, MDX-1106) 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.

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

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

7.3.2 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Ipilimumab (MDX-010, NSCs 732442 and 720801) (03/21/2016) (02/13/2017) (07/30/2018) (06/10/2019)

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 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[2] (Gr 3)***
		Colonic perforation[3]	
	Constipation		
Diarrhea			***Diarrhea (Gr 3)***
	Enterocolitis		
	Esophagitis		
		Ileus	

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%)**	
Nausea			***Nausea (Gr 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[2]		
	Guillain-Barre syndrome[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%)**	
	Headache		
	Myasthenia gravis[2]		
		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 tissue 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 adverse event:

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 adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.4 Expedited Reporting of Adverse Events

All serious adverse events that meet expedited reporting criteria defined in the reporting table below will be reported via the CTEP Adverse Event Reporting System, CTEP-AERS, accessed via the CTEP web site, https://eapps-ctep.nci.nih.gov/ctepaers/pages/task?rand=1390853489613

Submitting a report via CTEP-AERS serves as notification to NRG and satisfies NRG requirements for expedited adverse event reporting.

CTEP-AERS provides a radiation therapy-only pathway for events experienced that involve radiation therapy only. These events must be reported via the CTEP-AERS radiation therapy-only pathway.

In the rare event when Internet connectivity is disrupted, a 24-hour notification must be made to the NRG Regulatory Affairs by phone at 215-854-0770. An electronic report must be submitted immediately upon re-establishment of the Internet connection.

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

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

7.4.1 Expedited Reporting Methods

- CTEP-AERS-24 Hour Notification requires that a CTEP-AERS 24-hour notification is electronically submitted within 24 hours of learning of the adverse event. Each CTEP-AERS 24-hour notification must be followed by a complete report within 3 days. Supporting source documentation is requested by NRG as needed to complete adverse event review. When submitting supporting source documentation, include the protocol number, patient ID number, and CTEP-AERS ticket number on each page, and fax supporting documentation to the NRG Regulatory Affairs by phone at 215-854-0716.
- A serious adverse event that meets expedited reporting criteria outlined in the AE Reporting Tables but is assessed by the CTEP-AERS as "an action *not* recommended" must still be reported to fulfill NRG safety reporting obligations. Sites must bypass the "NOT recommended" assessment; the CTEP-AERS allows submission of all reports regardless of the results of the assessment.

7.4.2 Expedited Reporting Requirements for Adverse Events

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for

Adverse Events that Occur on Studies under a CTEP IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

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

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

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

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

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

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

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

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 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 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

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

Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

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

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

7.4.3 Reporting to the Site IRB/REB

Investigators will report serious adverse events to the local Institutional Review Board (IRB) or Research Ethics Board (REB) responsible for oversight of the patient according to institutional policy.

7.4.4 Secondary Malignancies **(02/13/2017)**

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 during or subsequent to treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. In addition, secondary malignancies following radiation therapy must 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.

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 reporting via CDUS unless otherwise specified.

8. REGISTRATION, STUDY ENTRY, AND WITHDRAWAL PROCEDURES

8.1 CTEP Investigator Registration Procedures **(05/22/2017)**

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

Registration requires the submission of:

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

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

questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

8.1.1 CTEP Associate Registration Procedures / CTEP-IAM Account

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

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

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

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

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

8.1.2 CTSU Registration Procedures **(02/13/2017)**

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

8.1.2.1 IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to: an active Federal Wide Assurance (FWA) number, an active roster affiliation with the Lead Network or a participating organization, a valid IRB approval and compliance with all protocol specific requirements.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for the study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRB Manager to indicate their intent to open the study locally. The

CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

8.1.2.2 Requirements for NRG-GY003 Site Registration: **(05/22/2017)**

- IRB approval letter (For sites not participating via the NCI CIRB; local IRB documentation, and IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration Of Exemption Form, or a combination is accepted)

8.1.2.3 Submitting Regulatory Documents: **(05/22/2017)**
Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission

When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

8.1.2.4 Checking Your Site's Registration Status: **(05/22/2017)**
You can verify your site registration status on the members' section of the CTSU website:

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

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

8.2 **Oncology Patient Enrollment Network (OPEN) (02/13/2017) (05/22/2017)**
Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7

basis. All site staff (NRG and CTSU Sites) will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' web site https://www.ctsu.org.

Prior to accessing OPEN site staff should verify the following:

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

Access requirements for OPEN:

- See Section 8.1.1 for information on obtaining a CTEP-IAM account.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the relevant Group or CTSU roster.
- To perform registrations on protocols for which you are a member of the NRG, you must have an equivalent 'Registrar' role on the NRG roster. Role assignments are handled through the Groups in which you are a member.
- To perform registrations to trials accessed via the CTSU mechanism (i.e., non-Lead Group registrations) you must have the role of Registrar on the CTSU roster. Site and/or Data Administrators can manage CTSU roster roles via the new Site Roles maintenance feature under RSS on the CTSU members' web site. This will allow them to assign staff the "Registrar" role.

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

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

8.3 Agent Ordering and Agent Accountability

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

one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

In general, sites may order initial agent supplies when a subject 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 (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime. Refer to the PMB's website for specific policies and guidelines related to agent management. **(05/22/2017)**

8.3.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol. **(05/22/2017)**

8.3.3 Investigator Brochure Availability **(05/22/2017)**
The current versions of the IBs for PMB-supplied agents will be accessible to site investigators and research staff 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. Questions about IB access may be directed to the PMB IB coordinator at IBCoordinator@mail.nih.gov.

8.3.4 Useful Links and Contacts **(05/22/2017)**

- 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 Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB IB Coordinator: IBCoordinator@mail.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)

9. DRUG INFORMATION

9.1 Nivolumab (BMS-936558, MDX1106), NSC #748726 (02/28/2019)

9.1.1 Classification: Anti-PD-1MAb

9.1.2 M.W.: 146,221 daltons

9.1.3 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.

9.1.4 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, non-pyrogenic, 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).

9.1.5 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.

9.1.6 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. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. All vials must come from the same batch.

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

9.1.8 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.

9.1.9 Route of Administration: Intravenous infusion over 30 minutes. Do not administer as an IV push or bolus injection.

9.1.10 Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, in-line filter. **(02/22/2016)**

9.1.11 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.

9.1.12 See Section 7.3.1 for CAEPR for BMS 936558 (Nivolumab)

9.2 Ipilimumab (MDX-010) (NSC 732442)

9.2.1 Chemical Name or Amino Acid Sequence**:** 4 polypeptide chains, 2 identical heavy chains with 447 amino acids and 2 identical light chains consisting of 215 amino acids.

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

9.2.3 Classification**:** Human monoclonal antibody

9.2.4 M.W.: 147,991 Daltons

9.2.5 Mode of Action**:** Ipilimumab is specific for the CTLA4 antigen expressed on a subset of activated T-cells. CTLA4 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 CTLA4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA4/B7 creates the interaction.

9.2.6 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.

9.2.7 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, non-pyrogenic, 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.

	Process C
Component	**200 mg/ 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.

9.2.8 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.

9.2.9 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.

9.2.10 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.

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

9.2.12 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).

9.2.13 Patient Care Implications**:** Monitor patients for immune-related adverse events, 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.

9.2.14 See Section 7.3.2 for CAEPR for Ipilimumab.

10. PATHOLOGY

No pathology is required for central pathology review, but pathology reports are required.

11. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

11.1 Reimbursement

See the Funding Sheet found on the CTSU web site (www.ctsu.org).

11.2 Translational Science (05/22/2017)

11.2.1 Specimen Requirements

11.2.1.1 Mandatory Specimen Requirements (05/22/2017) (09/07/2018)

The patient must give permission to participate in this **mandatory** study component. Participating sites are required to submit the patient's specimens as outlined below.

Required Specimen (Specimen Code)	Collection Time Point	Sites Ship Specimens To
ARCHIVAL FFPE		
FFPE Primary Tumor (FP01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to all treatment *(Preferred FFPE)*	NRG BB-Columbus within 8 weeks of registration[1]
FFPE Metastatic Tumor (FM01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to all treatment *(Optional if FP01, FRP01, FRM01, FPP01, or FPM01 is submitted)*	
FFPE Recurrent Primary Tumor (FRP01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to study treatment *(Optional if FP01, FM01, FRM01, FPP01, or FPM01 is submitted)*	
FFPE Recurrent Metastatic Tumor (FRM01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to study treatment *(Optional if FP01, FM01, FRP01, FPP01, or FPM01 is submitted)*	
FFPE Persistent Primary Tumor (FPP01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to study treatment *(Optional if FP01, FM01, FRP01, FRM01, or FPM01 is submitted)*	
FFPE Persistent Metastatic Tumor (FPM01)* 1st Choice: block 2nd Choice: 35 unstained slides (**3 charged, 4 µm**† and 32 charged, 5µm)	Prior to study treatment *(Optional if FP01, FM01, FRP01, FRM01, or FPP01 is submitted)*	

* A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the NRG BB-Columbus.

† **The 3 charged, 4µm, slides should be cut first as they are required to test the integrated biomarker.** If less than 35 slides are available, please contact BPCBank@nationwidechildrens.org and/or NRG-TR@nrgoncology.org.

1 NRG BB-Columbus / Protocol NRG-GY003, Nationwide Children's Hospital, 700 Children's Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2865, FAX: (614) 722-2897, Email: BPCBank@nationwidechildrens.org

11.2.1.2 Optional Specimen Requirements (05/22/2017) (07/31/2017) (09/07/2018)

If the patient gives permission to participate in this **optional** study component, then participating sites are required to submit the patient's specimens as outlined below. PLEASE NOTE that the Pre-Treatment Blood Collections were omitted from the following table in the Amendment #5 document in Error.

Required Specimen (Specimen Code)	Collection Time Point	Sites Ship Specimens To
PRE-TREATMENT BLOOD COLLECTIONS		
Pre-Treatment DNA Whole Blood (WB01) 7-10mL drawn into purple top (EDTA) tube(s)	Prior to study treatment	NRG BB-Columbus the day the specimen is collected[1]
Pre-treatment Serum (SB01) prepared from 7-10mL of blood drawn into plain red top tube(s)	Prior to study treatment	NRG BB-Columbus within 14 weeks of registration[1]
Pre-Treatment Buffy Coat (LB01) prepared from 7-10mL of blood drawn into purple top (EDTA) tube(s)		
6 WEEK BLOOD COLLECTIONS		
6 Week DNA Whole Blood (WB02) 7-10mL drawn into purple top (EDTA) tube(s)	6 weeks after starting study treatment	NRG BB-Columbus the day the specimen is collected[1]
6 Week Serum (SB02) prepared from 7-10mL of blood drawn into plain red top tube(s)		NRG BB-Columbus within 14 weeks of registration[1]
6 Week Buffy Coat (LB02) prepared from 7-10mL of blood drawn into purple top (EDTA) tube(s)		
12 WEEK BLOOD COLLECTIONS		
12 Week DNA Whole Blood (WB03) 7-10mL drawn into purple top (EDTA) tube(s)	12 weeks after starting study treatment	NRG BB-Columbus the day the specimen is collected[1]
12 Week Serum (SB03) prepared from 7-10mL of blood drawn into plain red top tube(s)		NRG BB-Columbus within 14 weeks of registration[1]
12 Week Buffy Coat (LB03) prepared from 7-10mL of blood drawn into purple top (EDTA) tube(s)		

1 NRG BB-Columbus / Protocol NRG-GY003, Nationwide Children's Hospital, 700 Children's Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2865, FAX: (614) 722-2897, Email: BPCBank@nationwidechildrens.org

11.2.2 Specimen Procedures

A detailed description of specimen procedures can be found in Appendix IV.

11.2.3 Laboratory Testing (05/22/2017) (09/07/2018)

Assay details are included in Appendix V. Specimens will be prioritized for testing as outlined below.

	Priority				
Specimen	**1**	**2**	**3**	**4**	**5**
Buffy Coat (LB01-LB03)	Transcript Profiling (11.2.3.5)	-	-	-	-
DNA (WB01-WB03)	TCR Repertoire (11.2.3.4)	-	-	-	-
FFPE (FP01, FM01, FRP01, FRM01, FPP01, FPM01)	PD-L1* (11.2.3.1)	Neo-antigen (11.2.3.3)	TIL IHC (11.2.3.2)	Tumor transcriptome (11.2.3.5)	TCR repertoire (11.2.3.4)
Serum (SB01-SB03)	TAA Responses (11.2.3.6)	-	-	-	-

11.2.3.1 PD-L1 Immunohistochemistry (05/22/2017) (09/07/2018)

Three 4µm charged sections of formalin-fixed, paraffin-embedded (FFPE) archival tumor will be **batch shipped by the NRG BB-Columbus** upon trial completion to *TBD* for immuno-histochemical analysis of PD-L1.

11.2.3.2 Infiltrating Lymphocytes Immunohistochemistry (05/22/2017) (09/07/2018)

Unstained sections of FFPE archival tumor will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Daniel Powell for IHC analysis of tumor infiltrating lymphocytes (TILs). Markers to be examined include CD3, CD4, CD8, FoxP3, CD16, CD68, CD137, OX40, GITR, CD40, IDO, LAG3, TIM3, B7-H3, B7-H4, PD-1, CTLA-4, and VISTA. Quantification of cells in the tumor and invasive margin will be included, provided the invasive margin can be identified.

Daniel J Powell Jr, PhD
University of Pennsylvania
3400 Civic Center Blvd, Bldg 421
Smilow Ctr, Rm 08-103
Philadelphia, PA 19104-5156
Phone: 215-573-4783
Email: poda@mail.med.upenn.edu

11.2.3.3 Neo-antigen Assessment (05/22/2017) (09/07/2018)

Ten 5µm charged sections of FFPE archival tumor collected pre-treatment will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin for DNA extraction and next-generation whole exome sequencing to characterize the neo-antigen landscape.

Dr. Dmitriy Zamarin
Memorial Sloan Kettering Cancer Center
Immune Monitoring Facility
417 East 68th street, Z15-25
Phone: 646-888-2322
Email: zamarind@mskcc.org

11.2.3.4 T Cell Receptor (TCR) Repertoires in Peripheral Blood and Tumors (05/22/2017) (09/07/2018)

DNA isolated from whole blood collected pre-treatment and at 6- and 12-weeks will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin for TCR repertoire analysis. DNA extracted from tumors for neoantigen assessment will be also used for TCR analysis. If insufficient, ten 5µm charged sections of FFPE archival tumor will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin for deep sequencing of TCR CDR3 regions.

11.2.3.5 Immune Transcriptional Profiling inPeripheral Blood (05/22/2017) (09/07/2018)

Peripheral blood buffy coats collected pre-treatment and at 6- and 12-weeks will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin for analysis of transcriptional signatures of immune activation by RNA sequencing or similar platform. Amongst these, the expression of the known activating co-stimulatory receptors such as 4-1BB (CD137), OX40, GITR, CD40, and ICOS, as well as known immune inhibitory proteins such as PD-L1, indoleamine dioxygenase (IDO), B7-H3, B7-H4, LAG3, TIM-3, PD-1, CTLA-4, VISTA, and BTLA will be assessed.

11.2.3.6 Immune Transcriptional Profiling in Tumors (09/07/2018)

Five 5 µm charged sections will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin for analysis of transcriptional signatures of immune activation and correlation of immune signatures with signatures of specific tumor driver pathways such as WNT and MYC, which have been previously associated with lack of response. Immune deconvolution algorithms will be employed to delineate the composition of the tumor microenvironment; these data will be compared to the IHC data. Depending on RNA quality, gene expression assessment will be performed using either RNA sequencing, Affymetrix Clariom D microarray, or HTG EdgeSeq Oncology Biomarker panel, which includes 2560 transcripts associated with tumor driver pathways and microenvironment.

11.2.3.7 Tumor-Associated Antigen (TAA) Serologic Responses (05/22/2017) (09/07/2018)

Serum collected pretreatment and at 6- and 12-weeks of therapy will be **batch shipped by the NRG BB-Columbus** upon trial completion to Dr. Dmitriy Zamarin (address above) for assessment of TAA serologic responses.

11.2.4 Banking Specimens for Future Research

Details regarding the banking and use of specimens for future research can be found in Appendix IV.

11.3 Quality of Life
Not applicable.

12. DATA AND RECORDS

12.1 Data Management/Collection (02/13/2017)
Data collection for this study will be done exclusively through Medidata Rave®. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP-IAM account (check at https://eapps-ctep.nci.nih.give/iam/index.jsp) and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on either the LPO or participating organization roster at the enrolling site.

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

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

12.2 Summary of Data Submission (02/13/2017)
Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during the trial using Medidata Rave®. Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. See Section 7 for information about expedited and routine reporting.

For reporting of secondary cancers or other report forms available in Rave: Indicate form for reporting in Rave, timeframes; add if loading of the pathology report is required.

Summary of Data Submission: Refer to the NRG Oncology member website for the table of Required Forms and Materials.

12.3 Global Reporting/Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design

Primary Efficacy Analysis

Design Summary **(05/22/2017)**

Approximately 48 patients (24 within each arm) will be recruited during the first stage of accrual. The proportion of patients responding to therapy will be determined. If the observed proportion responding on the Nivolumab and Ipilimumab arm is greater than the observed proportion on the Nivolumab arm, then the trial may (with medical judgment indicating) continue with recruitment of a second stage of accrual. If the observed proportion responding to Nivolumab and Ipilimumab is equal to or less than the proportion responding to Nivolumab arm, then the trial should stop early and declare the combination regimen unworthy of further investigation. The trial may be amended and continue as a single arm study if there is evidence of activity in the Nivolumab arm, based on comparisons to historical data. For example, if there are three or more responses in (22-24) or 4 or more in (25-29) at the interim analysis, indicating that the upper bound of a 1-sided 90% CI is ≥25%, then the study could continue as a single arm study with nivolumab (see section 13.7.2).

If the randomized study proceeds to the second stage (with an approximate cumulative accrual of 48 patients per arm; 96 patients in total), the proportion responding in the Nivolumab plus Ipilimumab arm will be compared to the proportion responding in the Nivolumab alone arm, using a 2-stage Fisher's Exact Test that conditions on the event that the trial proceeded to the second stage (see below for further details). The study's overall desired unconditional probability of a type I error (i.e. a false positive study) is 15%. Operationally this would be like using a nominal critical p-value of 15% divided by the probability of going to the second stage when the null hypothesis is true (typical tests could reject Ho if the p-value is less than 33%). See below for more details.

Due to data discreteness, the study's actual alpha=0.114 and beta ≤ 0.196, assuming that exactly 24 patients enroll in each arm for each stage. These operating characteristics will change, depending on the realized accrual. An intent-to-treat analysis will be used for this study.

Design Details and Math Derivation

The study will randomize patients to two treatments, τ_0 (the reference group) and τ_1 (the experimental group) in Stage 1. The realized sample size for τ_0 is n_{01} and for τ_1 is n_{11}. The number of responses to τ_0 is X_0, and the number of responses to τ_1 is X_1. These random variables are assumed to be distributed as binomials:

$$X_0 \sim Bin(n_{01}, P_0)$$
$$X_1 \sim Bin(n_{11}, P_1)$$

If:

$$\frac{X_1}{n_{11}} > \frac{X_0}{n_{01}}$$

then the trial will proceed to Stage 2. Again randomize patients to the two treatments. Let the realized sample size for τ_0 in Stage 2 be n_{02} and for τ_1 be n_{12}. The number of responses to τ_0 is Y_0, and the number of responses to τ_1 is Y_1. These random variables are assumed to be distributed as binomials:

$$Y_0 \sim Bin(n_{02}, P_0)$$
$$Y_1 \sim Bin(n_{12}, P_1)$$

Define the following:

$$Z_1 = X_1 + Y_1$$
$$Z_0 = X_0 + Y_0$$

$$T = Z_1 + Z_0$$

The set, $\frac{X_1}{n_{11}} > \frac{X_0}{n_{01}}$, can be stated equivalently as:

$$X_1 \geq \left[\frac{X_0}{n_{01}} \times n_{11} + 1\right] = [a_0]$$

So, $a_0 = \frac{X_0}{n_{01}} \times n_{11} + 1$, and $[a_0]$ is the integer function of a_0.

Now the joint distribution, conditioned on $X_1 \geq [a_0]$, can be written as follows:

$$f(X_1, X_0, Y_1, Y_0 | X_1 \geq [a_0])$$
$$= P^{-1} I_{X_1 \geq [a_0]}(X_0, X_1) \binom{n_{11}}{X_1} \binom{n_{01}}{X_0} \binom{n_{12}}{Y_1} \binom{n_{02}}{Y_0} P_1^{X_1+Y_1} Q_1^{n_{11}+n_{12}-X_1-Y_1}$$
$$\times P_0^{X_0+Y_0} Q_0^{n_{01}+n_{02}-X_0-Y_0}$$

where I is an indicator function, $P = P(X_1 \geq [a_0])$, and $Q_i = 1 - P_i$ for $i = 0, 1$.

Rewriting in terms of X_1, X_0, Z_1, and Z_0 gives:

$$f(X_1, X_0, Z_1, Z_0 | X_1 \geq [a_0]) = P^{-1} \cdot I_{X_1 \geq [a_0]}(X_0, X_1) \binom{n_{11}}{X_1} \binom{n_{01}}{X_0} \binom{n_{12}}{Z_1 - X_1} \binom{n_{02}}{Z_0 - X_0} P_1^{Z_1} Q_1^{n_{11}+n_{12}-Z_1} \times P_0^{Z_0} Q_0^{n_{01}+n_{02}-Z_0}$$

Note that $[a_0] \leq X_1 \leq n_{11}$ and $Z_1 - n_{12} \leq X_1 \leq Z_1$. Conditional on $X_1 \geq [a_0]$, we know that $X_0 \leq n_{01} - 1$ since $0 \leq X_0 \leq [n_{01} n_{11}^{-1} X_1] - 1 = n_{01} - 1$ when $X_1 = n_{11}$. X_0 is also restricted by $Z_0 - n_{02} \leq X_0 \leq Z_0$ from the definition of Z_0.

So the mass function can be written strictly in terms of Z_1 and $T = Z_0 + Z_1$ by integrating out X_0 and X_1 from f over their restricted ranges. The above equation is rewritten in exponential format:

$$f(\cdot) = P^{-1} \cdot I \cdot exp(Z_1(\theta_1 - \theta_0) + (Z_0 + Z_1)\theta_0) \times \sum_{x_0 = max\{0, Z_0 - n_{02}\}}^{min\{Z_0, n_{01}-1\}} \binom{n_{01}}{x_0} \binom{n_{02}}{Z_0 - x_0} \sum_{x_1 = max\{[a_0], Z_1 - n_{12}\}}^{min\{n_{11}, Z_1\}} \binom{n_{11}}{x_1} \binom{n_{12}}{Z_1 - x_1}$$

where $\theta_i = ln\big(P_i/(1 - P_i)\big)$ is the log odds for treatment $i, i = 0,1$. It is important to note that the second summation is an "inner loop" that depends on each iteration of x_0 through the lower bound of x_1 (i.e. $max\{[a_0], Z_1 - n_{12}\}$). The sufficient statistics for θ_0 and $(\theta_1 - \theta_0)$ are $T = Z_0 + Z_1$ and Z_1, respectively. Substituting $T - Z_1$ for Z_0 and $\Delta = \theta_1 - \theta_0$, which is the log odds ratio for response on treatment τ_1 to τ_0, into the above function, we get:

$$f(Z_1, T) = P^{-1} \cdot I \cdot exp(Z_1 \Delta + T\theta_0) \times \sum_{x_0 = max\{0, T - Z_1 - n_{02}\}}^{min\{T - Z_1, n_{01}-1\}} \binom{n_{01}}{x_0} \binom{n_{02}}{T - Z_1 - x_0} \sum_{x_1 = max\{[a_0], Z_1 - n_{12}\}}^{min\{n_{11}, Z_1\}} \binom{n_{11}}{x_1} \binom{n_{12}}{Z_1 - x_1}.$$

The nuisance parameter, θ_0, is eliminated by conditioning on the realized value of T. That is:

$$f(Z_1 | T) = \frac{f(Z_1, T)}{f(T)}$$

The marginal distribution of T can be found by integrating Z_1 from the joint distribution listed above. Z_1 is restricted as follows:

$$1 \leq Z_1 \leq n_{11} + n_{12}$$
$$T - (n_{01} - 1 + n_{02}) \leq Z_1 \leq T$$

The first set of inequalities stems from the definition of Z_1 and remembering that the

minimal value of $X_1 = 1$. The second set of inequalities stems from the definition of T, Z_0, and remembering that $X_0 \leq n_{01} - 1$. Looking at the joint distribution of $f(Z_1, T)$, we note, based on summation over x_1, that $Z_1 \geq [a_0]$. A lower bound of x_0 is $T - Z_1 - n_{02}$. Accordingly, a lower bound for Z_1 is $[T - Z_1 - n_{02} + 1]$, which is recursive. Setting $Z_1 \geq T - Z_1 - n_{02} + 1$, we get $Z_1 \geq (T - n_{02} + 1)/2$. Finally, $Z_1 \geq \left[\frac{(T-n_{02}+1)}{2} + 0.5\right]$ since Z_1 is an integer. If this definition is not met, the summation over x_1 is 0, which means the value of $Z_1$is not an element of its sample space.

Therefore,

$$f(T) = \sum_{Z_1 = max\left\{1, T-(n_{01}-1+n_{02}), \left[\frac{(T-n_{02}+1)}{2}+0.5\right]\right\}}^{min\{n_{11}+n_{12}, T\}} f(Z_1, T)$$

$f(T)$ can also be thought of as a normalization constant, "C_N", for the conditional distribution of Z_1. That is, $f(T) = P^{-1} \cdot I \cdot exp(T\theta_0)C_N$.

$$f(Z_1|T) = C_N^{-1} \cdot exp(Z_1\Delta) \times \sum_{x_0 = max\{0, T-Z_1-n_{02}\}}^{min\{T-Z_1, n_{01}-1\}} \binom{n_{01}}{x_0}\binom{n_{02}}{T - Z_1 - x_0} \sum_{x_1 = max\{[a_0], Z_1-n_{12}\}}^{min\{n_{11}, Z_1\}} \binom{n_{11}}{x_1}\binom{n_{12}}{Z_1 - x_1}$$

The conditional distribution of $f(Z_1|T)$ is simpler than the joint distribution since the components containing the nuisance parameter, $P^{-1}exp(T\theta_0)$, drops out. The conditional distribution of $f(Z_1|T)$ only depends on Δ and the observable statistic, T.

The cumulative distribution function is given by $P(Z_1 \leq z_1|T) = F(z_1|T)$, and the survival function is provided with $P(Z_1 \geq z_1|T) = S(z_1|T) = 1 - F(z_1|T) + f(z_1|T)$.

<u>Calculating Unconditional Power Using $f(Z_1|T)$</u>

In most applications, the total number of responses is not fixed to T. Instead, the first and second stage sample sizes are set to a desired target (i.e. n_{ij} are fixed for $i = 0,1;\ j = 1,2$), and T is allowed to vary randomly so that attention can focus on the overall probability that $Z_1 \geq Z_c$ where Z_c is a critical value obtained from various survival distributions, $S(Z_c|T)$. One way of obtaining this probability is with:

$$Power = P(Z_1 \geq Z_c, X_1 \geq [a_0]) =$$

$$= \sum_{X_1 \geq [a_0], t \in \Omega_t} P(Z_1 \geq Z_c | X_1 = x_1, X_0 = x_0, T = t) P(T = t | X_1 = x_1, X_0 = x_0) P(X_1 = x_1, X_0 = x_0)$$

Specifically,

$$P(Z_1 \geq Z_c, X_1 \geq [a_0]) = \sum_{x_0=0}^{n_{01}-1} \left(\sum_{x_1=[a_0]}^{n_{11}} f_{X_0}(x_0) f_{X_1}(x_1) \left\{ \sum_{t=x_1+x_2}^{x_1+x_2+n_{02}+n_{12}} S(Z_c|t) f_T(t) \right\} \right)$$

where f_{X_i} are the marginal probability mass functions of X_i, $i = 0, 1$, and $f_T(t)$ is the conditional mass function of T when $X_1 = x_1, X_0 = x_0$. The f_{X_i} are binomial mass functions. The $f_T(t)$ probability can be found as follows:

$$f_T(t) = \sum_{y_0 = max\{0, t-x_1-x_0-n_{12}\}}^{min\{n_{02}, t-x_1-x_0\}} f_{Y_1}(t - x_1 - x_0 - y_0) f_{Y_0}(y_0).$$

Again, the f_{Y_i} are the binomial mass functions of Y_i.

Note that cases $X_1 < [a_0]$ do not contribute to power. If the study closes early, then it is assumed that the null hypothesis is not rejected.

Approach to an Analysis of the Design

The null hypothesis, $H_0: \Delta \leq 0$ is tested against $H_1: \Delta > 0$ using $Z_1 \geq Z_c$ where Z_c is a critical value obtained from $S(Z_c|T)$. Under the null hypothesis, $S(Z_c|T)$ provides the conditional probability of incorrectly rejecting H_0 which implicitly assumes also that $X_1 \geq [a_0]$ (i.e. $S(Z_c|T, X_1 \geq [a_0])$). Since the $max\{P(X_1 \geq [a_0])\} \leq 0.50$ over all values of $P_1 \ni P_1 = P_0$ when $n_{01} = n_{11}$, a more liberal critical value can be utilized from the conditional distribution. When it is desired to test the null hypothesis at an unconditional level of significance, α, then we can find a conditional Z_c so that $S(Z_c|T) \leq 2\alpha$. Then:

$$S(Z_c|T) \times max\{P(X_1 \geq [a_0])\} \leq 2\alpha \cdot max\{P(X_1 \geq [a_0])\} \leq 2\alpha \cdot \frac{1}{2} = \alpha$$

For example, if we want the unconditional level of significance to be 10%, we can find Z_c so that the conditional distribution gives $S(Z_c|T) \leq 0.20$ because the interim analysis will stop the study early and reject H_0 at least 50% of the time.

When $n_{01} \neq n_{11}$, it is not necessarily true that $max\{P(X_1 \geq [a_0])\} \leq 0.50$. In this case, a search for $max\{P(X_1 \geq [a_0])\}$ is necessary and a potentially more conservative critical value is needed.

In general, the conditional level of significance used to determine the critical value

for rejecting the null hypothesis will be found from the desired unconditional level of significance, α, and $max\{P(X_1 \geq [a_0])\}$. Then the smallest value of Z_1 will be found so that under the null hypothesis, $S(Z_1|T) \leq \alpha / max\{P(X_1 \geq [a_0])\}$. Given the discreteness of the dataset, this method is still conservative. That is to say, the true size of the test will be less than or equal to α.

Probabilities of rejecting H_0 can be explored under various P_0, P_1, and sample sizes in order to obtain a design with desired power through simulation or numeric computation as shown above.

Implementation:

Stage 1 will accrue 48 patients with approximately half receiving the reference, τ_0 and the others receiving the experimental therapy τ_1. If the proportion responding on the experimental therapy is greater than the proportion on the reference, then the study will enroll another 48 patients in Stage 2 in a like manner (assuming medical judgment indicates). The unconditional level of significance is 15%. If the study completes accrual to the second stage, then the conditional test will be conducted at no more than the 15%/ $max\{P(X_1 \geq [a_0])\}$level of significance.

Assuming that the targeted accruals are achieved, the study's operating characteristics will have a realized size (probability of a type I error) equal to about 10.4% (when $P_0 = P_1 = 0.2$) and power=80.4% when $P_0 = 0.2$ and $P_1 = 0.4$ (based on exact calculations with a conditional level of significance equal to 0.33881). The probability of early termination (PET) is 57.2% when $P_0 = P_1 = 0.2$ and 8.3% when $P_0 = 0.2$ and $P_1 = 0.4$. The minimum PET under the null hypothesis is 55.7% when $P_0 = P_1 = 0.5$.

13.1.1 Stratification
Patients will be stratified by their most recent platinum-free interval (PFI) according to PFI < 6 months and 6 ≤ PFI < 12 months.

13.1.2 Randomization
Treatment randomization will be conducted by using permuted blocks within each stratum. The permuted blocks will be of size 2 (e.g. 01|10|10|01|10 etc.).

13.1.3 Total Accrual
First stage accrual will target 48 patients (24 in each arm). The second stage of accrual will target an additional 48 patients (96 cumulative).

13.1.4 Justification of Design:
Assuming that the targeted accruals are achieved, the study's operating characteristics will have a realized size (probability of a type I error) equal to about 10.4% (when $P_0 = P_1 = 0.2$) and power=80.4% when $P_0 = 0.2$ and $P_1 = 0.4$ (based on exact calculations). The probability of early termination (PET) is 57.2% when $P_0 = P_1 = 0.2$ and 8.3% when $P_0 = 0.2$ and $P_1 = 0.4$. The minimum PET under the

null hypothesis is 55.7% when $P_0 = P_1 = 0.5$.

13.2 Study Endpoints

13.2.1 Primary Endpoints
The frequency and duration of objective tumor response within 6 months of study entry as assessed by modified RECIST v. 1.1 criteria.

13.2.2 Secondary Endpoints

- The hazard of having a disease progression or dying (PFS endpoint) by treatment.
- The duration of overall survival (OS) by treatment arm.
- The frequency and severity of adverse events as assessed by CTCAE.

13.2.3 Exploratory Endpoints

- Natural anti-tumor immunity in TIL and tumor cells assessed with IHC.
- Markers of "immunogenicity" as determined by the neo-epitope landscape using next-generation whole exome sequencing (NGS).
- Assessing the impact of biomarkers on tumor response, PFS, and OS.

13.3 Primary Objectives Study Design

13.3.1 Primary Hypothesis and Endpoints
Primary Hypothesis: Ho: $P_1 \leq P_0$ versus Ha: $P_1 > P_0$ where P_0 is the probability of response for the reference regimen (nivolumab) and P_1 is the probability of response for the combination regimen (nivolumab + ipilimumab).

The endpoint is tumor response within 6 months of study entry.

13.3.2 Definitions of Primary Endpoints and How These Will Be Analyzed
Tumor response is defined according to RECIST v. 1.1. It will be analyzed according to the methods outlined in section 14.1.

13.3.3 Sample Size and Power Calculations:
See section 13.1 for power calculations. The sample size is 48 patients randomized to the first stage, and another 48 randomized to the second stage.

13.4 Study Monitoring of Primary Objectives

As this is an intent-to-treat analysis, all randomized patients will be included in these analyses. For purpose of timely analysis, we will use tumor response in 6 months as the primary endpoint. If a patient remains on study for more than 6 months with

stable disease as the best response, then this patient will be considered as not responding to therapy.

The frequency of tumor response will be monitored on a weekly basis. If a patient is still on study and has not had a tumor response within 6 months of study randomization (e.g. has stable disease), this patient will be classified as having an unknown tumor response. Patients who do not respond within 6 months will be classified as "non-responders," so all patients should have their tumor responses determined by about 6 months of study suspension or closure, depending on institutional reporting.

An interim report will be given to the DMC once the first stage data is mature. Their task will be to provide a recommendation for early termination (i.e. study closure), continuation, or study amendment. Final study results will be initially reported to the Study Chair and Developmental Therapeutics Chair for review, then to the Cancer Therapeutics Evaluation Program (CTEP). Efficacy information is not routinely reported until the data are sufficiently mature.

13.5 Accrual Considerations

13.5.1 Accrual Rate: 6 patients per month. If accrual is too slow, then an amendment to the study will be considered.

13.5.2 Accrual Goal: 48 patients for stage 1, and 96 patients cumulatively for stage 2.

13.5.3 Study Duration: We anticipate 8 months of accrual for each stage. The first stage may take 11 months. The interim analysis may take up to 6 or 8 months of follow-up.

13.5.4 Estimated Duration for Completion of Primary Endpoint: All of the data should be in by 8 months after the closure of the second stage, so the primary endpoint completion date should be about 35 months after study opening.

If the study terminates early, the completion date should be about 19 months after study activation.

13.6 Dose Level Guidelines

See Schema.

13.7 Secondary or Exploratory Elements (including correlative science aims)

13.7.1 Secondary Hypotheses and Endpoints:
Ho: HR=1 versus Ha: HR<1 where HR is the hazard ratio of the combination regimen to the reference regimen. The endpoints include those for PFS and OS.

For toxicity, Ho: Odds Ratio of a severe toxicity is equal to one. The alternative

hypothesis is that the odds ratio of a severe toxicity is greater than one (treatment to reference regimen). Endpoints are adverse events classified as severe or not.

For Translational Research, the prognostic impact for the level of biomarker will be assessed by testing Ho: HR=1 versus Ha: HR ≠ 1 for biomarker level 1 to level 2 for PFS and OS (across both regimens). Subset analyses will be conducted within each treatment regimen. To assess the predictive value of a biomarker, we note in statistical terms, the hazard ratio for progression on the experimental treatment to the control treatment depends on the level of biomarker expression (high versus low). Using Peterson and George's notation,[93] let Delta1 be the hazard ratio for low levels of biomarker expression and Delta2 be the hazard ratio for the high levels of biomarker expression. Ho: $\Delta_1 / \Delta_2 = 1$ versus Ha: $\Delta_1/\Delta_2 \neq 1$.

13.7.2 Definitions of Secondary Endpoints and How These Will Be Analyzed

Secondary Endpoints:

An analysis on PFS and OS to assess the impact of treatment will be conducted using a stratified log-rank test. In order to obtain 80% power for detecting a 37.5% reduction in the hazard rate while testing at the 10% level of significance, it is necessary to observe 82 events. This would require 85% of the patients to have a PFS endpoint (82/96), which is feasible. It is probably not feasible to wait for this many events for OS, so the power of the study to detect a similar reduction in the hazard of death will likely be smaller. Given the imprecision of the estimate, the point estimate may be emphasized for an indication of promise.

If the study terminates early for lack of promise in the combination regimen (i.e. is considered uninteresting), consideration may be given for amending the study to further evaluate the nivolumab regimen against a historical control. If a 1-sided 90% CI on the probability of response excludes 25% as an element of the interval, then the study will recommend closing the study. However, if the 90% CI includes 25%, then the study could enroll an additional set of patients to evaluate nivolumab against a historically interesting RR of 10% versus 25%. Such a study could be powered at nearly 90% with 10% level of significance. **(05/22/2017)**

Adverse Event Monitoring

Patients who receive any therapy will be included in the analyses of adverse events. Only those who refuse all therapy according to the assigned regimen will be excluded from these analyses. Ineligible patients who receive any study therapy will be included.

Toxicities on this protocol will be reviewed before each semi-annual meeting and will also be reviewed by the Study Chairperson in conjunction with the Statistical and Data Center. In some instances, because of unexpectedly severe toxicity, the Statistics and Data Management Center in Buffalo may elect, after consultation with the Study Chair and the Chair of Developmental Therapeutics, to recommend early

closure of a study.

The frequency and severity of all toxicities are tabulated and summarized for review by the Study Chair, Developmental Therapeutics Committee, and the Data Monitoring Committee (DMC) in conjunction with each semi-annual meeting. In addition, all serious and/or unexpected events are communicated to the Study Chair, sponsor, and regulatory agencies as mandated in the protocol. These reports are reviewed by the Study Chair (or designated co-chair) within two working days for consideration of investigator notification, amendment, or immediate study suspension. If the study is suspended, all participating institutions will then receive notification of the toxicities and reason for study suspension. Under these circumstances, accrual cannot be re-activated until the study is reviewed by the DMC. However, patients currently receiving treatment may continue to receive treatment in accordance with protocol guidelines at the discretion of their physicians, unless directed otherwise.

Approximately 24 patients will be accrued to the combination regimen after the first stage. As part of the summary to be presented to the DMC before opening the trial to Stage 2, attention will be called and consideration will be given to closing the study for reasons of safety if more than 10% of the patients experience grade 4 or grade 5 treatment-related adverse events. The safety of the two arms will be compared according to organs or organ systems. Toxicities will be divided into severe versus non-severe events, and the rates of severe toxicities will assessed using an exact Chi-square test. Adverse events that are significant at the 5% level of significance will be highlighted in the presentation.

Translational Research

Translational research (TR) data can be fairly difficult to analyze statistically for various reasons including data that are highly skewed (non-normal) or of ordinal quality where differences between observations are not meaningful. It has become customary to dichotomize biomarker data to help overcome these difficulties and ease the interpretation of the results.

PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes (TILs) will be measured by quantitative immunohistochemistry (IHC). It is anticipated that data will be available by the percent staining positive (PP) and the staining intensity. A histoscore will be derived from these data (i.e. histoscore will be the product of the percent staining positive and the staining intensity). Depending on the distribution of the data, Kaplan-Meier curves of PFS and OS will be examined by staining intensity or high staining intensity versus low intensity. The PP and histoscore will also be used to construct Kaplan-Meier curves in terms of high versus low values. Cox models will be used to assess the potential impact of these values on the hazard of death or the hazard of the PFS endpoint (death or disease progression). The biomarker data may be analyzed in dichotomized form or as raw data. Deviance residual plots may be provided to explore potential relationships with the hazard. Relationships with response will be assessed using tables and possibly with logistic models.

For this study, biomarker data will be dichotomized (if feasible) at the median or, less commonly, whether or not expression is observed in the patients. Dichotomizing at the median tends to have an advantage by increasing the sensitivity of the analysis (relative to other cut points) when there is a significant association between the biomarker and clinical outcome.

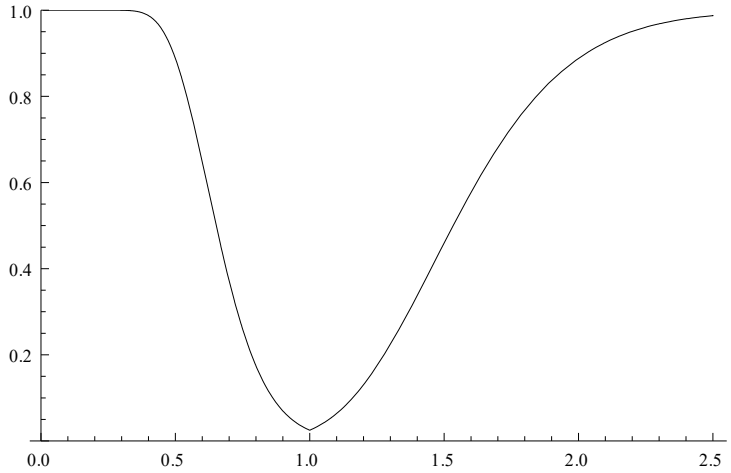
The marginal probability of detecting a biomarker's prognostic effects on the hazard of death or progression depends on its true hazard ratio, θ, the level of significance, α, and the number of events in the study, D, through the normal cumulative distribution function (cdf), provided by $\Phi(\cdot)$ as follows:

$$\text{Power} = \Phi\left(\frac{\sqrt{D}}{2}|\theta| - z_{\alpha/2}\right)$$

The equation above holds since we expect ½ of the patients to score high. This study is expecting about 84 PFS endpoints, so the probabilities of detection under this assumption are provided in Figure 11.2 (assuming a high proportion of patients participating in the TR aspect of this study).

Figure

Power as a function of the hazard ratio for patients with high levels of expression versus those with low levels of expression when assessed with the entire dataset (D=84)

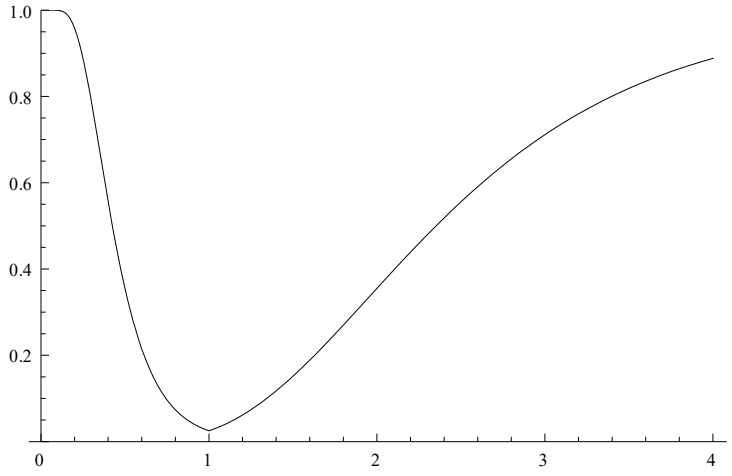


Often the predictive value of a biomarker is of considerable interest. Such a biomarker could help direct the physician towards one treatment over another because the effectiveness of the treatment depends on the level of expression of the biomarker. In statistical terms, the hazard ratio for progression on the experimental treatment to the control treatment depends on the level of biomarker expression

(high versus low). Using Peterson and George's notation,[93] let Delta1 be the hazard ratio for low levels of biomarker expression and Delta2 be the hazard ratio for the high levels of biomarker expression. When $\Delta_1 / \Delta_2 = 1$, the biomarker contains no predictive value for treatment effectiveness. The hazard ratios are the same regardless of the level of biomarker expression (however, the biomarker could still be prognostic).

Because these tests amount to tests of interaction between treatment and biomarker level, the amount of power is drastically reduced. All analyses will involve the full dataset and be exploratory (hypothesis generating) in nature. The equation for calculating power is similar to the one above except the relevant number of events is now only 21 (84 events for 2 treatments cut into four cells is 21.[93]). Note that roughly equal numbers of patients with high levels of biomarker expression are expected to be randomized to the treatments; also, the power calculations are robust as shown in Table 3 of publication.

Figure
Marginal power of tests for detecting predictive biomarkers.



TR analyses that examine the impact of changes in biomarker values over time on the hazard of progression will be adjusted with the Landmark Method.[94] This includes peripheral blood parameters after 6 and 12 weeks of therapy. Patients that progress before the last time of tissue collection (in the entire sample) will not be included in the analysis. The starting point will be adjusted from the date of entry. Because a number of patients (and some events) will be eliminated, the overall power of these analyses will not be as high as described previously. However, the powers listed previously should be fair approximations since the number of patients that progress should be few.

Biomarker measures of natural anti-tumor immunity include tumor infiltrating lymphocytes (TILs) and T cell repertoire analyses. TIL IHC analyses will be conducted in similar analysis as proposed for the PD-L1. T cell repertoire analyses are being finalized. Some of the procedures used for collecting biological data are

novel and could be subject to change before the trial opens. Therefore, details of the analysis are not fully presented yet.

13.7.3 Interim Analysis for All Other Endpoints (Goals):
There will also be an informal interim analysis to examine the toxicities of the regimens.

13.7.4 Power Calculations:
See section 13.1.

13.7.5 Expected Sample Size or Patient Cohorts:
There is one patient cohort accrued in 2 stages. The expected sample size is 48 to 96 patients, depending primarily on whether the trial opens to the second stage.

13.8 Exploratory Hypothesis and Endpoints

See section 13.7 which discusses exploratory analyses.

13.9 Gender/Ethnicity/Race Distribution

	Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1		1
Not Hispanic or Latino	95		95
Ethnic Category: Total of all	96		**96**
	Gender		
Racial Category	**Females**	**Males**	**Total**
American Indian or Alaskan Native	1		1
Asian	1		1
Black or African American	5		5
Native Hawaiian or other Pacific	0		0
White	89		89
Racial Category: Total of all subjects	96		**96**

14. EVALUATION CRITERIA

14.1 Antitumor Effect – Solid Tumors **(02/22/2016)**
Response and progression will be evaluated in this study using a slightly modified version of the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[95] Changes in the largest diameter (uni-dimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. The modification to these criteria minimizes the possibility that therapeutic agents that induce T cell infiltration into tumors as an early manifestation of anti-tumor effect could be erroneously interpreted as disease progression on imaging and result in premature discontinuation of a therapeutically effective agent. Therefore, **the**

protocol specifies continuation of treatment in cases of radiologic progression at the first 8 week (+/- 7 days) CT if all of the following criteria are satisfied:

- No decrease in performance status
- No requirement for immediate alternative treatment or urgent palliative treatment
- Progression limited to an increase of 40% in the sum of diameters of target lesions
- No more than 4 new lesions included in the sum.

For patients who continue treatment in the case of radiologic progression at the first 8 week (+/- 7 days) CT:

- The **8 week CT will serve as the new baseline** for subsequent radiologic assessments of disease. **(05/22/2017)**
- At any subsequent CT scan patients who have stable disease **as compared to the 8 week (+/- 7 days) CT scan** will be allowed to continue on study treatment.
- Patients who continue treatment in the case of radiologic progression at the first 8 week (+/- 7 days) CT, and later experience a PR or CR (as compared to baseline CT) will be recorded as delayed responses by the Statistics and Data Management Center.

14.1.1 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 ≥10 mm with CT scan, as ≥20 mm by chest x-ray, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in decimal fractions of centimeters.

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

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. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, and abdominal/pelvic masses (identified by physical exam and not CT or MRI), are considered as non-measurable.

Notes:
Bone lesions: Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft

tissue components, that can be evaluated by CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.

Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

14.1.2 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), but NOT lung.

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, subsequent image acquisitions should use the same type of scanner and follow the baseline imaging protocol as closely as possible. If possible, body scans should be performed with breath-hold scanning techniques.

NRG will not allow PET-CT use for RECIST 1.1 response criteria.

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

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

CA-125 (Ovarian, fallopian tube and primary peritoneal cancer trials): **CA125 cannot be used to assess response or progression in this study**. If CA125 is initially above the upper normal limit, it must normalize for a patient to be

considered in complete clinical response. Specific guidelines for CA-125 response (in recurrent ovarian cancer) have been published [*JNCI* 96:487-488, 2004]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria that are to be integrated with objective tumor assessment for use only in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

It is mandatory to obtain cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when measurable disease has met criteria for response or stable disease. This confirmation is necessary to differentiate response or stable disease versus progressive disease, as an effusion may be a side effect of the treatment.

14.1.3 Response Criteria

Determination of response should take into consideration all target (See 14.1.3.1) and non-target lesions (See 14.1.3.2) and, if appropriate, biomarkers (See 14.1.3.3).

14.1.3.1 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 progression).

Note: The protocol specifies continuation of treatment in cases of radiologic progression at the first 8 week (+/- 7 days) CT if all of the criteria in section 14.1 are satisfied.

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

14.1.3.2 Evaluation of Non-Target Lesions

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

Note: If CA-125 is initially above the upper normal limit, it must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) 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.

Not evaluable (NE): When at least one non-target lesion is not evaluated at a particular time point.

Although a clear progression of only "non-target" lesions 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).

14.1.3.3 Evaluation of Biomarkers

If serum CA-125 is initially above the upper normal limit, it must normalize for a patient to be considered in complete clinical response.

Progression **cannot** be based upon biomarkers, including serum CA-125 and HE4 for this study.

14.1.3.4 Evaluation of Best Overall (unconfirmed) Response

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

Time Point Response for Patients with Measurable Disease at baseline (i.e., Target Disease)

Target Lesions	Non-Target Lesions	Biomarker CA-125	New Lesions*	Time Point Response
CR	CR	Within normal limits	No	CR
CR	Non-CR/Non-PD	Any value	No	PR
CR	NE	Any value	No	PR
PR	Non-PD or NE	Any value	No	PR
SD	Non-PD or NE	Any value	No	SD
NE	Non-PD	Any value	No	NE
PD	Any	Any value	Yes or No	PD
Any	PD**	Any value	Yes or No	PD
Any	Any	Any value	Yes	PD

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Time Point Response for Patients with only Non-Measurable Disease at baseline (i.e., Non-Target Disease)

Non-Target Lesions	Biomarker CA-125	New Lesions*	Time Point Response
CR	Within normal limits	No	CR
CR	Above normal limits	No	Non-CR/non-PD*
Non-CR/non-PD	Any value	No	Non-CR/non-PD*
NE	Any value	No	NE
Unequivocal PD	Any value	Yes or No	PD
Any	Any value	Yes	PD

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion

** '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

14.1.3.5 Best Overall Confirmed Response

Confirmation of CR and PR for determination of best overall response is required for studies with a primary endpoint that includes response.

Confirmed CR and PR for best overall confirmed response

Time Point Response First time point	Time Point Response Subsequent time point	BEST overall confirmed response
CR	CR	C
CR	PR	SD, PD or PR*
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD

CR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
PR	CR	P
PR	PR	P
PR	SD	S
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
NE	NE	N E

*If a CR is *truly* met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR or SD, not CR at the first time point. Under these circumstances, the original CR should be changed to PR or SD and the best response is PR or SD.

In non-randomized trials where response is part of the primary endpoint, confirmation of CR or PR is needed to deem either one the "best overall response." **Responses (CR and PR) require confirmation at greater than or equal to 4 weeks from initial documentation.**

For this study, the minimum criteria for SD duration is 8 weeks.

Patients with a global deterioration of health status requiring discontinuation of treatment or die without objective evidence of disease progression at that time should be reported to be off study treatment due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

14.1.4 Duration of Response

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

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

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

14.1.5 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from study entry to time of progression or death, whichever occurs first.

Modified RECIST PFS makes allowances for greater increases in sum of dimensions at the 8th week evaluation as stated in Section 14.1.

14.1.6 Survival

Survival is defined as the duration of time from study entry to time of death or the date of last contact.

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APPENDIX I COLLABORATIVE AGREEMENT

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

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

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

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

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

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

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

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

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

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

Email: ncicteppubs@mail.nih.gov

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

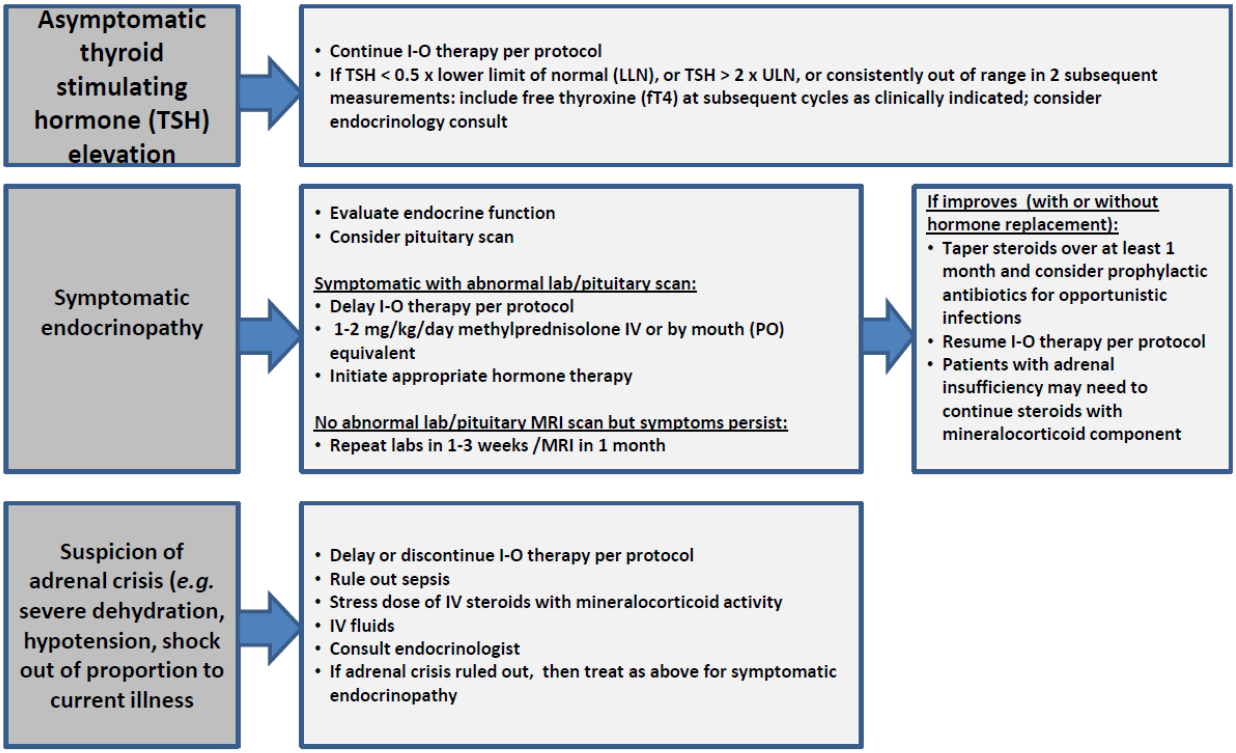
APPENDIX II PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

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

Endocrinopathy Management Algorithm

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



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

GI Adverse Event Management Algorithm

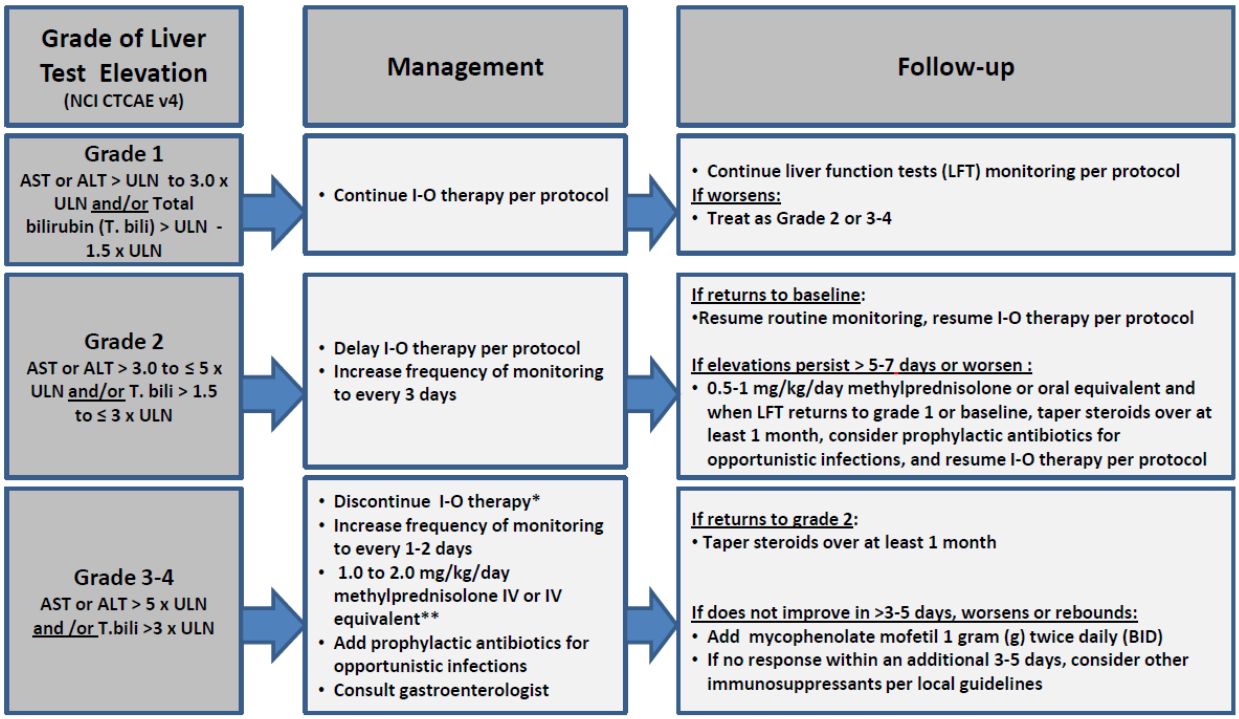
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

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

Hepatic Adverse Event Management Algorithm

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



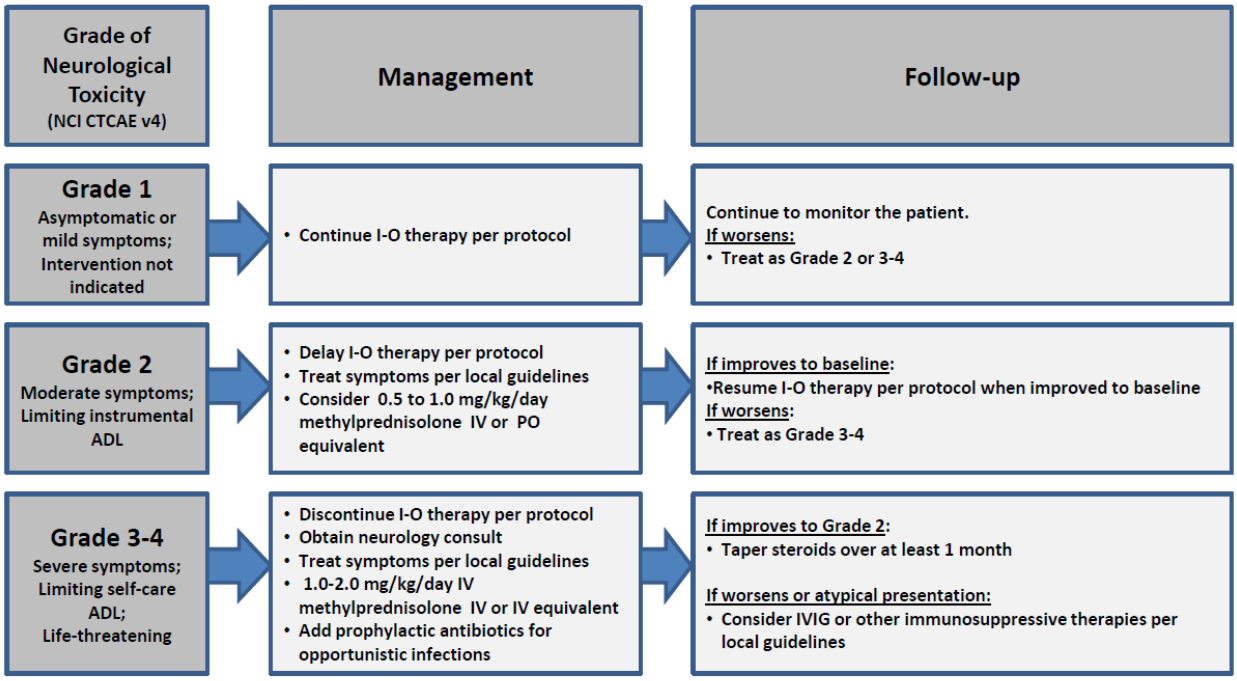
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

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

Neurological Adverse Event Management Algorithm

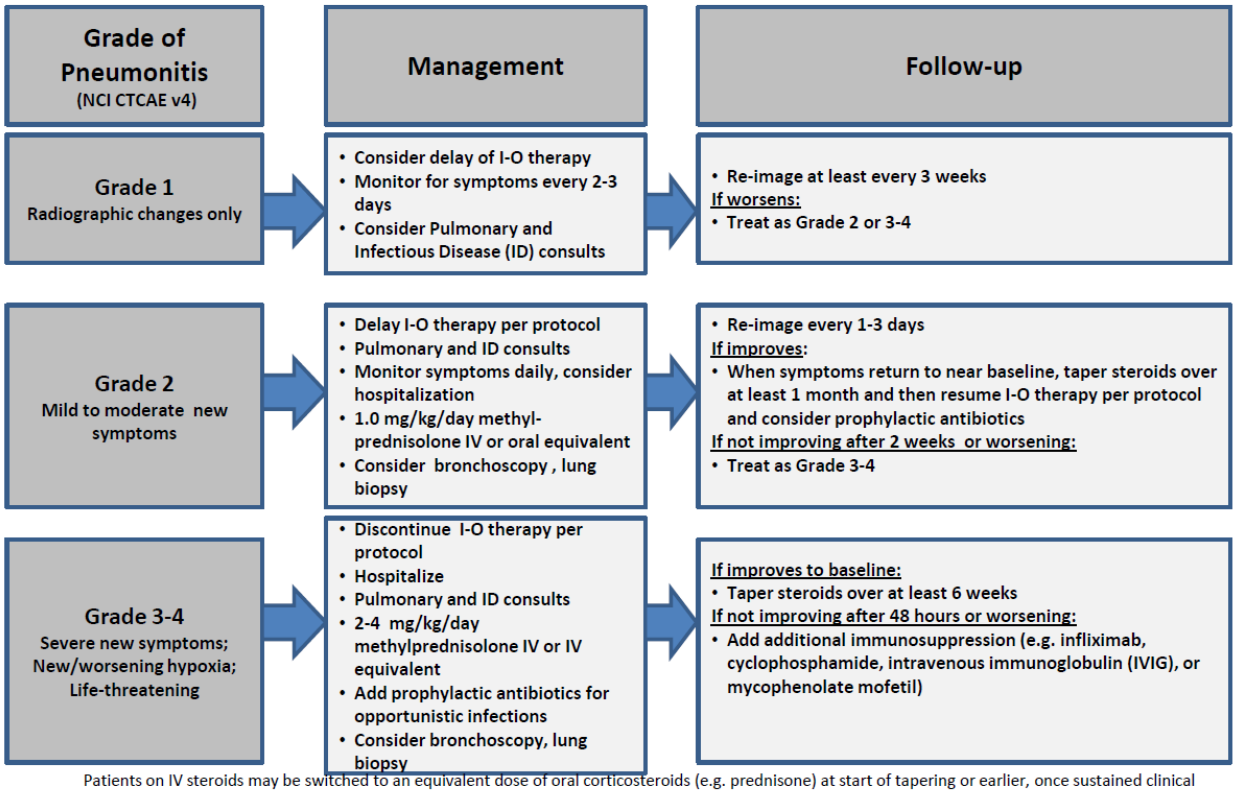
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

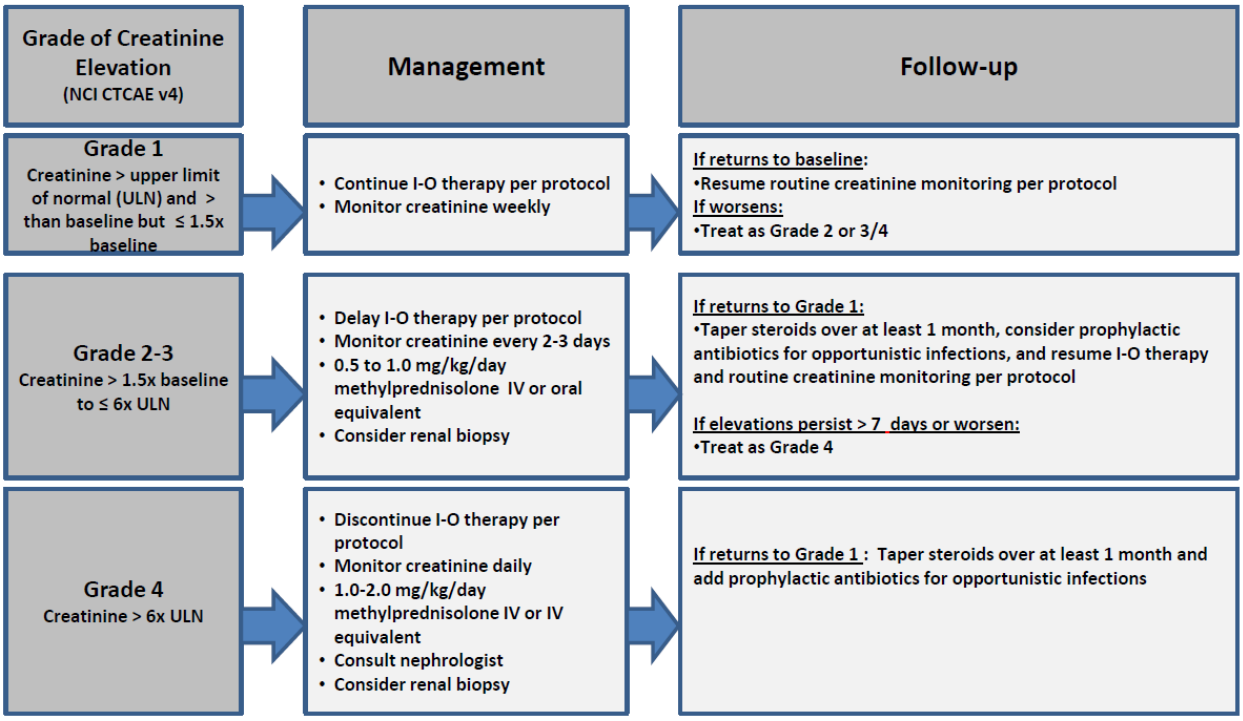
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

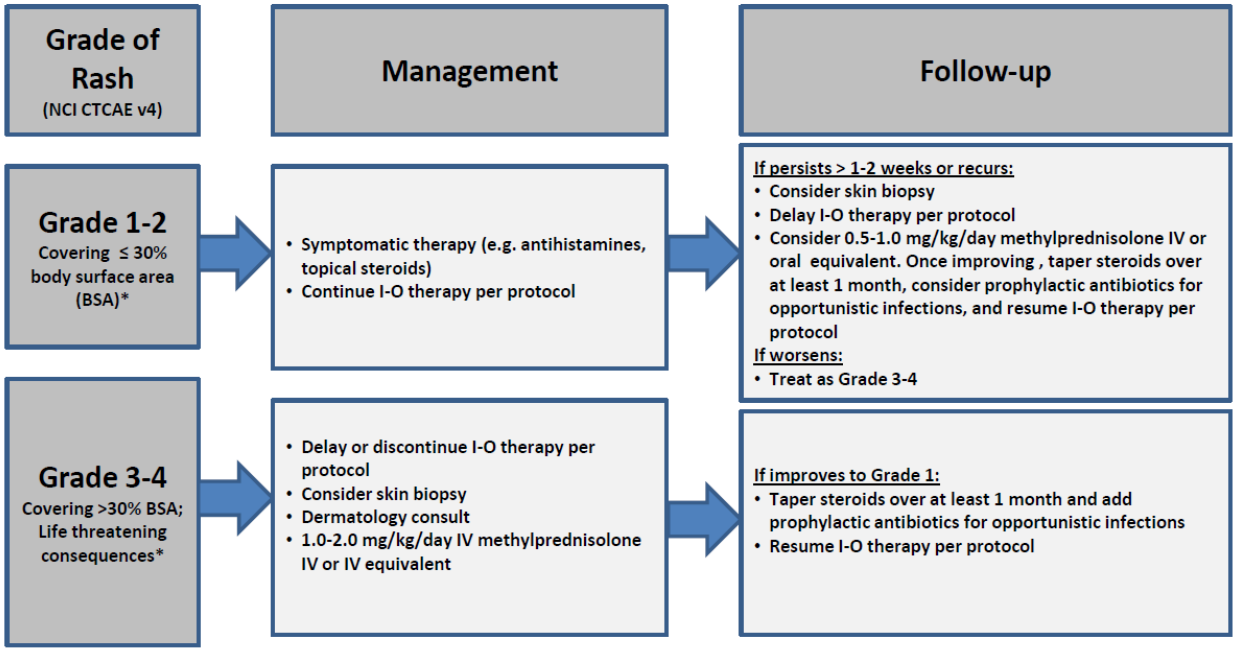
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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 IV TRANSLATIONAL SCIENCE SPECIMEN PROCEDURES (05/22/2017) (09/07/2018)

I. Obtaining a Bank ID for Translational Science Specimens

Only one Bank ID (# # # # - # # - G # # #) is assigned per patient. All translational science specimens and accompanying paperwork must be labeled with this coded patient number.

Translational science biospecimens should not be submitted until after patient registration and Bank ID assignment.

A Bank ID is automatically assigned once the Specimen Consent is completed and indicates that a patient has agreed to participate in the translational science component. If a patient has previously been assigned a Bank ID, please ensure the Bank ID appearing in Rave is the same as the previously assigned Bank ID.

Please contact User Support if you need assistance or have assigned more than one Bank ID to a patient (Email: support@nrgoncology.org).

II. Requesting Translational Science Specimen Kits

One single chamber kit will be provided per patient for the collection and shipment of frozen specimens.

Sites can order kits online via the Kit Management link https://ricapps.nationwidechildrens.org/KitManagement. Each site may order two kits per protocol per day (daily max = 6 kits). **(02/22/2016)**

Please contact the NRG BB-Columbus if you need assistance (Email: BPCBank@nationwidechildrens.org; Phone: 866-464-2262).

Be sure to plan ahead and allow time for kits to be shipped by ground transportation. Kits should arrive within 3-5 business days.

Note: Unused materials and kits should be returned to the NRG BB-Columbus.

III. FFPE Tissue Shipped to the NRG BB-Columbus

Formalin-fixed, paraffin embedded (FFPE) tissue should be the most representative of the specimen type (primary, metastatic, recurrent, persistent).

Only one block may be submitted per tissue type. If submitting sections, all sections must be cut sequentially from the same block.

All FFPE tissue must be submitted with the corresponding pathology report.

Mandatory FFPE Biospecimen Requirement

Every attempt should be made to provide a FFPE block; however, if a block cannot be provided on a permanent basis, then 35 unstained slides (**3 charged, 4 µm** and 32 charged, 5µm) should

be submitted. **Note: The 3 charged, 4µm, slides should be cut first as they are required to test the integrated biomarker. If less than 35 slides are available, please contact** BPCBank@nationwidechildrens.org **and/or** NRG-TR@nrgoncology.org**.**

Archival FFPE
Primary (FP01) and **metastatic (FM01)** tumor should be collected prior to all treatment.

Recurrent and **persistent** tumor should be collected prior to the study treatment. Recurrent or persistent tumor collected from the site of primary disease should be labeled **recurrent primary (FRP01)** or **persistent primary (FPP01)**, respectively. Recurrent or persistent tumor collected from a site other than the site of primary disease (e.g., lymph node) should be labeled **recurrent metastatic (FRM01)** or **persistent metastatic (FPM01)**, respectively.

Completing Form TR for FFPE Biospecimens
The type of specimen (block, slides) should be specified on Form TR. If submitting slides, the slide type, thickness, and count should also be specified.

Note: Since the "Charged slides" and "Count" fields only accommodate one value, "Count" should be entered as the total count of slides (35) at a "Thickness" of 5 µm. To note submission of the 3 charged, 4µm sections required to test the integrated biomarker, check "Other" for "Items shipped" (in addition to "Slides") and specify "three at 4 µm." **(02/22/2016)**

Labeling FFPE Tissue
A waterproof permanent marker or printed label should be used to label each translational science specimen with:

Bank ID (# # # # - # # - G # # #)
protocol number (NRG - GY - # # #)
specimen code (section 11.2.1)
collection date (mm/dd/yyyy)
surgical pathology accession number
block number

Note: If labeling slides, only label on the top, front portion of the slide. Do not place a label on the back of the slide or over the tissue. The label must fit on the slide and should not be wrapped around the slide or hang over the edge.

IV. Whole Blood Shipped to the NRG BB-Columbus

1. Label the lavender/purple top (K2EDTA) collection tube(s) as described below. Multiple tubes may be used to collect the required amount.
2. Draw 7-10mL of blood into the labeled lavender/purple top tube(s). A minimum of 3mL is needed for processing.
3. Immediately after collection, gently invert the tube 5-10 times to mix the blood and K2EDTA.
4. Whole blood specimens should be refrigerated (4°C) until the specimens can be shipped.

Ship whole blood to the NRG BB-Columbus the day the specimen is collected. If the whole blood absolutely cannot be shipped the day it is collected, the tube(s) should be refrigerated (4°C) until the specimen can be shipped.

Labeling Whole Blood
A waterproof permanent marker or printed label should be used to label each translational science specimen with:

Bank ID (# # # # - # # - G # # #)
protocol number (NRG - GY - # # #)
specimen code (WB # #)
collection date (mm/dd/yyyy)

V. Serum Shipped to the NRG BB-Columbus

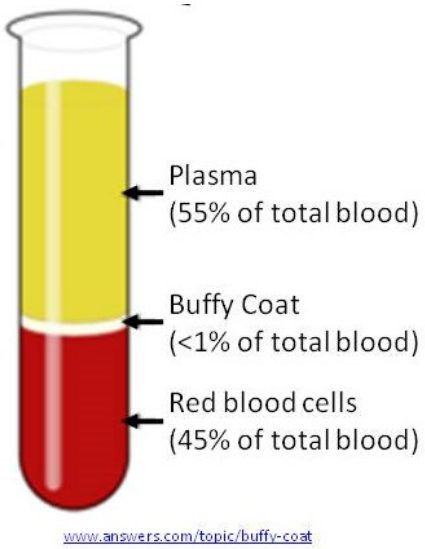
1. Label cryovials and a 15mL conical tube as described below. Use 2mL cryovials if serum will be shipped to the NRG BB-Columbus.
2. Draw 7-10mL of blood into red top tube(s).
3. Allow the blood to clot at 4°C (or in a bucket with ice) for at least 30 minutes but no longer than 3 hours.
4. Centrifuge the blood at 1000g for 15 minutes at 4ºC (preferred) or room temperature to separate the serum (top, straw-colored layer) from the red blood cells (bottom, red layer).
5. Transfer the serum into a 15mL conical tube and gently mix.
6. Quickly, evenly dispense (aliquot) the serum into the pre-labeled cryovials and cap the tubes securely. Place a minimum of 0.25mL into each cryovial.
7. Immediately **freeze the serum in an upright position** in a -70ºC to -80ºC freezer or by direct exposure with dry ice until ready to ship. If a -70ºC to -80ºC freezer is not available for storage, store and ship on dry ice within 24 hours of collection.

Labeling Serum
A waterproof permanent marker or printed label should be used to label each translational science specimen with:

Bank ID (# # # # - # # - G # # #)
protocol number (NRG - GY - # # #)
specimen code (SB # #)
collection date (mm/dd/yyyy)

VI. Buffy Coat Specimens Shipped to the NRG BB-Columbus

1. Label a cryovial and a 15mL conical tube as described below. Use a 2mL cryovial if buffy coat will be shipped to the NRG BB-Columbus.
2. Draw 7-10mL of blood into lavender/purple top (K2EDTA) tube(s).
3. Immediately after collection, gently invert the blood collection tube 5-10 times to mix the blood and K2EDTA.
4. Centrifuge the blood at 1000g for 15 minutes at 4ºC (preferred) or room temperature to separate the plasma (top, straw-colored layer) from the buffy coat (middle, white layer) and red blood cells (bottom, red layer).



5. Leaving a small amount of plasma above the buffy coat layer, remove the plasma and discard.
6. Transfer the buffy coat layer to a cryovial. Avoid drawing up any red blood cells.
7. Centrifuge the buffy coat at 1000g for 10 minutes at 4ºC (preferred) or room temperature to pellet the cells.
   **If a cryovial centrifuge is not available, place the cryovial in a clean 15mL conical tube and centrifuge the cryovial in the 15mL conical tube.*
8. Carefully, remove most of the supernatant.
9. Immediately **freeze the buffy coat in an upright position** in a -70ºC to -80ºC freezer or by direct exposure with dry ice until ready to ship. If a -70ºC to -80ºC freezer is not available for storage, store and ship on dry ice within 24 hours of collection.

Labeling Buffy Coat Specimens
A waterproof permanent marker or printed label should be used to label each translational science specimen with:

Bank ID (# # # # - # # - G # # #)
protocol number (NRG - GY - # # #)
specimen code (LB # #)
collection date (mm/dd/yyyy)

VII. Submitting Form TR

An electronically completed copy of Form TR must accompany each specimen shipped to the NRG BB-Columbus. Handwritten forms will not be accepted.

Note: A copy does not need to be sent to the NRG BB-Columbus if specimens are not collected.

Form TR should be printed from the Translational Research Form screen in Rave using the **"PDF File" link at the top of the form**. Clicking this link will generate a PDF of Form TR in a "SEDES style" format. Do not use the "Printable Version" or "View PDF" links at the bottom of

the form or any other method to print the form, as these formats will not be accepted.

Retain a printout of the completed form for your records.

Please contact User Support if you need assistance (Email: support@nrgoncology.org).

VIII. Shipping Translational Science Specimens

Translational science biospecimens should not be shipped until after patient registration and Bank ID assignment.

An electronically completed copy of Form TR must be included for each translational science specimen.

A. FFPE Tissue

FFPE tissue, an electronically completed copy of Form TR, and a copy of the corresponding pathology report should be shipped using your own container at your own expense to:

NRG BB-Columbus / Protocol NRG-GY003
Nationwide Children's Hospital
700 Children's Dr, WA1340
Columbus, OH 43205
Phone: 614-722-2865
FAX: 614-722-2897
Email: BPCBank@nationwidechildrens.org

Do not ship FFPE tissue for Saturday delivery.

B. Frozen Specimens

Frozen specimens should be shipped using the specimen kit provided to the NRG BB-Columbus (address above).

Frozen specimens should be shipped **Monday through Thursday for Tuesday through Friday delivery**. Do not ship frozen specimens on Friday or the day before a holiday. Note: Saturday delivery is not available for frozen specimens.

Frozen specimens should be stored in an ultra-cold freezing/storage space (i.e., ultra-cold ≤-70ºC freezer, liquid nitrogen, or direct exposure with dry ice) until the specimens can be shipped.

Shipping Frozen Translational Science Specimens in a Single Chamber Kit

1. Pre-fill the kit chamber about 1/3 full with dry ice.
2. Place each frozen specimen type and time point in a separate zip-lock bag.
3. Place the zip-lock bags in the biohazard envelope containing absorbent material. Do not put more than 25 cryovials in a single chamber kit. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible before sealing both envelopes.
4. Place the Tyvek envelope containing the frozen specimens into the kit and fill the chamber to the top with dry ice.

5. Insert a copy of Form TR for each specimen.
6. Place the cover on top of the kit. Tape the outer box of the kit closed with filament or other durable sealing tape. Please do not tape the inner chamber.
7. Print a pre-paid FedEx air bill using the Kit link (https://ricapps.nationwidechildrens.org/KitManagement). Attach the air bill.
8. Attach the dry ice label (UN1845) and the Exempt Human Specimen sticker.
9. Arrange for FedEx pick-up through your site's usual procedure or by calling 800-238-5355.

C. Whole Blood

Whole blood specimens should be shipped Priority Overnight to the NRG BB-Columbus (address above).

Whole blood specimens can be shipped to the NRG BB-Columbus **Monday through Friday for Tuesday through Saturday delivery**. Do not ship whole blood the day before a holiday. Use your own shipping container to ship specimens via **FedEx priority overnight**.

When shipping whole blood specimens, **your site must comply with IATA standards** (www.iata.org). If you have questions regarding your shipment, contact the NRG BB-Columbus at BPCBank@nationwidechildrens.org or by phoning 866-464-2262.

To ship whole blood specimens you will need (1) a sturdy shipping container (e.g., a cardboard or styrofoam box), (2) a leak proof biohazard envelope with absorbent material*, (3) a puncture and pressure resistant envelope (e.g. Tyvek envelope), (4) an Exempt Human Specimen sticker, and (5) a pre-paid FedEx air bill.

**If you will be shipping whole blood specimens from more than one patient, please put each specimen in a separate plastic zip-lock bag before placing the specimens in the shipping bag. You may include up to four different blood specimens in one biohazard envelope.*

If you do not have these materials available at your site, you may order them from any supplier (e.g., Saf-T-Pak; Phone: 800-814-7484; Website: www.saftpak.com). The NRG BB-Columbus does not provide supplies for shipping whole blood.

Shipping Whole Blood Using Your Own Shipping Container

1. Place the whole blood specimen in a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the bag.
2. Wrap the biohazard envelope in bubble wrap or another padded material.
3. Place the padded tube(s) into a Tyvek envelope. Expel as much air as possible before sealing the envelope.
4. Place the Tyvek envelope in a sturdy shipping container (e.g., cardboard FedEx box).
5. Insert a copy of Form TR for each specimen.
6. Attach an Exempt Human Specimen sticker to the outside of the shipping container.
7. Print a pre-paid FedEx air bill using the Kit Management link (https://ricapps.nationwidechildrens.org/KitManagement). Attach the air bill.
8. Make arrangements for FedEx pick-up through your site's usual procedure or by calling 800-238-5355.

IX. Banking Translational Science Specimens for Future Research

Specimens will remain in the NRG BB-Columbus and made available for approved research projects if the patient has provided permission for the use of her specimens for future health research. The patient's choices will be recorded on the signed informed consent document and electronically via Specimen Consent. At the time of specimen selection for project distribution, the most recent consent information will be used.

Sites can amend a patient's choices regarding the future use of her specimens at any time if the patient changes her mind.

If the patient revokes permission to use her specimens, the NRG BB-Columbus will destroy or return any remaining specimens. The patient's specimens will not be used for any further research; however, any specimens distributed for research prior to revoking consent cannot be returned or destroyed. In addition, the patient cannot be removed from any research that has been done with her specimens distributed prior to revoking consent.

Note: If return of specimens is requested, shipping will be at the site's expense.

APPENDIX V – TRANSLATIONAL SCIENCE LABORATORY TESTING PROCEDURES (05/22/2017) (09/07/2018)

I. PD-L1 Immunohistochemistry

A. Rationale

PD-L1 expression has been previously associated with improved clinical benefit from therapies targeting the PD-1/PD-L1 pathway. The utility of PD-L1 expression as a predictive biomarker has been debated, as it is highly dynamic and can be up-regulated in response to immune activating factors. In this trial, archival, formalin-fixed, paraffin-embedded (FFPE) tumor will be evaluated by immunohistochemistry (IHC) for expression of PD-L1 as a predictive biomarker.

B. Laboratory Testing Procedures

FFPE tumor will be shipped from sites to the NRG BB-Columbus. The NRG BB-Columbus will batch ship unstained sections of tumor to TBD for PD-L1 IHC. PD-L1 IHC will be done using Bristol Meyers Squibb's (BMS) proprietary DAKO assay.

II. Infiltrating Lymphocytes Immunohistochemistry

A. Rationale

There is abundant evidence that native host anti-tumor cell mediated immune mechanisms play a role in controlling malignant progression of epithelial ovarian cancer (EOC), the majority of which exhibit high grade serous morphology. Investigators at the University of Pennsylvania (Penn) first discovered the impact of anti-tumor immune response on overall survival (OS) in patients with newly diagnosed advanced EOC, where the presence of CD3+ tumor-infiltrating lymphocytes (TILs; within tumor islets) was found to be a predictor of prolonged OS.(*1*) Others independently showed that the accumulation of TILs in ovarian cancers was associated with improved progression-free survival (PFS) and OS.(*2*) The accumulation of CD8+ TILs, but not CD4+ TILs, was shown to predict improved OS, suggesting a role for CD8+ T-cells in tumor control. It is now clear that TILs isolated from ovarian cancers can recognize autologous tumor and known tumor antigens *in vitro*,(*3-7*) can exhibit tumor-specific cytolytic activity *ex vivo*(*8, 9*) and are oligoclonal in composition.(*10, 11*) Thus, naturally arising T lymphocytes with anti-tumor potential exist in patients with EOC and underscore the potential for immunotherapy to affect the natural course of Mullerian duct carcinomas in order to improve patient outcome.

Until recently, the immuno-biology of spontaneous tumor-reactive T-cells in ovarian cancer was not well-defined, largely due to the difficulty in identification and interrogation of responses. Recently, our research elucidated CD137 (4-1BB), an activation induced TNF receptor family member, as a biomarker for bona fide naturally-occurring tumor-reactive T-cells in EOC.(*12*) CD137+ TILs, but not CD137- TILs, demonstrated HLA-dependent IFN-γ secretion in response to autologous tumor stimulation and were capable of controlling EOC outgrowth *in vivo*. These results have enabled rapid identification of tumor-reactive T-cells in this disease. We have now validated the existence of CD137+ TILs in ovarian cancer samples via analytical IHC, allowing for the quantification of tumor-reactive TILs in biopsied tumor specimens. Our results ascribe a previously unknown role for CD137 in the immuno-biology of cancer.

On the other hand, solid tumors may successfully evade immune attack by blocking effector T-cell trafficking into tumor islets and inhibiting effector T-cell activation. There are two important parallel mechanisms of tumor induced cell mediated immunologic tolerance in ovarian cancer

which may operate in a complementary fashion and represent candidate therapeutic targets to reverse spontaneous T-cell mediated anti-tumor immunity. First, in general, activation of T-cells requires binding of the T-cell receptor (TCR) to antigen in association with major histocompatibility (MHC; HLA) molecules, plus co-stimulation by antigen-presenting cells (APCs).(*13*) Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a negative regulatory homologue of CD28 found on activated T-cells and, by competing with CD28 for binding to CD86 and CD80 on the APC, functions as a checkpoint to prevent auto-immunity. CTLA-4 is also expressed ubiquitously on a naturally-occurring subpopulation of $CD4^+$ regulatory T-cells (Treg) constitutively expressing FOXP3 and CD25 (the IL-2 receptor-α chain) and thought to inhibit autoimmune pathology, mediate transplantation tolerance, impede anti-tumor immunity *in vivo*.(*14*) The paradigm of Treg-mediated immunosuppression in human cancer was originally defined in EOC where it was shown that human tumor-infiltrating Treg cells suppressed tumor-specific T-cell immunity and contributed to growth of human tumors *in vivo*(*15*). We therefore hypothesize that those immune therapies that enhance CD8+ T-cell accumulation and activity while reducing the population of Treg cells will demonstrate anti-tumor activity.

A second mechanism involves signaling through the negative regulatory co-stimulatory T-cell receptor, human programmed death-1 (PD-1, CD279)(*16, 17*). PD-1 is highly expressed on activated T-cells. The principal ligands for PD-1 are PD-L1 (B7-H1, CD274),(*17, 18*) expressed on tumor cells, antigen-presenting cells and dendritic cells and PD-L2 (B7-DC, CD273),(*19, 20*) commonly expressed on endothelial cells. Disruption of PD-1/PD-L1 interaction is a rational strategy for immunotherapy in patients with advanced EOC. Nivolumab is a humanized monoclonal antibody binding to PD-1 and blocking the interaction between PD-1 and its ligands, thus inhibiting PD-1 signal transduction in activated T-cells. A phase I dose-finding trial involving 296 patients with multiple non-gynecologic tumors response in 42 patients with pretreatment tumor specimens was associated with tumor expression of PD-L1 by IHC analysis, with no responses in 17 patients bearing PD-L1 (-) tumors and 9 (36%) responses in 25 patients with PD-L1 (+) tumors. This provides the rationale for the study of both PD-L1 and PD-1 expression as prognostic biomarkers of response.

Still, other markers have shown prognostic value in ovarian cancer. In particular, markers of T cell activation (e.g. CD45RO, TIA-1), B cells (CD20), MHC (HLA class I and II), and macrophages (CD68) have shown prognostic value (21) but there role as biomarkers of response to therapy are unknown. 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 minimally both memory helper and activated effector cells would be needed to sustain long-term responses. Accordingly, we have place emphasis on understanding the relationships of the tumor, cellular infiltrate, and immunologic milieu surrounding each tumor in the context of immune checkpoint inhibition.

B. Laboratory Testing Procedures

For exploratory TIL IHC biomarker studies, standard operating procedures (SOPs) have been produced or are under validation by the Pathology Core Laboratory in Penn's Department of Pathology and Laboratory Medicine. If required, detailed SOPs will be provided at the time of specimen distribution and analysis.

Briefly, we anticipate up to 96 pre-treatment tumor specimens to be available for study. IHC will

be conducted using FFPE tumors collected at the time of surgical resection or from biopsy. After heat-induced antigen retrieval (Bond ER2, 20 minutes), tumor slides will be stained with antibody on a Leica Bond III automated staining system using Bond Polymer Refine Detection (Leica Microsystems AR9800). Intensity of staining will be scored on a 0-3+ scale, with both the percent tumor cells or macrophages staining positive and the cellular pattern (membrane versus cytoplasm) analyzed by two GYN pathologists previously trained in pathologic techniques. Each reader will be blinded to patient information and provide a pathologic grade from 0-3+ depending on the level of staining. Furthermore, analyzers will count the number of positive staining cells per section. Each pathologic grade and number of positive cells will be determined for the both the stroma and the tumors.

Collectively, we will explore the prognostic and future therapy implications of measuring markers with roles in immune function and activity – e.g., PD-1, PD-L1, CTLA-4, CD3, CD4, CD8, FOXP3, CD45RO, TAI-1, CD137, HLA class I and Class II, CD68, and indoleamine-2,3-dioxygenase (IDO). Other immune checkpoints, such as LAG-3 and TIM-3, may serve as additional immune inhibitory mechanisms, which may dampen the overall immune response. As such, FFPE will be evaluated for the presence of TILs and immune markers including CD16, OX40, GITR, CD40, LAG3, TIM3, B7-H3, B7-H4 and VISTA.

C. References

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III. Identification of genetic determinants of response (09/07/2018)

A. Rationale

Studies in other cancers have identified the overall tumor mutational burden (TMB) [4], overall HLA diversity of the host [5], and presence of certain genomic alterations [10, 12, 13] to be associated with response to PD-1 blockade. To study these parameters as predictors of response in EOC, DNA will be isolated from archival tissue and matched blood and subjected to whole exome sequencing (WES) using Illumina HiSeq sequencing.

B. Laboratory Testing Procedures

1. Pre-treatment FFPE tumor will be processed for DNA isolation and confirmation of concentration and purity by OD 260/280.
2. Matched DNA extracted from peripheral blood will be used for matched normal DNA for whole exome sequencing and HLA typing (ATHLATES (http://www.broadinstitute.org/scientific-community/science/projects/viral-genomics/athlates) [2].
3. WES of tumor and matched normal DNA will be performed using the SureSelect Human All Exon 50MB kit (Agilent) for exon capture and sequencing on the HiSeq 2000 platform (Illumina) to a goal of 150X coverage. FFPE samples will be sequenced by the commercial sequencing facility of the Broad Institute (Broad Institute, Genomics Platform, Cambridge, MA). Raw FASTQ files will be generated for subsequent analysis at MSKCC.
4. Raw sequencing data are mapped to the human reference genome (hg37). Alignment, base-quality score recalibration, duplicate-read removal, and exclusion of germline variants will

be performed using the Genome Analysis Toolkit (GATK) [3]. Known single nucleotide polymorphisms (SNPs) will be eliminated [4-6], keeping only novel mutations and those in COSMIC. Mutations will be annotated using SnpEffect [7].

5. Mutations will be analyzed using four callers: Somatic Sniper [8], VarScan [9], Strelka [10] and MuTect [11], then filtered by allelic frequency of each alteration in tumor (>10%) and normal (<3%), depth of coverage (≥7X) and call quality and considered positive if called by ≥2 callers. All calls made by only one caller will be manually reviewed using IGV [12].
6. To assess putative tumor neoantigens, exonic stretches containing each non-synonymous mutation are used to generate pairs of 17 amino acid strings: one with the mutation and onecorresponding to the wild-type sequence. A "sliding window" approach is then used to identify the nonamer within each mutant 17-mer with maximal binding affinity to any patient-specific MHC class I allele using NetMHCpan [13]. The binding affinity of each mutated and corresponding WT nonamer will be examined with patient-specific MHC I alleles.
7. Presence or absence of putative neoantigens will be further correlated with PFS by using Log-Rank test.

IV. T Cell Receptor (TCR) repertoires

A. Rationale (05/22/2017) (09/07/2018)

Since Cancer Testes (CT) antigens may not be present in all patients and may not be the immunodominant antigens that will determine tumor sensitivity to the immune system, the analysis of overall changes in T cell receptor repertoires during therapy may help to determine whether certain dominant T cell clones emerge or persist in response to therapy. Cha et al. reported that maintenance of specific T cell clonotypes in blood of patients with advanced melanoma treated with ipilimumab was associated with improved survival [14]. Studies of immune checkpoint blockade in other cancers have demonstrated that high intratumoral TCR clonality prior to treatment was associated with clinical benefit [7, 9]. In addition, those patients with low baseline peripheral blood TCR clonality tended to have better progression free and overall survival, along with a more pronounced expansion of tumor-associated TCR clones in the peripheral blood after the initiation of therapy. To assess whether baseline TCR diversity as well as dynamic changes in TCR diversity could predict clinical benefit, TCR repertoires will be assessed in archival tumor samples. Similarly, TCR repertoires will be assessed in the peripheral blood at baseline, and 6 and 12 weeks post-therapy initiation. DNA will be extracted from tumors and peripheral blood using Qiagen kits and will be sent for deep sequencing of CDR3 regions to Adaptive Biotechnologies. Rearranged TCRbeta CDR3 sequences will be amplified and sequenced using Adaptive Biotechnologies' immunoSEQ platform. To assess whether baseline TCR diversity as well as dynamic changes in TCR diversity could predict clinical benefit, TCR repertoires will be assessed in archival tumor samples. Similarly, TCR repertoires will be assessed in the peripheral blood at baseline, and 6 and 12 weeks post-therapy initiation.

B. Laboratory Testing Procedures

1. DNA will be isolated from FFPE tumor using the Qiagen QIAamp DNA FFPE Tissue kit. DNA will be isolated from whole blood by the NRG BB-Columbus.
2. Isolated DNA will be tested for concentration and purity by spectrophotometry with OD measurement at 260 and 280nm.
3. Per guidelines for the immunoSEQ Assay, 7-8ug of genomic DNA will be shipped to Adaptive Biotechnologies for deep level sequencing.

4. The information derived from these studies will be used to determine the relative T cell density, clonality, and clonal overlap in FFPE tumor obtained pre-treatment. Based on prior experience, for tumor and blood T cell repertoire analyses, rare clonotypes (bottom 5%) will be avoided. PFS will be correlated with increase in antigen-specific immune responses and with persistence or increase in the number of dominant TCR clones and the degree of increase in the dominant TCR clones. Each of the parameters will be treated as a continuous variable. Relative change in each biomarker from pre-treatment to 6 and 12 weeks will be calculated.

V. Transcriptomic analysis of tumor microenvironment (09/07/2018)

A. Rationale

Prior studies of gene expression in epithelial ovarian cancer revealed immune transcriptional subtype to be associated with improved prognosis. Studies from different cancer types treated with immune checkpoint blockade have identified type I and type II IFN signatures to be correlated with clinical benefit. Finally, transcriptional signatures indicative of activation of specific tumor driver pathways, such as Myc and beta-catenin have been associated with immunotherapy resistance. We will use transcriptional analysis of the tumors to analyze several parameters. Using bioinformatic deconvolution (e.g. CIBERSORT), predominance of specific immune cell subtypes will be calculated in each sample. In addition, we will focus on the expression of genes related to cytotoxic T cell function, as well as the known genes related to immune inhibition, such as immune checkpoints (e.g. PD-1/PD-L1, LAG-3, VISTA) and indoleamine 2,3-dioxygenase (IDO). We will also characterize the expression of SWI/SNF components to establish correlation with data generated by WES. Finally, we will analyze the expression of putative antigenic targets recognized by the immune system. These include the known cancer germline antigens, putative neoantigens identified by WES, and endogenous retroviral sequences (ERVs), which have been previously demonstrated to guide tumor immune recognition through activation of type I IFN pathway [11, 29]. Expression of these genes will be correlated with T cell infiltration and type I and type II IFN signatures.

B. Laboratory testing procedures

RNA extracted from the archival tissue will undergo quality control using Agilent RNA pico chip, processed for sequencing using Illumina Tru-Seq Stranded RNA kit, followed by amplification, hybrid capture, and Illumina sequencing. If FFPE RNA does not meet quality controls for RNAseq, alternative methods for transcriptome analysis will be utilized. These include Clariom D microarray (Thermo Fisher) and targeted RT-PCR panels (available from HTG or Nanostring) focusing on immune-related gene expression.

VI. Peripheral Blood Immune Transcriptional Profiling

A. Rationale

Peripheral blood biomarkers have been previously reported to be associated with outcomes of immunotherapy. The MSKCC research group has recently demonstrated that high percentages of peripheral blood myeloid derived suppressor cells (MDSC) have been associated with poor prognosis and poor response to ipilimumab in metastatic melanoma [16]. In addition, in studies performed by our research group and others, clinical benefit from CTLA-4 blockade was shown to be associated with an increase in absolute lymphocyte counts and with sustained upregulation of the co-stimulatory molecule inducible costimulator (ICOS) on the surface of T cells [17, 18].

Increases in T cells expressing HLA-DR, ICOS, and ki67 was similarly observed in patients with malignant melanoma treated with ipilimumab/nivolumab combination [19]. Emerging from these data, in animal models, targeting of ICOS in combination with CTLA-4 blockade resulted in significant enhancement in therapeutic efficacy [20].

To gain a broad understanding of the immune changes happening in peripheral blood in response to therapy and to extend the peripheral blood analyses to more sites, transcriptional profiling of the peripheral blood will be performed by the Nanostring platform using the nCounter PanCancer Immune Profiling Panel, which consists of 770 genes related to angiogenesis, inflammation, and innate and adaptive immune response.

B. Laboratory Testing Procedures

1. Peripheral blood buffy coats will be isolated, frozen on site, and shipped to NRG BB-Columbus.
2. One aliquot of pre-treatment and 6 and 12 week buffy coats will be batch shipped to Dr. Dmitriy Zamarin (MSKCC).
3. RNA isolation will be performed using Qiagen RNeasy kit.
4. Isolated RNA will be tested for concentration and purity by spectrophotometry with OD measurement at 260 and 280nm.
5. RNA will be analyzed on the Nanostring nCounter platform at MSKCC using the PanCancer Immune Profiling Panel.
6. By using unsupervised clustering, associations between specific gene signatures present at baseline and on treatment with PFS will be determined. For the genes that demonstrate the most significant changes, in univariate analyses, the expression of individual selected gene will be treated as a continuous variable and will be correlated with PFS by using minimal p value approach.

VII. Tumor-Associated Antigen (TAA) Serologic Responses

A. Rationale

Expression of cancer-testis (CT) antigens in a large percentage of gynecologic malignancies has been reported in multiple studies. Monitoring for early responses to CT antigens and TAA such as NY-ESO1, MAGE 4, SOX2, and p53 may help to identify the patients that are more likely to derive benefit from therapy. Serologic responses to the antigens above will be assessed by ELISA in pre-treatment blood, as well as the blood collected at 6- and 12 weeks after starting study treatment.

B. Laboratory Testing Procedures

1. Human serum will be isolated, frozen on site, and shipped to NRG BB-Columbus.
2. Aliquots of serum will be batch shipped to Dr. Dmitriy Zamarin (MSKCC) for analysis.
3. Validated ELISAs using diluted sera (1:100, 1:200, 1:400, and 1:800) and 30ng of recombinant antigen proteins will be used. Recombinant proteins have been purchased commercially and each lot is tested with specific antibody to ensure reproducibility.
4. For analysis, concentration of antibody to each antigen will be treated as a continuous variable. Relative change in each biomarker from pre- to post-treatment at 6 and 12 weeks will be calculated. Univariate analyses for each biomarker using a minimum p value approach will be used to calculate an optimal cutoff value, using PFS as the outcome variable.

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APPENDIX VI—STUDY PARTICIPANT WALLET CARD (07/31/2017)

INFORMATION ON POSSIBLE DRUG INTERACTIONS You are enrolled on a clinical trial using the experimental agents Nivolumab and Ipilimumab**.** This clinical trial is sponsored by the NCI. Nivolumab and Ipilimumab may interact with other medications. Because of this, it is very important to: ➢ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➢ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial. ➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/ for a list of drugs to avoid, or contact your study doctor. Your study doctor's name is _____ and can be contacted at _____.