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FOX CHASE CANCER CENTER
TEMPLE HEALTH

Depletion of Myeloid Derived Suppressor Cells to Enhance anti PD-1 Therapy

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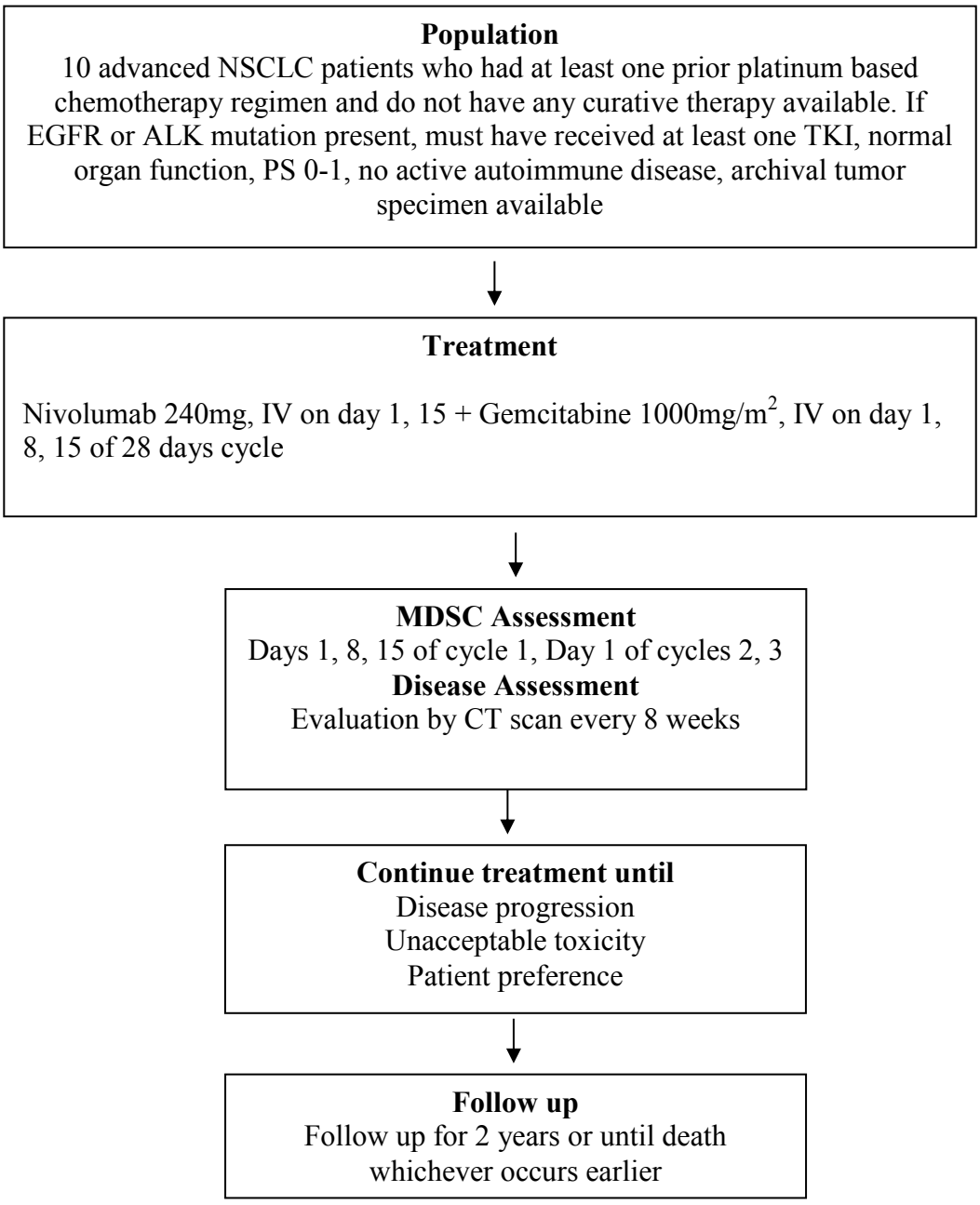

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1.0 Introduction

1.1. Non-small cell lung cancer

Lung cancer is the most common form of cancer worldwide. In the United States in 2015, there will be an estimated 213,380 new cases of lung cancer and 160,390 deaths (Jemal et al. 2015). Non-small cell lung cancer (NSCLC) accounts for >80% of lung cancer diagnoses. Most patients with NSCLC will present with metastatic disease or develop metastases after initial, potentially curative, treatment with surgery or chemoradiotherapy.

Treatment of advanced NSCLC has become increasingly complex in the past 10 years. Approximately 15% of patients have "activating mutations" that can be targeted with specific agents, usually tyrosine kinase inhibitors (TKI). These mutations include EGFR activating mutations, ALK and ROS translocations amongst others. Though such patients usually have excellent initial responses to therapy, the overwhelming majority eventually develops progressive disease and will be candidates for additional therapy. The median survival for these patients is approximately 30 months. Patients with advanced disease that is not characterized by activating mutations or in which there is no targeted therapy for the mutation is not available (e.g. k-ras, insertion 20 EGFR etc), are often initially treated with a platinum-based doublet chemotherapy regimen. The median survival with such regimens is typically 10-14 months with virtually all patients ultimately dying of disease. Once a patient has relapsed after platinum-based chemotherapy, second-line therapy is considered. There are 3 approved second-line treatment options for NSCLC in patients with prior platinum-based chemotherapy: docetaxel , pemetrexed, and erlotinib. A randomized, phase 3 trial of second-line therapy with docetaxel produced a statistically significant improvement in survival compared with best supportive care. (Fossella et al. 2000, Shepherd et al. 2000). A phase 3 trial of pemetrexed compared with docetaxel demonstrated similar response rates (RR), progression-free survival (PFS), and overall survival (OS) for the 2 treatments. (Hanna et al. 2004). While a formal proof of non-inferiority was not achieved, the efficacy and toxicity data were sufficient to support full approval of pemetrexed in this setting. Erlotinib, which is also approved for use in second-line NSCLC, was studied in patients with NSCLC previously treated with at least 1 chemotherapy; this study demonstrated a survival advantage compared with placebo (Shepherd et al. 2005) and led to approval of erlotinib as a second- and third-line therapy. In the second-line setting, RR and survival continue to be low (Shepherd et al. 2000, Hanna et al. 2004). Docetaxel has demonstrated phase 3 RR of 5% to 11% and a median survival of 5 to 8 months in second-line NSCLC patients. Pemetrexed is comparable, with a RR of 9.1% and a median survival of 8.3 months in a similar population. In a large phase 3, placebo-controlled trial, erlotinib and placebo demonstrated RR of 8.9% and <1% and median survival of 6.7 and 4.7 months, respectively (Shepherd et al. 2005). In 2014 the anti-VEGF-receptor agent ramacirumab was approved in combination with docetaxel for the second line treatment of NSCLC, though the degree of benefit is quite modest.

1.2. Immunotherapy in NSCLC

Immunotherapy, specifically targeting of T-cell checkpoints has emerged as an important therapeutic approach in non-small cell lung cancer. The CTLA-4 inhibitor ipilumamab, approved for therapy in melanoma, appeared to provide some benefit in addition to standard chemotherapy in a randomized phase II study.[1] More remarkably, are the reports of activity of anti-PD1 and PD-L1 antibodies with significant and occasional durable responses in heavily pretreated disease. [2 3] Nivolumab is a fully humanized, IgG4 (kappa) isotype monoclonal antibody (mAb) that binds the programmed death receptor-1 (PD-1). PD-1 is a transmembrane protein primarily expressed on activated immune cells. In its usual function, the binding of PD-1 (found on activated T-cells) to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) inhibits T-cell proliferation and activation. Upregulation of PD-1 ligands can occur in tumors and is thought to serve as a means of immune evasion by the tumor. Nivolumab blocks the interaction of the PD-1 T-cell receptor with its ligands, potentially enabling the reactivation of immunosurveillance and cancer eradication.
Nivolumab was approved by the US Food and Drug Administration in March 2015 for the treatment of patients with metastatic squamous non-small cell lung cancer with progression on or after platinum-based chemotherapy. A Phase II (NCT01721759) study evaluated nivolumab in patients refractory squamous lung cancer, progressive after 2 or more lines of chemotherapy. This study demonstrated an objective response rate of 15%, stable disease rate of 26% with median duration of response for stable disease patients of 6 months. Median overall survival was 8.2 months (95% CI 6.1-10.9 months) and overall survival was 41% at 1 year. A phase III trial evaluating patients with squamous cell carcinoma of the lung (NCT01642004) has been reported. In interim analysis, patients randomized to nivolumab demonstrated an improvement in median overall survival of 9.2 months compared to those treated with docetaxel (p-0.00025).[4] Similar results have been reported for non-squamous carcinoma, leading to approval in that group of patients as well. More recently, the dosing schedule has been changed from 3 mg/kg to a flat dose of 240 mg every 3 weeks.

In most studies, response rates have been higher for patients with strong PD-L1 expression by immunohistochemistry. For example in a pooled analysis of responses to pembroluzimab, patients with high levels of expression of PD-L1 had response rates of 39%, though responses were seen for patients who were negative (16%).[5] While these results are encouraging, it is clear that current immunotherapeutics only benefit a minority of patients. A logical approach to improving efficacy is to combine anti-PD-1 therapy with other agents that can enhance immune function. This has already been done with possible benefit with the combination of anti-PD-1 therapies with anti-CTLA4 agents. However, this approach is likely to be ultimately limited by toxicity and expense. Another approach is to target other aspects of immunity that may lead to suppression of the tumor immune response.

Tumor initiation and progression are associated with inflammation and immune dysfunction. A link between these two areas is the myeloid derived suppressor cell (MDSC).[6] It has been demonstrated that patients with non-small cell lung cancer

(NSCLC) show elevated levels of MDSCs that act as powerful immune suppressors via multiple pathways. These include preventing activation of $CD4^+$ and $CD8^+$ T-cells, inducing T-regulatory cells, polarizing immunity towards a type-2 tumor-promoting phenotype, and blocking natural killer cell cytotoxicity.[7] MDSCs originate in the bone marrow as $Gr1^+CD11b^+CD31^+$ hematopoietic precursor cells that under normal conditions differentiate into mature dendritic cells, macrophages, or granulocytes. In the presence of tumor-derived and host-produced factors, differentiation is blocked and MDSCs accumulate.[8] These cells have been shown to inhibit T-cell proliferation and cytotoxic lymphocytes in an MHC- and antigen-independent manner. [9] Myeloid cells expressing similar markers in humans have been found to be increased fivefold in patients with head and neck squamous cell cancer, renal cell cancer, breast cancer, and NSCLC.[10] Observations that pro-inflammatory cytokines both induce MDSCs and promote tumor progression suggest that chronic inflammation increases the number of MDSCs, inhibiting anti-tumor immunity, and promoting tumor growth.[1] Depletion of MDSCs has been shown to reduce tumor progression and to improve immune-based cancer therapies.[12 13]

We have evaluated peripheral blood mononuclear cells (PBMCs) of 10 patients with NSCLC and 5 normal controls. These studies show that $CD11b^+CD33^+$ (MDSC) levels were quantified at 26.5±13% of PBMCs from NSCLC patients as compared to 11±6% of PBMCs from healthy patients. To determine if the MDSC population is suppressive, PBMCs from patients 3, 8, and 11 were activated in vitro with anti-CD3 and anti-CD28 mAbs. T-cell proliferation was assessed by ^{3}H-thymidine uptake. Depletion of MDSCs significantly increased T-cell proliferation.

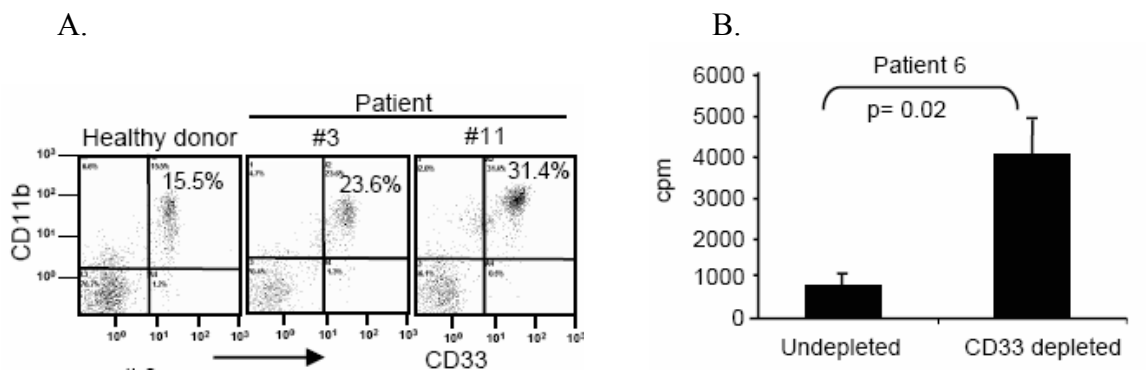

Fig. 1: Lung cancer patients have immunosuppressive $CD11b^+CD33^+$ (MDSCs) in their peripheral blood. *Panel A:* PBMC from healthy donor BC123104 and from NSCLC patients 3 and 11 were stained with mAbs for CD11b, CD33, and CD15, CD3, CD68, CD11c, DEC205, CD56, CD80, or isotype control mAbs (data not shown). Viable cells were gated and analyzed by flow cytometry. *Panel B*:PBMCs from lung cancer patient 6 were either depleted or not depleted for $CD11b^+CD33^+$ (MDSC) cells and activated with anti-CD3 and anti-CD28 mAbs. T-cell proliferation was assessed by ^{3}H-thymidine uptake. These data are representative of two to three independent experiments with PBMC from 5 healthy donors and 10 NSCLC patients.

Cytotoxic chemotherapy has traditionally been thought to suppress antitumor immunity. Most cytotoxics kill through apoptosis, typically thought to be

immunologically "bland" or to result in tolerance. However, several agents, most notably gemcitabine, can in fact selectively enhance anti-tumor immunity, in particular, T-cell mediated immunity. [14] Cytotoxic chemotherapy also has the potential to both increase and decrease MDSC numbers. A recent paper has demonstrated that cyclophosphamide and doxorubicin can increase MDSC and potentially decrease the efficacy of immune checkpoint inhibitors.[15] However, gemcitabine has reduced some MDSC in animal models.[16] [17] Gemcitabine has demonstrated benefit in a number of common malignancies, including NSCLC, breast, ovarian and pancreatic cancers. Given the activity of the drug as both a single agent and its potential to enhance immunotherapy, it is logical to explore its interaction with immunotherapeutics.

1.3. Study Hypothesis

We hypothesize that gemcitabine can deplete MDSCs in lung cancer patients and depletion of MDSCs will enhance T-cell activity and consequently the activity of PD-1 inhibitors.

1.4. Correlative Testing

T-cell activation assay

Since the hallmark of MDSC is their ability to suppress T cell activation, we will determine the effects by comparing the level of T cell activation using patient samples obtained before and after treatment. T cell activation studies will be performed *in vitro* with PBMC at specified time points.

These assays will be conducted as previously described.[21] Briefly, PBMC from each patient at each time point will be divided into two aliquots. One aliquot will be depleted for $CD11b^{+}CD33^{+}$ cells (MDSC) using Miltenyi magnetic beads. The number of T cells in each aliquot will then be determined by flow cytometry staining with antibodies to CD3. Both aliquots will then be activated by placing 10^5 T-cells/well in a 96 well round-bottom plates previously coated with antibodies to CD3 and CD28. These cultures will be incubated for 72 hrs and then pulsed with tritiated thymidine. Sixteen to 18 hrs later the cells will be harvested and counted in a scintillation counter. Since tritiated thymidine uptake is a direct measure of T cell activation, we anticipate that there will be minimal uptake of the radiolabeled thymidine in the total PBMC and higher levels of radiolabel in the MDSC depleted cultures. Since we will initially plate out the same number of T cells in each well, we can directly compare the level of T cell activation between the different treatments. As an alternative to assessing T cell proliferation by tritiated thymidine uptake, we could assess T cell proliferation by CFSE dilution assay, or measure IFNγ production by either ELISA or Elispot assay

2.0 Objectives

2.1. Primary Objective

- The primary objective of this proposal is to evaluate gemcitabine as a method of MDSC depletion.

2.2. Secondary Objectives

- Evaluate whether these measures result in enhanced T-cell activity and/or NK cell function and number.
- Determine the tolerability and clinical activity (including response rate and survival) of this approach.
- Correlate MDSC number with tumor PD-L1 expression.

3.0 Study Plan

3.1. Description of Study Design, Population and Duration of Study Therapy

This is a phase II trial with primary endpoint of evaluating the ability of gemcitabine to deplete MDSC number prior to administration of nivolumab. Patients who meet eligibility criteria (see section 4) will be enrolled. Patients will receive nivolumab 240 mg on days 1, 15 and gemcitabine 1000 mg/m^2 on days 1, 8, 15 days. Every 28 days will be considered one cycle.

3.2. MDSC Enumeration Method

Blood will be collected for PBMC isolation on day 1, 8, 15 of cycle 1 and day 1 of cycles 2, and 3 before drug administration. Granulocytic and monocytic MDSC in the blood will be quantified by flow cytometry using fluorescently tagged antibodies to the following markers of monocytic and granulocytic MDSC. All human MDSC express CD33 and CD11b and are either negative or very low expressers of HLA-DR. They are also negative for the lineage markers CD3, CD16, CD19, CD20, and CD56 [18, 19, 20, 21 22, 23, 24]. Monocytic MDSC also express IL-4Rα and CD14, [22, 27, 25] while granulocytic (or polymorphonuclear) MDSC express CD15 [26, 27]. The staining protocol will be as previously described[28, 21].This will be done by the Ostrand-Rosenberg lab at the University of Maryland Baltimore County. The blood samples will be handled and shipped by the Fox Chase Cancer Center Protocol Support Lab (PSL) according to the procedures described in the lab manual. The amount of blood to be shipped along with the procedures to be followed are specified in section 8.1

3.3. PD-L1 expression

Fresh or archival specimens of formalin fix tissue samples will be required for study entry (See Appendix III for details). Specimens may be from tumor material obtained from the primary or metastatic sites. They will be analyzed for PD-L1 expression to correlate with response and MDSC changes. Given the small sample numbers, this would be an exploratory analysis. The commercially available and FDA approved complementary diagnostic analysis for PD-L1 expression using the DAKO 28-8 antibody

will be performed. We will analyze by employing the previously used cutoff points of >1%, 5% and 10% tumor cell staining. In addition, we will also evaluate staining of tumor infiltrating lymphocytes.

4.0 Patient Selection Inclusion & Exclusion

4.1. Inclusion Criteria

4.1.1 Histologically confirmed diagnosis of non-small cell lung cancer (NSCLC). Patients should have stage IV disease (AJCC 7th edition), stage IIIb disease that is not amenable to potentially curative treatment (e.g. chemoradiotherapy) or unequivocal progression in a prior irradiated field. Measurable or evaluable disease is required.

4.1.2 Fresh/ archived tumor tissue available for molecular marker testing is required for entry. A tumor block or at least 5 unstained slides must be available. As an alternative FFPE cell block that is sufficient for histologic analysis is acceptable. . If a patient has had PD-L1 status previously determined with and FDA approved assay, they have met this requirement. Tissue is still requested (but not required) for further analysis.

4.1.3 Age $\geq$ 18 years.

4.1.4 ECOG performance status 0 or 1

4.1.5 Patients must have normal organ and marrow function as defined below

- White Blood Cells (WBC) $\geq$ 2,000/mcL
- Absolute neutrophil count(ANC) $\geq$ 1,500/mcL
- Platelets $\geq$ 100,000/mcL
- Hb $\geq$ 9g/dl
- Serum creatinine $\leq$ 1.5 x ULN

or

- creatinine clearance (CrCl) $\geq$ 50 mL/min (if using the Cockcroft-Gault formula below):

    - Female CrCl = {(140 - age in years) x weight in kg/ (72 x serum creatinine in mg/dL)} x 0.85
    - Male CrCl = {(140 - age in years) x weight in kg/ (72 x serum creatinine in mg/dL)}x1.0

- AST/SGOT $\leq$ 3 x ULN
- Total bilirubin,
    - If no known liver metastases:

  Total bilirubin $\leq$ 1.5 x institutional upper limit of normal (ULN) (except subjects with Gilbert Syndrome, who may have total bilirubin <3.0 mg/dl)

- If known metastasis:
  Total bilirubin ≤ 5 ULN

4.1.6 Negative serum pregnancy test result in Women of Child-bearing Potential (WOCBP)

4.1.7 Prior therapies:

- Patients without activating mutations and gene rearrangements should have received at least one prior chemotherapy regimen. Any number of prior therapies is allowed except for immunotherapy (e.g. anti PD-1, PD-L1, vaccines, CTLA-4 etc.),
- Patients with activating mutations and gene rearrangements with known documented benefit from tyrosine kinase inhibitors should have received and demonstrated progression with that inhibitor (e.g. EGFR del 19 mutation should have been treated with gefitinib, erlotinib or afatanib etc). ALK rearrangements should have been treated with an ALK inhibitor. Patients who have progressed on these agents should be assessed, if appropriate, for resistance mutations susceptible to approved agents and treated with that agent.
- No prior gemcitabine treatment

4.1.8 Ability to understand and willingness to sign a written informed consent and HIPAA consent document

4.2. Exclusion Criteria

4.2.1 Patients with active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger

4.2.2 Patients 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. Subjects are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if > 10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (eg, delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

4.2.3 As there is a potential for hepatic toxicity with nivolumab, drugs with predisposition to hepatotoxicity should be used with caution in patients treated with nivolumab-containing regimen.

4.2.4 Patients are excluded if they have active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if metastases have been treated and without clinical or radiologic evidence of progression for 14 days prior to initiation of treatment. An MRI within 14 days of commencing therapy is required for patients with a history of brain metastases.

4.2.5 There must be no requirement for immunosuppressive doses of systemic corticosteroids (> 10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.

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

4.2.7 Uncontrolled intercurrent illness that would increase the risk of toxicity or limit compliance with study requirements. This includes but is not limited to, uncontrolled infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.8 Known HIV-positive patients on combination antiretroviral therapy are ineligible because of the abnormal immune response that results from HIV disease.

4.2.9 Patients should be excluded if they are positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection

4.2.10 Patients who have had systemic (IV) cytotoxic chemotherapy or any other investigational agents within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. If a patient received an oral agent, treatment on study cannot commence at least five half-lives of the agent have elapsed.

4.2.11 Subjects with previous malignancies (except non-melanoma skin cancers, and in situ cancers such as the following: bladder, gastric, colon, cervical/dysplasia, melanoma, or breast) are excluded unless a complete remission was achieved at least 2 years prior to study entry and no additional therapy is required or anticipated to be required during the study period.

4.2.12 Other active malignancy requiring concurrent intervention.

4.2.13 Subjects with any history of interstitial lung disease or a history of > or = to grade 2 radiation pneumonitis.

4.2.14 Pregnant or breast feeding. Refer to section 4.4 for further details.

4.3. Inclusion of Women and Minorities

Men and women, regardless of race, ethnic group or sexual orientation are eligible for this study.

4.4. Pregnancy

The effects of nivolumab and gemcitabine on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because nivolumab and gemcitabine agents are known to be teratogenic, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception prior to study entry, and for the duration of treatment.

WOCBP is defined as follows: Any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or a bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea $\geq$ 12 consecutive months, or women on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone (FSH) level > 35 mIU/ml). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g. vasectomy), should be considered to be WOCBP.

Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

Prior to study enrollment, WOBCP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential. Women of childbearing potential (WOCBP) receiving nivolumab will be instructed to adhere to contraception for a period of 23 weeks after the last dose of investigational product

All WOCBP must have a negative pregnancy test within 24 hours prior to receiving the first dose of the investigational agent(s). If the pregnancy test is positive, the patient must not receive protocol treatment and must not be enrolled in the study.

Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product. Women who are not of childbearing

potential (ie, who are postmenopausal or surgically sterile as well as azoospermic men do not require contraception

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

- Male condoms with spermicide
- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena® by WOCBP subject or male subject's WOCBP partners participating in the study may use hormone based contraceptives as one of the acceptable methods of contraception since they will not be receiving study drug
- Nonhormonal IUDs, such as ParaGard®
- Tubal ligation
- Vasectomy
- Complete Abstinence*

*Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

Less effective Methods of Contraception

- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal Sponge
- Male condom without spermicide
- Progestin only pills by WOCBP subject and male subject's WOCBP partner
- Female Condom*

*A male and female condom must not be used together

4.5. Patient Registration

Participants may be registered from 8:00 am to 4:00 pm EST excluding holidays by emailing the Investigator-Sponsored Research Unit (ISRU) at: **FCCC.MONITOR@fccc.edu.** Eligible participants will be entered on study centrally once the following items have been received by email:

Completed registration form
Consent and HIPAA signature pages
Eligibility checklist

Following registration, participants must begin protocol treatment within 7 calendar days of registration. Issues that would cause treatment delays must be discussed with the Sponsor-Investigator. If a registered participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. For additional registration questions, please email FCCC.MONITOR@fccc.edu or call **(215) 728-5544.**

The FCCC ISRU will notify the site by email once registration is confirmed and the sequence number has been assigned. Participants must be registered and have received a sequence number prior to the initiation of treatment.

Exceptions to the current registration policies will not be permitted.

5.0 Treatment Plan

Treatment will be administered on an outpatient basis. Treatment will be administered as described below. Dose delays and modifications should only be done following protocol guidelines described in section 6.0. Missed days will not be made up. Gemcitabine should be administered first followed by nivolumab. The day1 of the treatment should be scheduled in such a way that blood sample collection day for MDSC enumeration does not fall on a Friday.

5.1. Treatment Administration

Agent	***Dose***	***Route***	***Schedule***	***Cycle Length***
Gemcitabine	1000mg/m^2	IV over 30 minutes	Day 1,8,15	4 weeks (28 days)
Nivolumab	240 mg	30 minutes IV infusion	Day 1, 15	

5.1.1. Nivolumab

Nivolumab will be given every two weeks beginning day 1 at a dose of 240 mg to be administered as a 30 minute IV infusion. Subjects may be dosed no less than 12 days from the previous dose of drug. There are no premedications recommended for nivolumab on the first treatment. There will be no dose modifications allowed.

Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to Protocol guidelines in section 6.1.5 Doses of nivolumab may be

interrupted, delayed, or discontinued depending on how well the subject tolerates the treatment.

Nivolumab Injection, 100 mg/10 mL (10 mg/mL Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab Injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 1 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

5.1.2. Gemcitabine

Gemcitabine 1000 mg/m2 IV over 30 minutes on day 1, 8 and 15 every 28 days. The dosing calculations should be based on the actual body weight at baseline. If the subject'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.

5.2. Concomitant Medications, Supportive Care, Excluded Therapies and Restrictions

Active prophylaxis and therapy, when available, should be considered per standard clinical indications. Examples include gastritis and nausea prophylaxis and therapy for acute esophagitis, enteritis and cystitis. Palliative local therapy, including palliative radiation therapy- and palliative surgical resection, to symptomatic non-target bone lesions, skin lesions, or CNS lesions is permitted prior to discontinuation of study treatment for subjects who do not have evidence of overall clinical or radiographic progression per RECIST 1.1.

Prohibited and/or Restricted Treatments

The following medications are prohibited during the study (unless utilized to treat a drug related adverse event):

- Immunosuppressive agents.
- Immunosuppressive doses of systemic corticosteroids

5.3. Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment will continue as long as the patient is benefitting (absence of progression and good tolerance of therapy) or until one of the following criteria applies:

- Unequivocal disease progression
- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse events
- Patient becomes pregnant
- Patient decides to withdraw from the study (In this event sponsor-investigator must be notified, reason for withdrawal should be documented and patient must be followed for disease progression and survival for the duration specified in the protocol)
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.

5.4. Duration of Follow up

A safety follow-up will be performed 30 days after discontinuation from study treatment. Patients removed from study for unacceptable adverse events that are related to the study treatment will be followed until resolution or stabilization of the adverse event. All patients who discontinue the study treatment due to reasons other than disease progression will be followed every 2 months for response and survival (secondary endpoints) until disease progression or death or 2 years whichever occurs first. Reasonable effort should be made to evaluate their disease by scan to determine disease progression by a clinic visit. Patients who undergo disease progression or start a new anti-cancer therapy will directly move into survival follow-up and will be followed once every 3 months by phone call for survival status until death or 2 years whichever occurs first.

5.5. Criteria for Discontinuation

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the medical record and case report form.

6.0 Dose Modifications

6.1. General Principles

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories (see current Investigator Brochure and Appendix I for citation examples).
Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab). Nivolumab must be delayed until treatment can resume.

6.1.1. Nivolumab administration should be delayed for the following:

1. Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:
   - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay

2. Any Grade ≥3 skin, drug-related AE
3. Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:
   - Grade 3 lymphopenia or leukopenia does not require dose delay.
   - If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
   - If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.
   - Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay. The Sponsor-Investigator should be consulted for such Grade ≥ 3 amylase or lipase abnormalities.
4. Any AE, laboratory abnormality, or intercurrent illness that in the judgment of the investigator, warrants delaying the dose of study medication.

Subjects who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated and resume nivolumab dosing when re-treatment criteria are met.

6.1.2. Criteria to Resume Treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT or total bilirubin
- Any drug-related liver function test (LFT) abnormality that meets the following criteria requires discontinuation (also see Hepatic Adverse Event Management Algorithm):
  - AST or ALT > 5-10x ULN for > 2 weeks
  - AST or ALT > 10x ULN
  - Total bilirubin > 5 x ULN
  - Concurrent AST or ALT > 3 x ULN **and** total bilirubin > 2 x ULN

- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed. Subjects with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment after discussion with the Sponsor-Investigator.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment if Sponsor-Investigator allows.

- Patients who received systemic corticosteroids for the management of any drug-related toxicity must be off corticosteroids or have tapered down to an equivalent dose of prednisone ≤ 10 mg/day.

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled time-point per protocol. However, if the treatment is delayed past the next scheduled time-point per protocol, the next scheduled time-point will be delayed until dosing resumes.
If treatment is delayed or interrupted for > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section (6.1.4).

6.1.3. Management Algorithms

Guidelines for the management of immune related events can be found in the current Investigator Brochure, in the approved United States Package Insert and Appendix I. Immuno-oncology agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity. Management algorithms have been developed to assist investigators in assessing and managing the following groups of AEs:
Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, Neurological.

For subjects expected to require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider recommendations provided in the algorithms. These algorithms are found in the Nivolumab IB [and in Appendix I] of this protocol. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

6.1.4. Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period (section 6.1.2) OR requires systemic treatment
- Any Grade ≥ 2 drug-related pneumonitis or interstitial lung disease that does not resolve to dose delay and systemic steroids (also see Pulmonary Adverse Event Management Algorithm)
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement **do not** require discontinuation

  - Grade 3 drug-related laboratory abnormalities **do not** require treatment discontinuation except those noted below
    - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
    - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

    Total bilirubin > 5 x ULN

    Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
  - Grade 4 lymphopenia or leucopenia
  - Grade 4 neutropenia ≤ 7 days
  - Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Sponsor-Investigator [as allowed by protocol]
- Any dosing interruption lasting > 6 weeks with the following exceptions:
  - Dosing delays or interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Sponsor-Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted or delayed
  - Dosing interruptions or delays lasting > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Sponsor-Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal-Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness that in the judgment of the Sponsor-Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

6.1.5. Treatment of Nivolumab Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash,

pruritis, arthralgia, hypo- or hypertension, bronchospasm, or other symptoms of allergic-like reactions.

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 appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated) - Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours) - Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject 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 subject 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 subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or 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 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). **Grade 4**: (life threatening; pressor or ventilatory support indicated). - Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the treating Physician is comfortable that the symptoms will not recur. Nivolumab will be permanently

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

In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.2. Gemcitabine

6.2.1. General guidelines

If more than one of the dose modifications apply, use the most stringent (i.e., the greatest dose reduction). Gemcitabine will be continued when nivolumab is held for toxicities. If nivolumab is permanently discontinued for excessive toxicity gemcitabine will continue as monotherapy. All dose reductions are permanent

6.2.2. Dose Modifications

The following dose levels are used for dose modifications. If toxicity is seen at dose level -2, then gemcitabine will be permanently discontinued.

Dose Level	Gemcitabine
0	1000 mg/m^2
-1	750 mg/m^2
-2	500 mg/m^2

6.2.3. Hematologic Toxicity

1. For ANC <1500 or platelets <100000 on day 1, delay treatment with gemcitabine until ANC ≥1,500 and platelets ≥100,000, then resume at the previous doses. If treatment is delayed for ≥ 3 weeks, discontinue gemcitabine.
2. For platelets <25,000, decrease gemcitabine by one dose level for all subsequent doses.
3. For febrile neutropenia occurring at any time during a cycle, reduce gemcitabine by one dose level for all subsequent doses.
4. For ANC 500-999 or platelets 50,000 – 74,999 on day 8 or 15, decrease gemcitabine by one dose level for the current and all subsequent doses.
5. For ANC <500 or platelets <50,000 on day 8 or 15, skip gemcitabine and decrease gemcitabine by one dose level for all subsequent doses.
6. For a second episode of thrombocytopenia <25,000 or febrile neutropenia, decrease gemcitabine by one further dose level for all subsequent doses.

6.2.4. Nephrotoxicity

If creatinine clearance is < 45 ml/min, delay gemcitabine until CrCl ≥ 45 ml/min. When CrCl improves to ≥ 45 ml/min, resume gemcitabine at the previous dose level; if treatment is delayed / interrupted for ≥ 3 weeks, discontinue gemcitabine.

6.2.5. Pulmonary Toxicity

Gemcitabine may rarely cause pulmonary toxicity characterized by dyspnea and interstitial pneumonitis. The pneumonitis will usually respond to steroids. Other causes of dyspnea should be excluded (i.e., anemia, cardiac, COPD exacerbation, etc.) before concluding that pulmonary toxicity is drug associated. In addition, given the association of nivolumab with pulmonary toxicity, the algorithm for management of pulmonary toxicity due to nivolumab should be followed unless there is compelling evidence that the toxicity is due to gemcitabine.

- For grade 2 pulmonary toxicity due to gemcitabine, hold protocol treatment. Patients may be retreated with a two level dose reduction in gemcitabine dose on the next and all subsequent cycles at the physician's discretion if pulmonary symptoms improve to grade 0-1 within 3 weeks.
- For grade 3 and 4 pulmonary toxicity, discontinue protocol therapy.

6.2.6. Skin Toxicity attributed to gemcitabine

- **Grade 2:** Decrease gemcitabine by one dose level.
- **Grade 3 or 4**: Discontinue gemcitabine.

6.2.7. Hypersensitivity Reactions attributed to gemcitabine

For grade 3 allergic or anaphylaxis reactions thought to be due to gemcitabine, discontinue gemcitabine.

6.2.8. Other Non-Hematologic

Grade 3 Toxicity (not described above): Delay until toxicity improves to ≤ grade 2, then resume treatment with one dose level reduction. If treatment is delayed for > 3 weeks, discontinue gemcitabine.
For recurrent other non-hematologic grade 3 toxicity: Discontinue all protocol therapy.
Grade 4 Toxicity (not described above): Discontinue gemcitabine.

7.0 Study Agent Information

7.1. Nivolumab

Nivolumab will be supplied by BMS as investigational supply. Please refer to the FDA approved package insert and investigational brochure for additional information.

7.1.1. Product description

Product Description:(Other names = MDX-1106, ONO-4538, anti-PD-1)

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/ Label Type	Secondary Packaging (Qty) /Label Type	Appearance	Storage Conditions (per label)
Nivolumab (BMS-936558-01)* Injection drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL	100 mg/Vial (10 mg/mL).	Carton of 5 or 10 vials	10cc Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals.	Clear to opalescent, colorless to pale yellow liquid. May contain particles	**BMS-936558-01 Injection** must be stored at 2 to 8 degrees C (36 to 46 degrees F) and protected from light and freezing

*Nivolumab may be labeled as BMS-936558-01 Solution for Injection

7.1.2. Storage

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.
For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for "Recommended Storage and Use Conditions"

7.1.3. Route of administration

Intra-venous

7.2. Gemcitabine

7.2.1. Product description

Gemcitabine HCl (2'2'-difluorodeoxicytidine) is a nucleoside analogue that exhibits antitumor activity. It is commercially available and supplied as a lyophilized powder in sterile glass vials containing 200 mg or 1000 mg of active drug as the hydrochloride salt (expressed as the free base), mannitol, and sodium acetate.

7.2.2. Availability

Gemcitabine is available commercially. Please refer to the FDA approved package insert for additional information.

7.2.3. Solution preparation

The appropriate dose of gemcitabine should be diluted in 250 mL of normal saline (maximum concentration of 40 mg/mL) and administered intravenously as a constant infusion over 30 minutes. Normal saline is the only diluent approved; do not use other diluents. Nothing else should be added to the bag. Since gemcitabine is NOT a vesicant, extravasation should be handled according to local hospital policy concerning extravasation of drugs.

7.2.4. Storage requirements

The intact vials should be stored at controlled room temperature between 59°-86°F (15°-30°C). The intact vials are stable for 3 years at controlled room temperature. Reconstituted solutions should be used within 24 hours; unused portions should be discarded.

7.2.5. Route of administration

Intra-venous

7.2.6. Toxicity

The DLT is myelosuppression. Other possible clinical toxicities include fever, chills, nausea, vomiting, diarrhea, constipation, skin rash or confluent erythema, edema, paresthesia, fatigue, anorexia, headache, muscle aches, cough, rhinitis, insomnia, sweating, drowsiness, hypotension, and mucositis.

Dyspnea on exertion or at rest has occasionally been reported after gemcitabine therapy. Non-progressive liver function abnormalities and dip-stick-positive proteinuria, or hematuria may occur. Episodes of hemolytic uremic syndrome have been reported in patients treated with gemcitabine. There were no reports of injection site necrosis with gemcitabine in US trials. There may be some local irritation and injection site pain due to the low pH of the solution (pH≈3).

7.3. Drug Ordering, Storage and Handling

Following submission and approval of the required regulatory documents, participation in the study initiation meeting and receipt of the site activation letter from the FCCC ISRU, the initial order may be placed. Drug order forms and ordering procedure will be presented at the site initiation meeting.

7.4. Destruction of Drug

At the time of study closure, the unused and expired study drug will be destroyed at the site per Institutional SOPs unless otherwise specified. Expired drugs may be destroyed during the study as per institutional SOP.

7.5. Records to be kept at Site; Dispensing and Accountability

It is the responsibility of the Site Principal Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number.
- Dates and initials of person responsible for each investigational product inventory entry/movement.
- Amount dispensed to and returned by each patient, including unique patient identifiers.
- Amount transferred to another area for dispensing or storage.
- Non-study disposition (e.g., lost, wasted, broken).

8.0 Correlative /Special Studies

8.1. T- cell activation assay

8.1.1. Outcome measure

T-cell activation assay will be performed as explained in section 1.4.

8.1.2. Collection and handling of specimen

The blood samples will be collected and handled by Protocol Support Lab (PSL) according to the instructions specified in their lab manual

8.1.3. Specimen

At the time of registration, the investigator will contact the Immune Monitoring Facility (Dr. Kerry Campbell) lab to confirm dates that they can receive specimen for analysis. A minimum of 7ml green top tube of blood will be drawn and kept at room temperature.

8.1.4. Site(s) performing correlative study

Immune Monitoring Facility, Fox Chase Cancer Center

8.2. Optional Blood and tissue for future research and Banking

Archival tissue will be collected and used for genetic testing in consenting patients. Blood specimen will be collected from consenting patients to isolate plasma and sera for banking for future research by PSL according to the instructions specified in the lab manual. Blood will be drawn on cycle 1 day 1 pre-treatment, every 2 cycles (at the start of cycle 3, 5 and so on), and at the time of discontinuation.

9.0 Study Calendar

					Beyond Cycle 3, Cycle 2 and 3 will be repeated in the same order (except blood for MDSCs)								
		Cycle[K] 1			Cycle 2			Cycle 3			Safety Follow-up[L]	Follow-up[M]	Survival Follow-up[N]
	Pre-treatment	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15			
Informed Consent And HIPAA[A]	X												
Demographics	X												
Medical History	X										X	X	
Concurrent Medications	X	X			X			X			X	X	
Physical Examination	X	X			X			X			X	X	
Vital Signs[B]	X[C]	X			X			X			X	X	
Performance Status	X[C]	X			X			X			X		
Weight	X	X			X			X			X		
Height	X												
CBC w/differential, plts	X[C]	X	X	X	X	X	X	X	X	X	X		
Serum chemistry[D]	X[C]	X	X	X	X	X	X	X	X	X	X		
Thyroid (TFT)[P]		X						X					
Serum Pregnancy test[E]	X	X			X			X					
EKG (as clinically indicated)	X												
Radiologic Tumor assessment[F]	X[J]							X				X	
Other imaging[G]	X[J]							X				X	
Archival or fresh biopsy	X												
Nivolumab		X		X	X		X	X		X			
Gemcitabine		X	X	X	X	X	X	X	X	X			
Adverse Event		X	X	X	X	X	X	X	X	X	X	X	
Blood for MDSC quantification[H]		X	X	X	X			X					
Survival status												X	X
Sera and plasma for banking		X[I]						X			X		

Footnotes

A. Informed consent must be signed within 30 days of registration. If signature is outside that window the patient must sign a new consent. Following registration treatment must begin within 7 calendar days.
B. Temperature, Pulse, Respiratory rate, Blood Pressure
C. Should be done within a week of C1D1
D. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
E. Only for Women of Child Bearing Potential. Should be done within 24 hours of day 1 of every cycle.
F. CT scan of chest. Must be done before treating the patient and disease status must be determined before treatment.
G. As clinically indicated other imaging may be done, for example CT of abdomen if abdominal disease is indicated
H. Should be collected prior to drug administration on day 1, 8, 15 of cycle 1, and day 1 of cycles 2 and 3 only.
I. Sera and plasma to be collected before treatment on C1D1 for banking for future research
J. Must be done 28 days before C1D1
K. Each cycle is 28 days. All treatment visits have +/1 day window. Day 1 of each treatment cycle should be scheduled such that blood sample collection for MDSC quantification should not fall on a Friday.
L. 30 days after study discontinuation
M. Patients who discontinue without disease progression will be followed every 2 months until 2 years or disease progression or death whichever occurs earlier. Once disease progression is documented the patients will move to survival follow-up
N. Survival follow-up once in 3 months via phone call.
O. To be performed every other cycle i.e. on cycle 1,3,5 and so on.

10.0 Adverse Events

10.1. Definitions

Adverse Events (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, treatment or procedure regardless of whether it is considered related to the medical treatment or procedure *(NCI CTEP Guidelines March 28, 2011)*

Serious Adverse Event (SAE) is an AE that is fatal or life threatening, requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours), persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly/ birth defect. Important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent any of the above outcomes. A "life-threatening" adverse event places the patient at immediate risk of death in the judgment of the investigator or sponsor.

Although pregnancy, overdose, potential drug-induced liver injury (DILI) and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs.

Potential drug induced liver injury is defined as:

1) AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)
   AND
2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),
   AND
3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

10.1.1. Severity Rating

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.4.0) or study specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.4.0, severity is expressed in numerical grade using the following definitions:

- Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL.
- Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE

10.1.2. Attribution/Relationship to study drug

- Definite – clearly related
- Probable – likely related
- Possible – may be related
- Unlikely – doubtfully related
- Unrelated – clearly not related

10.1.3. Expectedness

An Expected Adverse Event is one where the specificity or severity is consistent with the current information available from the resources.

An Unexpected Adverse Event is one where the nature, severity, or frequency of the event is related to participation in the research is not consistent with either:

1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts: or

2. The expected natural progression of any underlying disease, disorder, or condition of the subject (s) experiencing the adverse event and the subjects(s) predisposing risk factor profile for the adverse event.

10.2. Recording and Reporting Responsibilities

10.2.1. Investigative Site Recording Responsibilities:

1. Upon identification of an AE or SAE, the site investigator will utilize the above definitions to properly classify the event. Each category listed above must be recorded for each event.

2. All AEs and SAEs will be recorded in the "AE case report forms" (CRF) and in progress reports with details about the grade and attribution of each episode, action taken with respect to the study drug, and the patient's outcome will be recorded in the CRF. All events will be recorded on case report forms for the duration of the study until they resolve.

3. All reportable SAEs will be recorded on the FDA MedWatch form 3500a. After submitting the initial report it may be necessary to submit follow up reports to the Sponsor should the event require further investigation.

10.2.2. Investigative Site Reporting Responsibilities:

1. The investigator/ site is responsible to report all SAEs that occur on or after the first day of study treatment to the sponsor within 24 hours of becoming aware of the event. All subsequent SAEs must be reported for up to 30 days after the last treatment.

   Each investigator is responsible to report all AEs/SAEs to their local IRB following guidelines set by that IRB. The FCCC OCR reserves the right to request an event be reported to the IRB at their discretion. Copies of events reviewed by the IRB must be sent by email to **SAE.FCCC@fccc.edu**.

2. If the investigator or IRB feels the event warrants a revision to the informed consent that was not already initiated by the OCR, draft revisions will be made in track changes and submitted to the OCR for consideration. Any consent revisions must receive OCR approval **prior** to submission to the IRB.

3. Any investigator who is in doubt of whether a particular AE needs to be reported is directed to call the Study Monitor for confirmation with the Sponsor-Investigator

4. If the results of an investigator or OCR investigation show an adverse event not initially determined to be reportable is so reportable, the investigator will report the event following the above guidelines based on the date the determination is made.

5. Copies of all related correspondence and reporting documents must be submitted to the ISRU and will be maintained in the trial master file.

Participating sites should report events to:

Investigator-Sponsored Research Unit
Office of Clinical Research
Fox Chase Cancer Center
SAE.FCCC@fccc.edu

10.2.3. Sponsor Reporting Responsibilities:

1. Adverse events which meet all of the following criteria must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event.
    a. Unexpected (in terms of nature, severity, or frequency) given
        i. (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and
        ii. (b) the characteristics of the subject population being studied;
    b. Possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
    c. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.
2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the OCR for each site's IRB of record along with the report of the adverse event.
3. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study at OCR.
4. SAEs that are related, unexpected, fatal, or life-threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions are as directed by FDA guidelines (http://www.fda.gov/medwatch/index.html). Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

Food and Drug Administration:
Telephone 1-800-FDA-1088
Fax 1-332-FDA-0178
http://www.fda.gov/medwatch/report.htm

SAE and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies must be reported on a Pregnancy Surveillance Form (Appendix II).

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

- An SAE report should be completed for any event where doubt exists regarding its seriousness.
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

10.3. Pregnancy

All WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

In the event of a confirmed pregnancy in a patient participating in the study, the site Investigator must immediately notify the Fox Chase Cancer Center Study Monitor who will notify Dr. Martin J Edelman and BMS.

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The sponsor-investigator must immediately notify Worldwide.Safety@bms.com of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

10.4. Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

10.5. Secondary Malignancy

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

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

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

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

10.6. Second Malignancy

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

10.7. Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

11.0 Measures of Effect

The patients will be re-evaluated every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained ≥ 4 weeks following initial documentation of objective response. Assessment of response is based upon RECIST 1.1 with the modifications described by Nishino et al. Though "pseudoprogression" in lung cancer is uncommon, it has been documented. Therefore, patients may continue on therapy if they are felt to be clinically benefitting and will be withdrawn if there is clinical deterioration. Additionally, new lesions will be added to the sum of the prior lesions and will not automatically be considered disease progression.

11.1. Treatment beyond disease progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD. Subjects will be permitted to continue treatment beyond initial RECIST 1.1 defined PD as long as they meet the following criteria determined by the investigator:

- Investigator-assessed clinical benefit
- Tolerance of study drug
- Stable performance status

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases).

A radiographic assessment/ scan should be performed within 6 weeks of initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced

by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with nivolumab.

For the subjects who continue nivolumab study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Nivolumab treatment should be discontinued permanently upon documentation of further progression.

New lesions are considered measureable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least15 mm). Any new lesion considered non-measureable at the time of initial progression may become measureable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions that become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

11.2. Definitions

Evaluable for adverse events. All patients will be evaluable for adverse events from the time of their first treatment

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

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

11.3. 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 $\geq$20 mm by chest x-ray, as $\geq$10 mm with CT scan, or $\geq$10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

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

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

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

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

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

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

11.4. 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.

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).

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

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

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response

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

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

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

Cytology and Histology: If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology. These techniques can be used to differentiate between PR and CR in rare cases (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.5. Response Criteria

11.5.1. Evaluation of Target Lesions

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

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

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

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

11.5.2. Evaluation of Non-Target Lesions

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

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

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

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

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

11.5.3. Evaluation of Best Overall Response

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

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

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

For patients not evaluable

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

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

Non-Measurable Disease (i.e., Non-Target Disease)

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

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.6. 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: measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest

measurements recorded since the treatment started, including the baseline measurements.

11.7. Progression-Free Survival

PFS is defined as the duration of time from first dose of the study drug to time of progression or death, whichever occurs first.

12.0 Statistical Considerations

The primary goal is to evaluate the efficacy of the combination therapy in suppressing MDSCs by comparing day 1 (baseline- prior to any treatment) with samples collected on day 1 of cycle 2, with each patient serving as his/her own control. Ten (10) patients will be treated with the combination therapy beginning in the first four week cycle (two administrations of nivolumab). A paired t-test, (one-sided; $\alpha = 0.05$) or Wilcoxon signed rank test will be used in this small pilot study to compare baseline to day 1 of cycle 2 changes in MDSC measurements. MDSC data may be transformed before analysis if appropriate. Efficacy analyses will be conducted using data from evaluable patients. It is anticipated that the addition of gemcitabine will produce at least a 50% reduction in the number of MDSCs circulating in the blood. Table 1 displays the detectable mean pre- to post-treatment reductions in MDSC values with 10 paired samples over a range of potential within-subject correlations.

Table 1. Detectable mean pre- to post-treatment reduction in MDSC measurements with 80% power using a paired t-test (1-sided; α=0.05) assuming unit standard deviation for individual measurements and 10 paired samples over a range of potential within-subject correlations.

Within-Subject Correlation	Standard deviation of change over time	Detectable mean pre- to post-treatment reduction in MDSC values
0.5	1.00	0.85
0.6	0.89	0.76
0.7	0.77	0.66
0.8	0.63	0.54
0.9	0.45	0.38

12.1. Analysis of Secondary and Exploratory Endpoints

Patient characteristics and biomarker measurements at baseline and day 1 of subsequent cycles will be summarized using standard methods (e.g., binomial proportions, means, medians, 95% confidence intervals). Secondary and exploratory endpoints will include response rate (determined with RECIST 1.1 and with modified immune RECIST, as described by Nishino), progression free and overall survival. Response rates will be computed and reported with 2-sided 95% confidence intervals. Overall survival (OS) is defined as the time between the date of first dose of experimental therapy and death from all causes. Progression-free survival (PFS) is defined as the time between the date of first dose of experimental therapy and disease progression or death from any cause, whichever comes first. Overall survival and progression-free survival will be characterized by the

Kaplan-Meier method. For calculation of OS, patients alive at last follow-up will be censored. For PFS analyses, patients alive and progression free at last follow-up will be censored. Adverse events will be tabulated by type, severity.

Since the hallmark of MDSC is their ability to suppress T cell activation, we will determine the effects of the treatment by comparing the level of T cell activation using patient samples obtained before and after treatment. These assays will be conducted as previously described. [21] T cell proliferation will be assessed by tritiated thymidine uptake or CFSE dilution assay; T cell function will be measured by IFNγ production by either ELISA or Elispot assay. Baseline to day 1 of subsequent cycles changes in the level of T cell proliferation and T cell function will be compared using one-sided Wilcoxon signed rank tests (α=0.05).

A series of correlative analyses will be conducted between pairs of biomarkers (e.g., MDSC number and tumor PD-L1 expression) measured at specific time points as well as baseline to follow-up changes in biomarker values. We will use Spearman's rank correlation coefficient to quantify these associations. With a two-tailed test (α=0.05) we will have 80% power to detect true correlations of > 0.76 (or<-0.76). Correlations much weaker than these are unlikely to be biologically and clinically useful.

12.2. Sample Size/Accrual Rate

Ten (10) patients with paired samples will be required. Therefore, a total of 10 evaluable patients will be enrolled. If a patient is not evaluable (i.e. there is not an adequate paired sample, that patient will be replaced and not considered in the final primary analysis. It is anticipated that there will be a 10% inevaluable rate, therefore, up to 11 patients may be enrolled. Accrual rate for this study is estimated to be 1-2 patients per month.

13.0 Data and Safety Monitoring Plan

13.1. Monitoring Plan

FCCC ISRU will monitor the medical and study records of each participant accrued throughout the course of the study. In addition, the ISRU will collect and report data to the study Sponsor-Investigator who will review the data on a regular basis at a rate dependent on subject accrual. All serious adverse events (SAEs) will be reviewed on a real time basis first by the study site PI and subsequently by the ISRU and Sponsor-Investigator as applicable.

13.2. Data & Safety Monitoring Board

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed by the Fox Chase Cancer Center Data & Safety Monitoring Board (FCCC DSMB). In this capacity the FCCC DSMB will serve as an advisory committee to the Sponsor-Investigator. The FCCC DSMB will review those aspects of this trial that are outlined in the responsibilities section of the Data & Safety Monitoring Plan (DSMP). If the committee decides that changes should be

made to this trial, it will make recommendations in writing to the Sponsor-Investigator, the Associate Director of Clinical Research, and the Protocol Management Executive Committee, which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Sponsor-Investigator before they are implemented. These changes may include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

14.0 Administrative

This study will be conducted in accordance with local, state and Federal regulations and according to accepted good clinical practice guidelines.

14.1. Data Reporting

The FCCC Study Monitor will request case report forms to be completed within 2 weeks of the protocol visit. Participating sites are responsible to respond to queries prior to the next scheduled monitoring visit

The ISRU is responsible for compiling and submitting data to the Sponsor-Investigator and statistician on an ongoing basis for monitoring as described in the DSMP and reporting to the Data and Safety Monitoring Board.

All patient information will be stored in an EDC system accessible only to the study team members for the purpose of entering, reviewing and analyzing data. Any paper records, such as case report files, produced will be stored in a secure location.

The ISRU is responsible for distributing and tracking review of all IND Action Letters, Safety Reports, study specific Serious Adverse Events

14.2. Retention of Records

Time points for the retention of records are described in detail in the contract between the grantor and the OCR and passed on to the participating site. Please refer to the study specific terms for specific time points. In all cases the Study Monitor must be notified of any plans to move records to an offsite location prior to doing so.

14.3. Study Agents

Any study agent supplied through the OCR from the manufacturer or a third party distributor may not be used for any purpose outside the scope of this protocol. The agent may not be transferred to any party not participating in the clinical trial.

14.4. Informed Consent

The IRB approved informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the

study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, they will be provided in a certified translation of the local language.

Original signed consent forms must be filed in each patient's study file or medical record with a copy in the study file.

At the time of disease progression patient must sign "treatment beyond disease progression" informed consent before next treatment with nivolumab

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Appendix I:

MANAGEMENT ALGORITHMS

Management for immune related Adverse Events

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity.

Pulmonary Adverse Events

Pulmonary AEs have been observed following treatment with nivolumab. The frequency of pulmonary AEs may be greater with nivolumab combination therapies than with nivolumab monotherapy. The majority of cases reported were Grade 1 or 2, and subjects presented with either asymptomatic radiographic changes (eg. focal ground glass opacities and patchy infiltrates) or with symptoms of dyspnea, cough, or fever. Subjects with reported Grade 3 or 4 pulmonary AEs were noted to have more severe symptoms, more extensive radiographic findings, and hypoxia.

Pulmonary Adverse Event Management Algorithm

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

Grade of Pneumonitis	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider bronchoscopy , lung	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics

	biopsy	<u>If not improving after 2 weeks or worsening:</u> • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung y	<u>If improves to baseline:</u> • Taper steroids over at least 6 weeks <u>If not improving after 48 hours or worsening:</u> • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

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

<u>Gastrointestinal Adverse Events</u>

Gastrointestinal AEs have been observed following treatment with nivolumab.
Most cases of diarrhea were of low grade (Grade 1-2). Colitis occurred less frequently than diarrhea. High-grade cases of diarrhea and colitis were managed with corticosteroids and, in all cases, the events resolved.

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	Management	Follow-up
Grade 1 <u>Diarrhea</u>:< 4 stools/day over baseline; <u>Colitis</u>: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately <u>If worsens:</u> • 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

Hepatic Adverse Events

Hepatic AEs, including elevated liver function tests (LFTs) and, infrequently, DILI, have been observed following treatment with nivolumab and nivolumab in combination with ipilimumab. Most cases were of low or moderate grade. Higher-grade hepatic AEs, including DILI, were managed with corticosteroids (with or without mycophenolate mofetil) and, in almost all cases, the events resolved.

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.

Grade of Liver Test Elevation	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: • Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

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

*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.

Skin Adverse Events

Rash and pruritus were the most common skin AEs observed following treatment with nivolumab. The rash was typically focal with a maculopapular appearance occurring on the trunk, back, or extremities. Most cases have been of low or moderate grade. In some cases, rash and pruritus resolved without intervention. Topical corticosteroids have been used for some cases of rash. Anti-histamines have been used for some cases of pruritus. More severe cases responded to systemic corticosteroids.

Skin Adverse Event Management Algorithm

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

Grade of Rash	Management	Follow-up
Grade 1-2 Covering ≤ 30% body surface area (BSA)	• Symptomatic therapy (e.g. antihistamines, topical steroids) • Continue I-O therapy per protocol	If persists > 1-2 weeks or recurs: • Consider skin biopsy • Delay I-O therapy per protocol • Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol If worsens: • Treat as Grade 3-4
Grade 3-4 Covering >30% BSA; Life threatening consequences	• Delay or discontinue I-O therapy per protocol • Consider skin biopsy • Dermatology consult • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent	If improves to Grade 1: • Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections • Resume I-O therapy per protocol

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 Events

Elevated creatinine and biopsy-confirmed tubulointerstitial nephritis and allergic nephritis have been infrequently observed following treatment with nivolumab. The frequency of renal AEs may be greater with nivolumab combination therapies than with nivolumab monotherapy. Most cases were Grade 2 or 3 and based on creatinine elevation. Subjects with a history of RCC or prior nephrectomy did not appear to be at higher risk. Events were managed with corticosteroids and, in all cases, renal function partially or fully improved.

Renal Adverse Event Management Algorithm

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

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

	methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	

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.

Neurologic Adverse Events

Neurologic AEs have been uncommonly observed following treatment with nivolumab. Neurologic AEs can manifest as central abnormalities (eg, aseptic meningitis, encephalopathy, or encephalitis) or peripheral sensory/motor neuropathies (eg, Guillain-Barre Syndrome, myasthenia gravis complicated with sepsis and fatality).

Neurological Adverse Event Management Algorithm

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

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

	methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	immunosuppressive therapies per local guidelines

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

Endocrinopathies

Endocrinopathies have been observed following treatment with nivolumab. Most cases were of low or moderate grade. The events have typically been identified through either routine periodic monitoring of specific laboratories (eg, TSH) or as part of a work-up for associated symptoms (eg, fatigue). Events may occur within weeks of beginning treatment, but also have been noted to occur after many months (while still on treatment). More than 1 endocrine organ may be involved (eg, hypophysitis [pituitary inflammation] may need to be evaluated at the time adrenal insufficiency or thyroid disorder is suspected). Moderate- to high-grade cases were managed with hormone replacement therapy and, in some cases, with the addition of corticosteroids. In some cases, nivolumab treatment was held until adequate hormone replacement was provided.

Endocrinopathy Management Algorithm

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

Asymptomatic thyroid stimulating hormone (TSH) elevation	• Continue I-O therapy per protocol • If TSH < 0.5 x lower limit of normal (LLN), or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include free thyroxine (fT4) at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathy	• Evaluate endocrine function • Consider pituitary scan <u>Symptomatic with abnormal lab/pituitary scan</u>: • Delay I-O therapy per protocol	<u>If improves (with or without hormone replacement)</u>: • Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections

	• 1-2 mg/kg/day methylprednisolone IV or by mouth (PO) equivalent • Initiate appropriate hormone therapy <u>No abnormal lab/pituitary MRI scan but symptoms persist</u>: • Repeat labs in 1-3 weeks /MRI in 1 month	• Resume I-O therapy per protocol • Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness	• Delay or discontinue I-O therapy per protocol • Rule out sepsis • Stress dose of IV steroids with mineralocorticoid activity • IV fluids • Consult endocrinologist • If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy	

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.

Appendix II

Pregnancy Surveillance Form - Quick Reference Guide

The Pregnancy Surveillance Form will be completed for all prospective and retrospective reports of pregnancy and pregnancy outcomes (live births: normal or abnormal, fetal death, neonatal death etc). It functions as a data collection and query tool to report pregnancies and related pregnancy information. AE/SAEs for all subjects/patients reported in association with the pregnancy (obstetric complications, maternal medical complications, etc) are to be reported separately on the clinical or non-interventional SAE form or spontaneous AE/SAE form.

Prospective reports of pregnancy exposure: are data acquired **prior** to the knowledge of the pregnancy outcome or prior to the detection of a congenital malformation at prenatal examination (e.g. foetal ultrasound, serum markers).

Retrospective reports of pregnancy exposure: are data acquired **after** the outcome of the pregnancy is known or after the detection of a congenital malformation on prenatal test.

For PROSPECTIVE REPORTS of pregnancy from Clinical/Non-interventional Studies or Spontaneous reports: Part I is completed and submitted when the pregnancy is first reported. Part II and Part III are forwarded for completion based on the estimated date of delivery in accordance with the CARES follow-up procedure for pregnancy reports. An additional Part III page can also be forwarded subsequently to collect additional significant follow-up information or if there is a new latent adverse event associated with the pregnancy outcome that needs to be reported.

For RETROSPECTIVE REPORTS of pregnancy with known (reported) outcomes from Non-interventional or Spontaneous reports: Parts I, II and III will be completed and submitted with all relevant data on the pregnancy and outcome. An additional Part III page can also be forwarded subsequently to collect additional significant follow-up information or if there is a new latent adverse event associated with the pregnancy outcome that needs to be reported.

All Pages Header Information

- For studies the "Patient Identifier" is the same as that used throughout the CRF, and populated with the **protocol,** site and subject numbers i.e. **CV131-345**-234-1134
- For spontaneous reports, enter local country number (if applicable) at the top left and/or enter a patient identifier (i.e. initials) if available or leave blank
- Parts I, II and III will be completed with all appropriate identifying header information on each page.

Part I - Page 1

Complete all questions for "PREGNANCY" as the only adverse event; other SAEs reported in association with the pregnancy (obstetric complications, maternal medical complications etc) are reported separately either on the clinical / non-interventional study SAE form or the Spontaneous AE/SAE forms.

Part I - Page 2 : Medication:

- Include each medication reported as a separate entry
- Indicate if the drug was associated with maternal or paternal exposure
- Indicate if the drug was specifically identified as a non - study medication or study medication by the investigator or reporter. Study medications include the medications under study (for non-interventional studies), the Investigational Medicinal Product (IMP), comparator medications and background therapy identified in the protocol.

"Pregnancy Related to Medication" Column:
Check whether or not the pregnancy was related to the medication.

Dosing Information: For route and period(s) of drug exposure, use the codes indicated at the bottom of the page. For period(s) of drug exposure, include all that apply.

Part I - Page 3: Prenatal Diagnostic Testing: Indicate if the results are baseline by checking under "baseline"; otherwise leave this box blank when providing the relevant details. Specify the test results (including any relevant units or other data), use the space below this section to describe results in more detail if needed.

Part II - Pregnancy Outcome: Complete delivery and outcome data as requested at the top of the page. If the outcome involved multiple gestations, please complete a separate outcome form for each fetus/infant. If the pregnancy/outcome involved labor or delivery complications, obstetric complications, or maternal medical conditions, briefly specify them.

NOTE: If any complications reported above meet the definition of an SAE (or an AE for non-study patients) they should be reported separately on either the clinical or non-interventional SAE form or the spontaneous AE/SAE form.

- If the outcome is "live birth- normal" check this box, and proceed to the next page.
- For any adverse outcome (live birth abnormal, fetal or neonatal death) complete all requested information to the fullest extent possible. A detailed causality assessment by the investigator is required for any reports from trials and must be provided as noted at the bottom of this page.

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Document owner: Sharon Mains-Reid

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I
(Antepartum Information)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

BMS RECEIPT DATE (BMS USE ONLY) D D M M M Y Y

GPV&E RECEIPT DATE (BMS USE ONLY) D D M M M Y Y

REPORT TYPE: ☐ SPONTANEOUS OR ☐ STUDY ☐ INITIAL REPORT OR ☐ FOLLOW-UP REPORT

COUNTRY

EVENT: PREGNANCY ☐ MATERNAL DRUG EXPOSURE OR ☐ PATERNAL DRUG EXPOSURE ☐ PROSPECTIVE REPORT OR ☐ RETROSPECTIVE REPORT

WERE THERE ANY ADDITIONAL MATERNAL/PATERNAL ADVERSE EVENTS ? ☐ NO ☐ YES

IF YES, REPORT THE ADVERSE EVENTS APPROPRIATELY (FOR STUDIES, REFER TO STUDY-SPECIFIC INSTRUCTIONS)

MATERNAL INFORMATION DATE OF BIRTH: D D M M M Y Y Y Y

AGE AT CONCEPTION:

HEIGHT: ☐ inches ☐ cm

WEIGHT: ☐ lb ☐ kg

RACE: ☐ WHITE ☐ BLACK ☐ ASIAN ☐ AMERICAN INDIAN OR ALASKAN NATIVE ☐ NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER ☐ OTHER RACE:

NUMBER OF PREGNANCIES INCLUDING THIS ONE

NUMBER OF BIRTHS

NUMBER OF LIVING CHILDREN

ONSET DATE LAST MENSTRUAL PERIOD (LMP): D D M M M Y Y

APPROXIMATE DATE OF CONCEPTION: D D M M M Y Y

ESTIMATED DATE OF DELIVERY: D D M M M Y Y

DATE PREGNANCY WAS CONFIRMED: D D M M M Y Y

TEST METHOD: ☐ SERUM ☐ URINE

ESTIMATED GESTATIONAL AGE WHEN PREGNANCY DIAGNOSED: WEEKS

DETERMINED BY: ☐ FETAL ULTRASOUND ☐ DATE FROM LMP

CONTRACEPTION AT TIME OF CONCEPTION: ☐ NO ☐ YES ☐ UNKNOWN (IF YES, SPECIFY)

RELEVANT MATERNAL MEDICAL HISTORY/RISK FACTORS	DATE OF ONSET D D M M M Y Y	IF APPLICABLE SPECIFY PERTINENT DETAILS

PATERNAL INFORMATION: AGE YEARS DATE OF BIRTH: D D M M M Y Y Y Y

RELEVANT PATERNAL MEDICAL HISTORY/RISK FACTORS	DATE OF ONSET D D M M M Y Y	IF APPLICABLE SPECIFY PERTINENT DETAILS

Alterations to this form are not permitted unless otherwise specified in the governing Procedural Document.

Original - Bristol-Myers Squibb; Copy - Retained by Investigator

Appendix III

Tumor Tissue Specimens

Pre-treatment tumor tissue specimens in the form of a paraffin embedded block or a minimum of 5 unstained slides, with a single section on positively charged slides will be submitted for central PD-L1 immunohistochemistry (IHC) assessment. These biopsy samples should be excisional, incisional, punch or core needle. Specimens that are obtained by needle that are sufficient for histologic analysis are appropriate. PD-L1 stained tissue sections will be assessed by a pathologist and scored as PD-L1 positive if membrane staining is observed. If the PD-L1 status has been previously assessed than the patient is considered to have satisfied the requirement for a tumor specimen. A tumor sample is still requested for further testing, but not required.

These tumor samples, as well as any solitary lesions that may have been surgically resected from subjects following an initial response may also be assessed for the expression of other immune related genes, RNAs and/or proteins, as well as, the presence of immune cell populations using a variety of methodologies inclusive of, but not limited to immunohistochemistry (IHC), qRT-PCR, genetic mutation detection and fluorescent in-situ hybridization (FISH). These tumor tissue biomarkers include, but are not limited to PD-1, PD-L2, tumor infiltrating lymphocytes (TILs) or subpopulations of TILs and a Th1 immune mRNA expression signature. In addition, other methods of measuring tumor PD-L1 expression may also be assessed. Tissue from the resected solitary lesions may be assessed for residual tumor cells and for markers expected to accompany tumor shrinkage in this study, including, but not limited to TILs and subsets thereof.