THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Phase 2 Study of Nivolumab in Patients with Primary Myelofibrosis, Post-Essential Thrombocythemia Myelofibrosis, or Post-Polycythemia Vera Myelofibrosis 2014-0962

Core Protocol Information

Short Title	Phase 2 Study of Nivolumab in Patients with Myelofibrosis
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Which Committee will review this protocol?

(●) The Clinical Research Committee - (CRC)

Protocol Body

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Phase 2 Study of Nivolumab in Patients with Primary Myelofibrosis, Post-Essential Thrombocythemia Myelofibrosis, or Post-Polycythemia Vera Myelofibrosis

1.0 HYPOTHESIS AND OBJECTIVES

Hypothesis
We hypothesize that blocking the PD-1/PD-L1 immune checkpoint with nivolumab may be an effective therapy for patients with Primary Myelofibrosis, Post-Essential Thrombocythemia Myelofibrosis, or Post-Polycythemia Vera Myelofibrosis (all collectively referred to as Myelofibrosis [MF])

Objectives
Primary:

- to determine the efficacy of nivolumab as therapy for MF

Secondary:

- to determine the safety of nivolumab as therapy for patients with MF

Exploratory:

- to explore time to response and duration of response
- to assess changes in symptom burden
- to explore changes in bone marrow fibrosis
- to explore changes in JAK2V617F (or other molecular marker) allele burden or changes in cytogenetic abnormalities

2.0 BACKGROUND AND RATIONALE

2.1 Myelofibrosis

Myelofibrosis (MF) is a rare clonal proliferative disorder of a pluripotent stem cell. This clone subsequently induces fibrogenic cytokines and/or growth factors in the marrow, which stimulate the deposition of extracellular matrix proteins by polyclonal fibroblasts. Megakaryocytic hyperplasia-dysplasia is frequently observed. Invasion of the blood stream and colonization of extramedullary sites ensues, resulting in organomegaly and splenomegaly. Extensive marrow fibrosis and osteosclerosis may be observed in advanced MF.

The entity of MF can be either idiopathic (primary) or representative of end-stage myeloproliferative diseases such as polycythemia vera (PV) or essential thrombocythemia (ET). MF occurs in about 15-25% of patients with PV and in 5-10% of patients with ET. In the early cellular phase of MF with minimal marrow fibrosis, the differential diagnosis includes Philadelphia-positive chronic myeloid leukemia (CML), PV, and ET that must be distinguished based on cytogenetics and clinicopathologic features. Other entities that can induce myelophthisis include myelodysplastic syndrome (MDS), metastatic malignancies, lymphoma, Hodgkin's disease, and plasma cell dyscrasias. MF must be differentiated from acute megakaryocytic leukemia (AML, M7 of the French-American-British classification) and MDS

with fibrosis. In acute megakaryocytic leukemia patients usually present with severe constitutional symptoms and pancytopenia but without organomegaly or peripheral blood myelophthisis.

The clinical picture of MF involves constitutional symptoms (e.g., cachexia, night sweats, fatigue, fever), splenomegaly, anisopoikilocytosis with teardrop erythrocytes, progressive anemia, immature myeloid and erythroid precursors in the peripheral blood, elevated lactate dehydrogenase (LDH) levels, and fibrosis of the marrow (as evaluated by reticulin and trichome [collagen] stains). The leukoerythroblastic picture is postulated to be related to both the intramedullary sinusoidal marrow and splenic hematopoiesis.

The disease generally occurs in adults, with the median age of 65 years. In 40% of the patients, constitutional symptoms are present, including fever, weight loss, nocturnal sweating, pruritus, and bone pain. Splenomegaly is present in 85% of the patients at diagnosis and is massive in 10%. Hematologic disease features include anemia in 50% to 70% at diagnosis and 25% will have severe anemia with hemoglobin level < 8.0 g/dL. Approximately half of the patients present with an elevated white cell count (WBC), 28% with thrombocytosis (platelet count > 500 x 10^9/L), and 37% with thrombocytopenia (platelet count < 150 x 10^9/L). Circulating blast cells are present in one-third of the patients.

Complications of MF are varied. Thrombotic obliteration of intrahepatic veins and splenomegaly may lead to portal hypertension; severe cases may be associated with ascites and/or variceal bleeding. Left upper quadrant pain may herald splenic infarction; episodes are usually self-limited and may persist for several days. Supportive care measures such as analgesics and hydration are usually sufficient; refractory cases may require splenectomy or irradiation. Extramedullary hematopoiesis (EMH) may occur in locations other than the liver or spleen; involvement of such sites may be managed by low-dose irradiation. Liver involvement is associated with increased levels of plasma alkaline phosphatase. Clinical manifestations of EMH include cardiac tamponade, papular skin nodules, pleural effusions, and spinal cord compression.

Autoimmune phenomena have been observed, including Coomb's positive autoimmune hemolytic anemia, nephrotic syndrome, antinuclear antibodies, rheumatoid factor, and lupus-type anticoagulant. Postulated etiologies include clonality of the lymphoid population or activation by abnormal monocyte-macrophage interaction with the immune system.

Adverse prognostic factors for survival include older age and anemia (hemoglobin < 10 g/dL). The etiology for the latter finding is usually multifactorial and related both to marrow failure and hypersplenism. Poor prognosis has also been correlated with leukocytosis, leukopenia, circulating blasts, increased numbers of granulocyte precursors, thrombocytopenia, abnormal karyotype, and hypercatabolic symptoms. The course of the disease is highly variable. Median survival from time of diagnosis ranges from 5 to 6 years. Progressive marrow failure, transformation into acute myeloid leukemia, and portal hypertension lead to demise.

Several therapies are available for MF, however no one therapy has demonstrated an ability to produce sustained remissions. In addition, currently available therapies are often limited by their

myelosuppressive effects. Ruxolitinib, a JAK1/2 inhibitor, produces symptom and spleen-size responses, but may result in anemia and/or thrombocytopenia in a significant proportion of patients. Hydroxyurea is another commonly used agent in the proliferative phases of the disease. Interferon-alpha had yielded hematologic responses and reductions in splenomegaly (definitions varying among studies) especially those with proliferative phase; however, this agent tends to be poorly tolerated. Agents used for the management of anemia include androgens and/or erythropoietin. Splenectomy and/or splenic irradiation have been used to manage symptomatic splenomegaly. Splenectomy has been associated with risk of leukemia transformation in some series, and splenic irradiation can result in severe myelosuppression. Patients with an intact quality of life and no threatening hematologic abnormalities, such as erythrocytosis or thrombocytosis, have usually been considered to not require any therapy.

2.2 Nivolumab

Nivolumab (BMS-936558) is a fully human, IgG4 (kappa) isotype, monoclonal antibody that binds PD-1. Blockade of the PD-1 pathway by nivolumab was studied using the mixed lymphocyte reaction (MLR). PD-1 blockade resulted in a reproducible enhancement of both proliferation and IFN-γ release in the MLR. The effect of nivolumab on antigen-specific recall response was investigated using a CMV-restimulation assay with human peripheral blood mononuclear cells (PBMCs), and was evaluated by ELISA. These data indicated that nivolumab, versus an isotype-matched control antibody, augmented IFN-γ secretion from CMV-specific memory T cells in a dose-dependent manner. PD-1 blockade by nivolumab is therefore considered a promising immunotherapeutic strategy.

2.3 Rationale

Expression of PD-L1 has been found in patients with myeloid malignancies (MDS, CMML), suggesting that nivolumab may be active in these patients (Yang et al. 2014). While expression of PD-L1 in MF has not yet been tested, dysregulated JAK-STAT signaling in hematopoietic cells and subsequent increased expression of cytokines in MF likely has downstream effects on T-cell development and differentiation. Furthermore, immunomodulatory drugs such as IFN-alpha (Quintas-Cardama 2013) and lenalidomide (Quintas-Cardama 2009) are to date the only agents that have been shown to reduce the mutant clone in patients with myeloproliferative neoplasms (including MF), suggesting that modulation of the immune response has the potential to eradicate disease. The dose schedule proposed is based on previous safety data from in-human trials and protocols being developed for other hematological malignancies.

3.0 BACKGROUND DRUG INFORMATION

The PK, clinical activity, and safety of nivolumab have been assessed in completed Phase 1 and ongoing Phase 2 and 3 studies sponsored by Bristol-Myers Squibb Company (BMS) in subjects with non-small cell lung cancer (NSCLC), melanoma, and clear-cell renal cell carcinoma (RCC) in addition to other tumor types. The current Phase 3 clinical program focuses on squamous and nonsquamous NSCLC, malignant melanoma, and RCC. Clinical activity and safety information presented in the Nivolumab Investigator Brochure (IB) (Appendix F) focuses primarily on that obtained from CA209063 (Phase 2 study in refractory squamous NSCLC), CA209037 (Phase 3 study in melanoma), MDX1106-03 (also known as CA209003; Phase 1 study in metastatic

NSCLC, colorectal cancer (CRC), melanoma, RCC, or metastatic castrate-resistant prostate cancer [mCRPC]), CA209010 (Phase 2 study in advanced/metastatic clear-cell RCC). Data are also provided from Phase 1 studies CA209004 (also known as MDX1106-04), CA209012, CA209016, CA209038, and CA209039. Nivolumab is being investigated both as monotherapy and in combination with chemotherapy, targeted therapies, and other immunotherapies.

3.1 Clinical Pharmacokinetics

The single-dose pharmacokinetics (PK) of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The multiple-dose PK of nivolumab was linear with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve over the dosing interval (AUC [TAU]) in the range of 0.1 mg/kg to 10 mg/kg. Both elimination and distribution of nivolumab in the dose range studied appear to be independent of dose in the dose-ranging studies, while the end of infusion and minimum serum concentration (Cmin) after the first dose were approximately dose proportional. Based on population PK (PPK) results (preliminary data), clearance of nivolumab is independent of dose in the dose range (0.1 mg/kg to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights and, therefore, is appropriate for future clinical trials with nivolumab.

3.2 Clinical Efficacy

Nivolumab has demonstrated clinical activity as monotherapy and as combination therapy with ipilimumab in several tumor types, including RCC, melanoma, NSCLC, and some lymphomas. The majority of responses were durable and exceeded 6 months (Please Refer to Section 5.4 of Nivolumab IB).

3.3 Clinical Safety

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 4,000 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. The only exception is pulmonary inflammation adverse events (AEs), which may be numerically greater in subjects with NSCLC, because in some cases, it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. In several ongoing clinical trials, the safety of nivolumab in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies is being explored. Most studies are ongoing and, as such, the safety profile of nivolumab combinations continues to evolve. The most advanced combination under development is nivolumab + ipilimumab in subjects with melanoma. Thus far, the combination of both agents results in a safety profile with similar types of AEs as either agent alone, but in some cases with a greater frequency.

For a complete review of clinical information, please refer to the nivolumab Investigator Brochure (Appendix F).

3.4 Rationale for Proposed Dose

The 3 mg/kg dose is being carried forward in all hematologic malignancy trials. The rationale is based upon PD results in patients with solid tumors, including in ongoing Phase 1 study

CA209003 in NSCLC. Anti-tumor activity was observed in NSCLC subjects at dose levels of 1, 3 and 10 mg/kg every 2 weeks. Anti-tumor activity appeared to approach a plateau at dose levels of 3 mg/kg and above. Consistent with these observations, the results of exposure response analyses showed that the probability of a tumor response tended to approach a plateau for trough concentrations produced by 3 and 10 mg/kg administered every 2 weeks. Nivolumab was adequately tolerated up to 10 mg/kg, the highest dose level tested, and no maximum tolerated dose (MTD) was identified. While the spectrum, frequency, and severity of -related AEs were generally similar across the dose levels tested, the 10 mg/kg dose level had numerically higher rates of Grade 3/4 drug-related SAEs and AEs leading to discontinuation. Based on these observations, a dose of 3 mg/kg every 2 weeks was chosen for further study.

Specific clinical findings among the 306 subjects with melanoma treated in MDX1106-03 study that contributed to the selection of the Phase 2 dose were as follows:

- Receptor occupancy was maintained at high levels across the dose range 0.1 to 10 mg/kg and over multiple cycles of drug administration.
- In exposure-response analysis, the probability of response in melanoma, lung, and RCC subjects increased over the range of steady-state trough concentrations observed, but appeared to plateau at the ≥3 mg/kg Q2W dose.
- There was a greater percent of objective responses observed in NSCLC subjects treated with 3 mg/kg (24.3%) and 10 mg/kg (20.3%) nivolumab than with 1 mg/kg (3%) nivolumab.
- The nature, frequency, and severity of adverse events (AEs) were similar across the dose range 0.1 to 10 mg/kg and across tumor types.

Integrated assessment of data from studies MDX1106-03 and CA209066 indicate that 3 mg/kg Q2W nivolumab offers an acceptable benefit-risk in this population, based on the consistency of ORR, DOR, other efficacy endpoints, and the safety profile.

PRODUCT INFORMATION TABLE:
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	10-cc 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

The dosing calculations should be based on the actual body weight. 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. Please see Appendix 1 "sample of drug ordering and pharmacy reference material" for more details. Unused/expired study drug will be destroyed per institutional policy.

4.0 PATIENT ELIGIBILITY CRITERIA

Inclusion criteria

1. Diagnosis of MF (either primary or post essential thrombocythemia/polycythemia vera) requiring therapy, including those previously treated and relapsed or refractory, or if newly diagnosed, with intermediate-1 or -2 or high risk according to International Prognostic Scoring System (IPSS).
2. Previously treated with ruxolitinib (unless not a good candidate for ruxolitinib therapy in the judgment of treating physician)^
3. Palpable splenomegaly or hepatomegaly of more than or equal to 5 cm below left or right, respectively, costal margin on physical exam
4. Understanding and voluntary signing an IRB-approved informed consent form.
5. No prior history of immune check point modulator therapy
6. Age ≥ 18 years at the time of signing the informed consent.
7. Disease-free of other malignancies.
8. ECOG performance status 0 to 2.
9. Negative pregnancy test in females of childbearing potential (FCBP)† Male patients with female partners of child-bearing potential and female patients of childbearing potential are required to use two forms of acceptable contraception, including one barrier method, during their participation in the study and for 23 weeks (for females) or 31 weeks (for males) following the last dose of study medication. Acceptable forms of contraception include 1 highly effective method such as an intrauterine device (IUD), hormonal (birth control pills, injections, or implants), tubal ligation, or partner's vasectomy and at least 1 additional approved barrier method such as a latex condom, diaphragm, or cervical cap plus spermicide. Female patients of childbearing potential must not be breast-feeding or planning to breast feed and must have a negative pregnancy test within 24 hours before first study treatment.
10. Adequate organ function as demonstrated by the following:
    - Direct bilirubin ≤ 1.5 x upper limit of normal (ULN)
    - Serum creatinine ≤ 1.5 x ULN
    - AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN (unless considered to be related to MF or patient has known history of Gilberts, in which case it must be ≤5 x ULN)

^ Must be documented in medical records (e.g. patient with grade 3 or 4 thrombocytopenia, or severe PRBC transfusion dependency)

† A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Exclusion Criteria

1. Use of any other standard or experimental therapy for MF within 14 days of starting study therapy.
2. Lack of recovery from all toxicity from previous therapy to grade 1 or baseline.
3. Any concurrent severe and/or uncontrolled medical conditions that could increase the patient's risk for toxicity while in the study or that could confound discrimination between disease- and study treatment-related toxicities.
4. Documented history of a cerebral vascular event (stroke or transient ischemic attack), unstable angina, myocardial infarction, or cardiac symptoms consistent with New York Heart Association Class III-IV within 6 months prior to their first dose of the study drugs
5. Patients who are currently receiving chronic (>14 days) treatment with corticosteroids at a dose ≥ 10 mg of prednisone (or its glucocorticoid equivalent) per day, or any other chronic immunosuppressive treatment that cannot be discontinued prior to starting study drug
6. Patients with autoimmune diseases are excluded: Patients with a history of Inflammatory Bowel Disease (including Crohn's disease and ulcerative colitis) are excluded from this study as are patients with a history of autoimmune disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], Systemic Lupus Erythematosus, autoimmune vasculitis [e.g., Wegener's Granulomatosis]).
7. Pregnant or breast feeding (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive β-HCG laboratory test.
8. Known positive for HIV or infectious hepatitis, type A, B or C.
9. The use of dietary supplements or herbal medications within 7 days of starting study therapy.

5.0 TREATMENT PLAN

On this study 4 weeks of therapy is considered one cycle of therapy. Nivolumab will be given IV in outpatient setting at 3 mg/kg approximately every 2 weeks (every 14 days +/- 2 days; patients may be dosed no less than 12 days from the previous dose of drug) for 8 doses followed by a maintenance regimen of one dose every 12 weeks. Nivolumab should be administered as an IV infusion over 60 minutes through an intravenous line containing a sterile, nonpyrogenic in-line filter (0.2- to 1.2-micron, low-protein binding polyethersulfone membrane). Patients experiencing clinical benefit will continue therapy for 4 years unless progression of disease or toxicity warranting discontinuation of therapy is observed.

5.1 Dose Modification

Dose reductions or dose escalations are not permitted.

5.2 Dose Delay Criteria

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. Dose delay criteria apply for all drug-related adverse events. Dose delays are also permitted for patient safety (e.g. unrelated medical issues, or life events) at the discretion of the PI.

Nivolumab administration should be delayed for the following:

- Grade 2 pneumonitis
- Grade 2 or 3 colitis
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 and up to 5 times upper limit of normal (ULN) or direct bilirubin greater than 1.5 and up to 3 times ULN
- Creatinine greater than 1.5 and up to 6 times ULN or greater than 1.5 times baseline
- Any other severe or Grade 3 treatment-related adverse reactions

5.3 Criteria to Resume Treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value. Subjects in whom a dose was held for a non-study drug-related AE may also resume treatment as acceptable and as indicated by the patient's condition and at the discretion of the treating physician.

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 > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

5.4 Management Algorithms

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. 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 Appendix G of this protocol. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

5.5 Discontinuation Criteria

Treatment should be permanently discontinued for the following study drug related events:

- Any life-threatening or Grade 4 adverse reaction
- Grade 3 or 4 pneumonitis
- Grade 4 colitis
- AST or ALT greater than 5 times ULN or direct bilirubin greater than 3 times ULN
- Creatinine greater than 6 times ULN
- Any severe or Grade 3 treatment-related adverse reaction that recurs
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 treatment-related adverse reactions that do not recover to Grade 1 or resolve within 12 weeks after last dose of therapy
- Any dosing interruption lasting > 6 weeks with the following exceptions:
    - Dosing interruptions > 6 weeks but not exceeding 12 weeks 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 Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
    - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 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 which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

5.6 Treatment of Nivolumab Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (v4.03) 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 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor 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).

5.3 Concomitant Therapy

Supportive measures consistent with optimal patient care may be given throughout the study. Concomitant medications will be captured in the medical record and will not be recorded on the case report form.

- Therapies considered necessary for the patient's well-being may be administered at the discretion of the investigator. These therapies include, but are not limited to antibiotics, analgesics, antihistamines, or other medications. Guidance for growth factors and transfusions of red blood cells, platelets, or fresh frozen plasma are described below:
- Packed red blood cells (RBCs): Transfusions of 2 units of packed RBCs should be considered for a decline in hemoglobin to <8 g/dl or symptoms of cardiovascular compromise. The following transfusion thresholds and guidelines are recommended for study participants:
    - hemoglobin <8 g/dl, 2 units of packed RBCs; hemoglobin <7, three units of packed RBCs

- Platelets: Six to 10 units of random donor platelets or one cytopheresis unit of single donor platelets should be administered to all subjects with signs of hemostatic failure (i.e., bleeding or petechiae) or life-threatening thrombocytopenia (i.e., platelet count < 10,000/μ L)
- Growth factor use with epoetin, darbepoetin, or granulocyte colony-stimulating factor must be terminated at least 14 days before initiation of study treatment and may not be used as part of supportive therapy while the patient is on study, except if in patients best interest after approval of PI (e.g., neutropenic fever)
- Patients should not receive androgens or steroids for treatment of their anemia or thrombocytopenia as part of supportive therapy
    - Patients may receive hydrocortisone prophylactically to prevent transfusion reactions but chronic systemic steroid (≥10 mg/day prednisone) are not allowed.

- Over-the-counter herbal preparations or dietary supplements, are not allowed throughout the trial. Patients should stop using these herbal medications at least 7 days prior to the first dose of study medication.

5.4 Correlative studies

Tumor tissue, blood samples and bone marrow aspirate will be collected on a separate IRB approved laboratory protocol (PA13-0291) for immune monitoring as previously published, under the supervision of the Immunotherapy Platform. Participation in this protocol is optional. In bone marrow biopsies immunohistochemical studies will be performed to evaluate disease and immunological cell markers such as CD4 and CD8 T cells. In peripheral blood, we will also evaluate malignant and immune cell populations including but not limited to CD4 and CD8 T cells in pre and post therapy samples.

Peripheral blood will be collected under an IRB-approved laboratory protocol for testing of biomarkers described in this clinical protocol at the following time points:
• At screening: 20cc
• Prior to each dose of study drug 20cc
• At progression/discontinuation of protocol participation 20cc

5.5 Pretreatment Evaluation

- History and physical exam including vital signs, body weight and height, and spleen and liver measurements. This also includes a transfusion history for 3 months prior to day 1 and concomitant medication notation (within 7 days of study day 1)
- CBC and differential, electrolytes (Na, K, Cl, HCO3), creatinine, uric acid, BUN, glucose, total and direct bilirubin, and amylase, lipase, SGPT and SGOT, PT/PTT, INR (within 7 days of study day 1).
- Bone marrow biopsy and aspirate (within 1 month of study day 1, or within 3 months of study day 1 if patient was not on any therapy).
- Pregnancy test (for FCBP only) within 24 hours of study day 1
- Myeloproliferative Neoplasm - Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) questionnaire (within 7 days of study day 1).

5.6 Evaluation During Study

- Directed history and physical examination including vital signs, body weight, and spleen and liver measurements on Days 1, 7, and 14 of Cycle 1, then on Day 1 and Day 14 (+/- 2 days) of Cycles 2 – 4, then on Day 1 of Cycle 5, and then on Day 1 (+/- 7 days) of every 3rd cycle after that (Cycles 8, 11, 14 and so on). This also includes a transfusion history and concomitant medication notation.
- CBC and differential, electrolytes (Na, K, Cl, HCO3), creatinine, BUN, glucose, total bilirubin, SGOT and SGPT, amylase and lipase on Days 1, 7, and 14 of Cycle 1, then on Day 1 and Day 14 of Cycles 2 – 4 (+/- 2 days), then on Day 1 of Cycle 5, and then on Day 1 (+/- 7 days) of every 3rd cycle after that (Cycles 8, 11, 14 and so on).
- Bone marrow aspiration and biopsy after 4 cycles, and if deemed appropriate by the investigator, then every 12 weeks.
- Response assessment after 4 cycles of therapy, then every 12 weeks
- Pregnancy test after 2 and 4 cycles of therapy, then every 12 weeks
- Continuous assessment of AEs
- MPN-SAF TSS questionnaire on Day 1 and Day 14 (+/- 2 days), of cycles 1-4, then on Day 1 of Cycle 5 (+/- 7 days), then Day 1 (+/- 7 days) of every 3rd cycle after that.

Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory and history and physical exam assessments completed outside of schedule, occasional missed required research samples.

The patient will be contacted by the PI and his staff approximately 30, 60 and 100 days after discontinuation of study drug to assess for AE's, unless the patient receives further MF-directed treatment. Pregnancy test (for FCBP only) will be required approximately 30, 60 and 100 days after discontinuation of study drug.

5.7 Criteria for Removal from the Study

Treatment with study drugs is to be discontinued when any of the following occurs:

- Lack of therapeutic effect (any effort should be made to provide therapy to the patient in a safe way for at least 3-6 cycles for proper assessment of potential efficacy)
- Adverse event(s) that, in the judgment of the Investigator, may cause severe or permanent harm or which rule out continuation of study drug.
- Withdrawal of consent
- Lost to follow up
- Death
- Suspected pregnancy

6.0 CRITERIA FOR RESPONSE

Best overall response will be categorized according to the International Working Group Criteria (for all response categories, benefit must last at least for 12 weeks to qualify as a response) :

Complete remission (CR): Requires all of the following in the absence of both transfusion and growth factor support:

- Bone marrow: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF[†] and
- Peripheral blood: Hemoglobin ≥10 g/dL and <UNL; neutrophil count ≥ 1.0 × 10^9/L and <UNL;
- Platelet count ≥100 × 10^9/L and <UNL; <2% immature myeloid cells[‡] and
- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH

Partial remission (PR):

- Peripheral blood: Hemoglobin ≥10 g/dL and <UNL; neutrophil count ≥1 × 10^9/L and <UNL; platelet count ≥100 × 10^9/L and <UNL; <2% immature myeloid cells and

- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH or

- Bone marrow: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF[†], and peripheral blood: Hemoglobin ≥8.5 but <10 g/dL and <UNL; neutrophil count ≥1 × 10^9/L and <UNL; platelet count ≥50, but <100 × 10^9/L and <UNL; <2% immature myeloid cells[‡] and
- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH

Clinical improvement (CI): The achievement of anemia, spleen or symptoms response without progressive disease or increase in severity of anemia, thrombocytopenia, or neutropenia[§]

Anemia response	Transfusion-independent patients: a ≥2.0 g/dL increase in hemoglobin level[‖] Transfusion-dependent patients: becoming transfusion-independent[¶]
Spleen response[#]	A baseline splenomegaly that is palpable at 5-10 cm, below the LCM, becomes not palpable or A baseline splenomegaly that is palpable at >10 cm, below the LCM, decreases by ≥50% A baseline splenomegaly that is palpable at <5 cm, below the LCM, is not eligible for spleen response
Symptoms response	A ≥50% reduction in the MPN-SAF TSS[††]

Progressive disease:

- Appearance of a new splenomegaly that is palpable at least 5 cm below the LCM or
- A ≥100% increase in palpable distance, below LCM, for baseline splenomegaly of 5-10 cm or
- A 50% increase in palpable distance, below LCM, for baseline splenomegaly of >10 cm or

- Leukemic transformation confirmed by a bone marrow blast count of ≥20% or
- A peripheral blood blast content of ≥20% associated with an absolute blast count of ≥1 × 10(9)/L that lasts for at least 2 weeks

Stable disease: Belonging to none of the above listed response categories

Relapse:

- No longer meeting criteria for at least CI after achieving CR, PR, or CI, or
- Loss of anemia response persisting for at least 1 month or
- Loss of spleen response persisting for at least 1 month
- Recommendations for assessing treatment-induced cytogenetic and molecular changes.

Cytogenetic remission	At least 10 metaphases must be analyzed for cytogenetic response evaluation and requires confirmation by repeat testing within 6 months window CR: eradication of a preexisting abnormality PR: ≥50% reduction in abnormal metaphases (partial response applies only to patients with at least ten abnormal metaphases at baseline)
Molecular remission	Molecular response evaluation must be analyzed in peripheral blood granulocytes and requires confirmation by repeat testing within 6 months window CR: Eradication of a pre-existing abnormality PR: ≥50% decrease in allele burden (partial response applies only to patients with at least 20% mutant allele burden at baseline)
Cytogenetic/molecular relapse	Re-emergence of a pre-existing cytogenetic or molecular abnormality that is confirmed by repeat testing

EMH, extramedullary hematopoiesis (no evidence of EMH implies the absence of pathology- or imaging study-proven nonhepatosplenic EMH); LCM, left costal margin; UNL, upper normal limit.

[*]Baseline and post treatment bone marrow slides are to be interpreted at one sitting by a central review process. Cytogenetic and molecular responses are not required for CR assignment.

[†]Grading of MF is according to the European classification: Thiele et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica.

2005;90:1128. It is underscored that the consensus definition of a CR bone marrow is to be used only in those patients in which all other criteria are met, including resolution of leukoerythroblastosis. It should also be noted that it was a particularly difficult task for the working group to reach a consensus regarding what represents a complete histologic remission.

[‡]*Immature myeloid cells constitute blasts + promyelocytes + myelocytes + metamyelocytes + nucleated red blood cells. In splenectomized patients, <5% immature myeloid cells is allowed.*

[§]*See above for definitions of anemia response, spleen response, and progressive disease. Increase in severity of anemia constitutes the occurrence of new transfusion dependency or a ≥20 g/L decrease in hemoglobin level from pretreatment baseline that lasts for at least 12 weeks. Increase in severity of thrombocytopenia or neutropenia is defined as a 2-grade decline, from pretreatment baseline, in platelet count or absolute neutrophil count, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. In addition, assignment to CI requires a minimum platelet count of ≥25 000 × 10(9)/L and absolute neutrophil count of ≥0.5 × 10(9)/L.*

[||]*Applicable only to patients with baseline hemoglobin of <100 g/L. In patients not meeting the strict criteria for transfusion dependency at the time of study enrollment (see as follows), but have received transfusions within the previous month, the pretransfusion hemoglobin level should be used as the baseline.*

[¶]*Transfusion dependency before study enrollment is defined as transfusions of at least 6 units of packed red blood cells (PRBC), in the 12 weeks prior to study enrollment, for a hemoglobin level of <85 g/L, in the absence of bleeding or treatment-induced anemia. In addition, the most recent transfusion episode must have occurred in the 28 days prior to study enrollment. Response in transfusion-dependent patients requires absence of any PRBC transfusions during any consecutive "rolling" 12-week interval during the treatment phase, capped by a hemoglobin level of ≥85 g/L.*

[#]*In splenectomized patients, palpable hepatomegaly is substituted with the same measurement strategy.*

[††]*Symptoms are evaluated by the MPN-SAF TSS. The MPN-SAF TSS is assessed by the patients themselves and this includes fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pain, abdominal discomfort, weight loss, and fevers. Scoring is from 0 (absent/as good as it can be) to 10 (worst imaginable/as bad as it can be) for each item. The MPN-SAF TSS is the summation of all the individual scores (0-100 scale). Symptoms response requires ≥50% reduction in the MPN-SAF TSS.*

[‡‡] *Baseline values for physical examination refer to pretreatment baseline and not to post treatment measurements.*

7.0 REGULATORY AND REPORTING REQUIREMENTS

7.1 Adverse Event Reporting for MD Anderson-Sponsored IND Protocols

All adverse events that are possible, probably or definitely related to the study treatment will be captured on the case report form. Any grade 3 or higher adverse event that is considered unrelated or unlikely related to the study treatment will also be recorded. Adverse events will be defined using NCI CTCAE version 4.03.

All adverse events occurring between the time the subject is consented and begins study drug will be considered a baseline event and recorded. Baseline adverse events will be recorded in the Clinical Oncology Research System (CORE). All baseline adverse events that increase in severity during the course of treatment will be captured as an adverse event in CORE.

7.2 Serious Adverse Event Reporting (SAE) for M. D. Anderson-Sponsored IND Protocols

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Any serious adverse event that occurs during the study period (up through 100 days after the last day drug is administered) will be followed until clinical recovery is complete and laboratory tests have returned to normal, until progression of the event has been stabilized, or until the PI determines there has been acceptable resolution of the event. The PI or designee will be responsible for assigning attribution for all SAEs.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32). Pregnancy, overdose and secondary malignancy will be handled as SAE.

- Important medical events as defined above may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures as per "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IRB, regardless of attribution (within 5 working days of knowledge of the event).
- All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 100 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 100 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA: Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32. It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Reporting by investigator to BMS: The investigator will inform BMS of any SAE within 24 hours of being aware of the event via email and/or fax. **All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:**

SAE Email Address: Worldwide.Safety@BMS.com
SAE Fax Number: 609-818-3804

The following SAEs are not subject to expedited reporting, but will be included in the annual report via the SAE log: Infection or cytopenias leading to hospitalization or prolongation of hospitalization; disease progression leading to death; life-threating AE, hospitalization or prolongation of hospitalization, or disability.

Adverse events will be documented in the medical record and entered into the case report form. PDMS/CORe will be used as the electronic case report form for this protocol. Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial. This form must be completed and supplied to BMS within 24 hours/1 business day at the latest on

the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up MDACC SAE reporting form.

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject or the female partner of a male subject occurring while the subject is on study drug, or within 30 days of the subject's last dose of study drug, are considered immediately reportable events. Study drug is to be discontinued immediately in a female patient and the patient instructed to return any unused portion of the study drug to the investigator(s). The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to BMS immediately by phone and facsimile using the MDACC AE report form. The female should be referred to an obstetrician-gynecologist experienced in reproductive toxicity for further evaluation and counseling.

8.0 DATA HANDLING

PDMS will be used as the database for this study. All adverse events will be reported in CORE.

9.0 STATISTICAL CONSIDERATIONS

This is a single-center phase II trial to determine the efficacy of nivolumab as a monotherapy in subjects with Primary, Post Polycythemia Vera, or Post Essential Thrombocythemia Myelofibrosis (PMF, post-PV MF, or post-ET MF). The primary endpoint is the objective response rate (ORR) after 8 doses of therapy (4 cycles), defined as CR (complete remission) + PR (partial remission) + CI (clinical improvement). Responses will be categorized according to the revised International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus criteria for myelofibrosis.

An optimum two-stage design proposed by Simon will be implemented. We assume a target ORR of 35%, and a response rate of 18% or lower will be considered not desirable. With a type I error rate of 7% and 80% power, we will enroll 16 patients in the first stage. If 3 or fewer patients achieve a response the trial will be stopped. If 4 or more out of the first 16 patients have a response, accrual will continue until a total of 40 patients have been enrolled. After the first 16 patients have enrolled, the study will not enroll new patient until enough responses (at least 4 responses out of 16 patients) are observed to warrant the continuation of the study. At the end of the study, if 11 or more out of these 40 patients achieve a response, the treatment of nivolumab will be considered efficacious and worth further investigation. Under this Simon's Optimum two-stage design, the probability of early termination is 68% if the true ORR is 18% and the expected sample size is 23.7 patients. Patients will be enrolled from MDACC and patients will be registered through the Clinical Oncology Research System (CORE). The estimated accrual rate is 1-2 patients per month. An interim analysis (response summary) will be submitted to the IND Monitor following the treatment of 16 and 40 patients.

The method of Thall, Simon and Estey will be used for toxicity monitoring for this study. Toxicity will be monitored among the 40 patients. Denote the probability of toxicity by p(T),

where toxicity is defined as Grade 3-4 clinically relevant non-hematologic toxicity or a serious adverse event that is at least possibly drug related (Common Terminology Criteria for Adverse Events CTCAE version 4.03) and occurs anytime during the treatment. We assume as a priori, p(T) ~ beta (0.6, 1.4). We will stop treating patients if Pr(p(T)> 0.30 | data) >0.9. That is, we will stop the trial for new patient enrollment if at any time during the study, we determine that there is more than 90% chance that the toxicity rate is more than 30%. This toxicity stopping rule will be applied by cohort size of 5, starting from the 5th patient. Stopping boundaries corresponding to this stopping rule are listed in table 8.a. The operating characteristics are summarized in Table 8.b. Multc Lean Desktop (version 2.0) was used to generate the toxicity stopping boundaries and the OC table.

Table 8.a Boundary table for toxicity monitoring

# of patients (in cohort of 5, starting from the 5th patient)	Stop the trial if there are this many patients with toxicities:
5	4-5
10	6-10
15	8-15
20	9-20
25	11-25
30	13-30
35	15-35

Table 8.b. Operating characteristics for toxicity monitoring

True toxicity rate	Prob(stop the trial early)	Average sample size
0.1	0.0006	40
0.2	0.021	39
0.3	0.186	36
0.4	0.583	27
0.5	0.905	17

Summary statistics will be provided for continuous variables. Frequency tables will be used to summarize categorical variables. The objective response rate (ORR) will be estimated along with the exact 95% confidence interval. Data from all subjects who receive any study drug will be included in the safety analyses. The severity of the toxicities will be graded according to the NCI CTCAE v4.03 whenever possible. We will follow standard reporting guidelines for adverse

events. Safety data will be summarized by category, severity and frequency. The proportion of patients with AEs will be estimated, along with the Bayesian 95% credible intervals.

Duration of response: Duration of response is defined as the date at which the subject's objective status is first noted to be a CR, PR or CI to the date of progression (no longer meeting criteria for either CR, PR or CI) is documented (if one has occurred). Patients who continue to respond as of the data cut-off date will be censored as of the date of their last assessment documenting continued response.

Time to response: The time to response is defined as the time from study registration to the first date at which the subject's objective status was classified as a response (CR, PR or CI). In subjects who do not achieve a response, time to response will be censored at the subject's last evaluation date.

The distribution for each of these event-time variables (duration of response and time to response) will be estimated by Kaplan-Meier curves.

Anemia: Descriptive statistics will be used to explore improvements in anemia and transfusion dependence. The following outcomes will be summarized: mean changes in hemoglobin at monthly intervals. The proportion of transfusion independent patients not requiring transfusions on study who experience an increase of 2 g/dL in their hemoglobin relative to baseline, the proportion of transfusion dependent patients (defined as requiring a transfusion of 2 units PRBCs monthly for 3 months (12 weeks) prior to starting the trial) who become transfusion independent (not requiring a transfusion of PRBCs over a period of 3 months (12 weeks) while on study), the proportion of transfusion dependent patients who become transfusion independent and have a 1 g/dL increase in hemoglobin relative to baseline, and the proportion of transfusion independent patients requiring a transfusion while on study.

JAK2V617F Allele burden: Change in JAK2V617 allele burden from baseline to each visit where it is measured.

Correlative Studies: Descriptive statistics including plots, mean, median and standard deviations will be used to summarize data. For continuous outcomes, t-test and ANOVA will be used to compare outcome measures across patient characteristics. Dunnett's and Tukey's test that properly adjust for multiplicity in multiple tests will be implemented. Pair-wise comparisons will be performed using pre- and post-therapy samples from each patient. The chi-square (c2) test or Fisher's exact test will be used to test the association between two categorical variables such as disease state and performance status.

9.0 REFERENCES

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APPENDIX 1 SAMPLE OF DRUG ORDERING AND PHARMACY REFERENCE MATERIAL

Nivolumab (BMS-936558) Pharmacy Reference Material

- Nivolumab has a concentration of 10mg/mL and is provided in a 10mL vial. Ten or five vials are provided in a carton.

Initial Orders

- *Following submission and approval of the required regulatory documents, a supply of nivolumab may be ordered ~~from~~ by completing a Drug Request Form provided by BMS for this specific trial. The first request may take place upon screening of the first patient*
- *The initial order should be limited to 20 vials. Allow 5 business days for shipment of drug from BMS receipt of the Drug Request Form. Drug is protocol specific, but not patient specific. All drug product will be shipped by courier in a temperature-controlled container. It is possible that sites may have more than one nivolumab clinical study ongoing at the same time. <u>It is imperative that only drug product designated for this protocol number be used for this study.</u>*
- *<u>Pharmacy supplies not provided by BMS:</u> Empty IV bags/containers, approved diluents, In-line filters and infusion tubing*

Re-Supply

- *Drug re-supply request form should be submitted electronically business days before the expected delivery date. Deliveries will be made Tuesday through Friday.*
- *When assessing need for resupply, institutions should keep in mind the number of vials used per treatment dose, and that shipments may take 14 business days from receipt of request. Drug is not patient-specific. Be sure to check with your pharmacy regarding existing investigational stock to assure optimal use of drug on hand.*

Drug Excursions

- *Drug excursions should be reported immediately to BMS on the form provided with the study-specific drug order form*

Please refer to the most recent version of the Investigator Brochure for additional information.

<u>Storage Conditions & Handling:</u>

- Store at 2-8°C (36-46°F), protect from light, freezing, and shaking.
- If any temperature excursions are encountered during storage, please report these to BMS for assessment via the Temperature Excursion Response Form.

- As with all injectable drugs, care should be taken when handling and preparing nivolumab. Whenever possible, nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique.
- Partially used vials should be disposed at the site following procedures for the disposal of anticancer drugs.

After final drug reconciliation, unused nivolumab vials should be disposed at the site following procedures for the disposal of anticancer drugs. For further information, please either discuss with your BMS CSR&O protocol manager or refer to your site IP Destruction policies and procedures

Use Time/Stability: Please refer to section 3.2.3 of the current Investigator's Brochure. The time of preparation should be noted in the Pharmacy Source documents [accountability logs] or in study files as required for investigator sponsored research [FDA and GCP]

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

Preparation and Administration:

1. Visually inspect the drug product solution for particulate matter and discoloration prior to administration. Discard if solution is cloudy, if there is pronounced discoloration (solution may have a pale-yellow color), or if there is foreign paticulate matter other than a few translucent-to-white, amorphous particles.

   *Note: Mix by **gently** inverting several times. **Do not** shake.*
2. Aseptically withdraw the required volume of nivolumab solution into a syringe, and dispense into an IV. bag. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall. **Do not** enter into each vial more than once. **Do not** administer study drug as an IV push or bolus injection
3. Add the appropriate volume of 0.9% Sodium Chloride Injection solution or 5% Dextrose Injection solution. *It is acceptable to add nivolumab solution from the vials into an appropriate pre-filled bag of diluent.*

   Note: Nivolumab infusion concentration must be at or above the minimum allowable concentration of 1 mg/mL.

   Note: It is not recommended that so-called "channel" or tube systems are used to transport prepared infusions of nivolumab.

   4. Attach the IV bag containing the nivolumab solution to the infusion set and filter. Nivolumab should be administered as an IV infusion over 60 minutes through an intravenous line containing a sterile, nonpyrogenic in-line filter (0.2- to 1.2-micron, low-protein binding polyethersulfone membrane).
5. At the end of the infusion period, flush the line with a sufficient quantity of approved diluents.

Example Dose Calculation [at 3mg/kg]:

Total dose should be calculated as follows (assuming total dose volume of 210 mL, 70 kg pt, dose of 3 mg/kg):

- Subject body weight in kg x 3 mg (for the 3 mg/kg cohort) = total dose (mg)
  70 kg x 3 mg/kg = 210 mg
- Total dose (mg) ÷ 10 mg/mL = Amount of solution to be withdrawn from vials
  210 mg ÷ 10 mg/mL = **21 mL**

Example of Total volume of solution to infuse (mL) for a minimum conc solution. – Volume of 10 mg/mL solution (mL) = Volume of Diluent (mL) to add

210 mL – 21 mL = 189 mL

Please note it is perfectly acceptable to dose Nivolumab at a higher drug concentrations, **as long as the total volume of diluted solution is <u>at or above</u> the minimum allowable concentration of 1 mg/mL, below is the calculation based on the above example. Please double check.**

Total dose in mg ÷ Total volume to infuse in mL = Overall drug concentration, mg/mL
210 mg ÷ 210mL = 1 mg/mL