Title: PD-1 Inhibition in Advanced Myeloproliferative Neoplasms

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PD-1 inhibition in advanced myeloproliferative neoplasms

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1.0 TRIAL SUMMARY

Abbreviated Title	PD-1 inhibition in MPN
Trial Phase	2
Clinical Indication	Myelofibrosis
Trial Type	Interventional therapeutic
Type of control	None
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	Single arm
Number of trial subjects	34 patients
Estimated Accrual Rate	17 patients per year (6 patients per year in each center)
Estimated enrollment period	24 months
Estimated duration of trial	36 months
Duration of Participation	Core study period of 18 weeks
Last Revision Date	05/04/16
BSRF Approval Date	05/05/16

2.0 TRIAL DESIGN

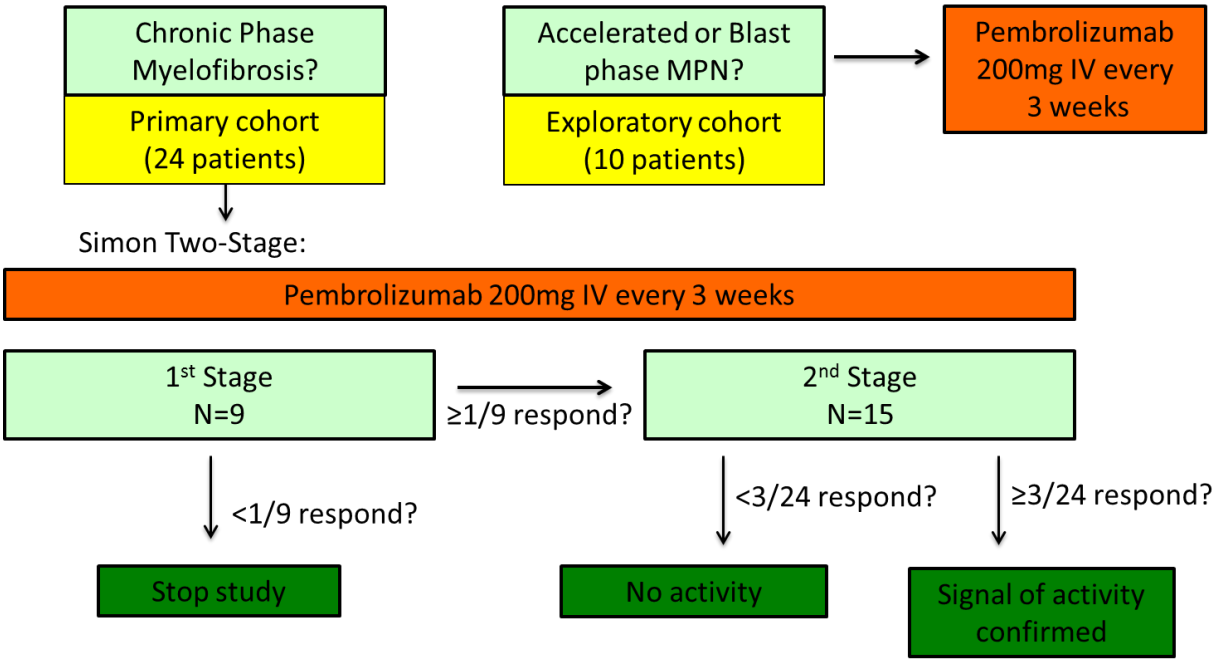
2.1 Trial Design

This is a multicenter, open-label, Phase 2 study to assess the efficacy, safety and tolerability of pembrolizumab in patients with chronic phase myelofibrosis (MF-CP) defined as either patients with primary myelofibrosis (PMF), post essential thrombocythemia (post-ET) or polycythemia vera (post-PV) myelofibrosis (primary cohort). We propose a Simon-two stage design for this study. We will test pembrolizumab at the FDA approved dose (in head and neck cancer) of 200mg dose administered via intravenous infusion over 30 minutes given every 3 weeks. Nine patients will be enrolled in the first stage of the Simon-two stage design, and 15 in the second stage. A treatment cycle is 3 weeks and the core study period is 6 cycles. Response assessment by established consensus criteria will be used to assess response after 6 cycles in order to determine if the trial will progress to the second stage and for the purpose of determining the primary endpoint. In addition, we will allow a maximum of ten patients with accelerated or blast phase disease (MPN-AP/BP) who are refractory or intolerant to conventional therapies such as decitabine, and in which hematopoietic stem cell transplant is not a therapeutic option (exploratory cohort), to enroll in the study as a separate exploratory cohort. These patients can be enrolled during stage 1 or 2 and they will be analyzed separately from the primary cohort population. The study will therefore enroll a maximum of 34 patients.

The study will enroll at 3 centers (Mount Sinai, NY; Montefiore, NY; Massachusetts General Hospital, MA).

Exploratory biomarkers will be obtained from enrolled patients at baseline, cycle 3 and cycle 7 and at 1 year of therapy. Patients that obtain at least a clinical improvement after 6 cycles of therapy can continue receiving pembrolizumab until evidence of disease progression, unacceptable toxicity, and patient or physician decision for a maximum of 2 years.

2.2 Trial Diagram



3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objectives & Hypotheses

1. **Objective:** To assess the efficacy of single agent pembrolizumab in patients with chronic phase myelofibrosis (MF-CP) comprising our primary cohort.

Hypothesis: Deregulated immune checkpoint inhibition allows for progression of myeloproliferative neoplasm cells and serves as a therapeutic target.

3.2 Secondary Objectives & Hypotheses

1. **Objective**: To assess the efficacy of single agent pembrolizumab in patients with accelerated or blast phase myelofibrosis (MPN-AP/BP) comprising our exploratory cohort.

Hypothesis: Deregulated immune checkpoint inhibition allows for evolution of the myeloproliferative neoplasm clone into acute leukemia.

3.3 Exploratory Objectives

1. **Objective:** To assess the expression of PD-1, PD-L1 on MPN cells and supporting stromal cells in the bone marrow and blood of patients being treated with pembrolizumab.

2. **Objective:** to evaluate whether treatment with pembrolizumab affects various immune pathways in the bone marrow of patients with advanced forms of myeloproliferative neoplasms.

3.4 Safety Objectives

1. **Objective**: To assess the safety and feasibility of single agent pembrolizumab in patients with chronic phase myelofibrosis (MF-CP) comprising our primary cohort and in patients with accelerated and or blast phase myelofibrosis (MPN-AP/BP) comprising our exploratory cohort.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses,

including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

The Philadelphia chromosome (Ph) negative myeloproliferative neoplasms (MPNs) are a heterogeneous group of myeloid malignancies involving the hematopoietic stem/progenitor cell and resulting in a hyper-cellular bone marrow with a tendency to develop reticulin/collagen fibrosis, increased risk of thrombotic and hemorrhagic complications, organomegaly secondary to extramedullary hematopoiesis, and evolution to acute leukemia. Myelofibrosis (MF) is the most concerning of the Ph-negative MPNs due to the significant symptom burden, consequences of bone marrow failure, rate of leukemic transformation, and resultant shortened median overall survival of 5-6 years. Hematopoietic stem cell transplantation is the only therapeutic option that offers the potential for cure but is limited in application by advanced patient age and competing comorbid conditions, lack of appropriate donor options, and often patient willingness. Treatment for MF is most often based on risk stratification by various systems including the Dynamic International Prognostic Scoring System (DIPSS) which allows a physician to a determine a treatment plan based on the specific clinical issues at hand (e.g. anemia, constitutional symptoms, symptomatic splenomegaly) considering the competing risks of treatment risk and disease risk [1]. Ruxolitinib (Jakafi, Incyte) a selective JAK1/2 oral tyrosine kinase inhibitor is the only FDA-approved therapy for intermediate/high risk MF patients with a relatively safe toxicity profile and proven efficacy in reducing splenomegaly and improving symptoms [2,3]. However, due to dose limiting thrombocytopenia, inability to alleviate anemia, loss of initial response, and failure to halt progression to acute leukemia, other therapies are desperately needed. A growing appreciation of the complex genetic (JAK2V617F, MPL, CALR, TET2, ASXL1, EZH2, IDH1/2) and epigenetic alterations [(p14/p16 hypermethylation, overexpression of histone deacetylase (HDAC)] contribute to the pathogenesis of the chronic phase (CP) of MF as well as the evolution to accelerated (AP) and blast phase (BP) [4]. Current experimental approaches include signaling pathway inhibition (JAK-STAT), histone deacetylase inhibition, DNMT1 inhibition, and telomerase inhibition and are moving ultimately towards combination therapy[5].

The role of deregulated immune function in the pathogenesis and maintenance of the malignant MPN clone has more recently been demonstrated. Elevation of circulating inflammatory cytokines (e.g. IL-6, TNFa) have been implicated in mediating MF symptoms and ruxolitinib treatment mostly through JAK1 inhibition leads to reduction of these levels and amelioration of constitutional symptoms [6]. Increased TGFb expression has also been linked to production of pathologic bone marrow fibrosis and promotion of the MF clone [7]. Recent reports in a related myeloid malignancy, myelodysplastic syndrome (MDS), show a potential role of programmed death 1 (PD-1) signaling (expression of PD-1 in bone marrow stromal cells and PD-L1 in MDS CD34+ cells) and gene expression of PD-1/PD-L1/CTLA4 was noted to be up-regulated in the setting of azacitdine therapy [8]. In fact, PD-L1 expression was more pronounced in patients with loss of response to this particular therapy providing a possible mechanism for resistance. Additionally, PD-L1 expression has been documented on myeloid cell lines, expression

appears to correlate with disease progression to acute leukemia from antecedent MDS, as well as provide prognostic significance in de novo AML [9-11].

The loss of anti-tumor immunity mediated by PD-1/PD-L1 signaling through aberrant expression of PD-L1 by the malignant cells has been well documented in solid tumors and serves as a novel and potent therapeutic target in many malignant disease types such as melanoma, lung cancer, lymphoma and others. The role of PD-1/PD-L1 pathway in myeloid derived malignancies remains poorly understood, but may offer an innovative approach at targeting the MPN hematopoietic stem cell and disrupting supporting signals from the stromal niche. Ongoing pre-clinical studies assessing the expression and function of PD-1 pathway in MPN primary cells is ongoing as a collaboration between the PI of this trial and Merck. These laboratory studies will provide insight into the role of PD-1 in escape of immune suppression of the malignant clone as well as progression and evolution of the malignant clone and the role the supporting bone marrow and splenic stroma may provide. However, it remains unclear at present if expression of PD-1 or PD-L1 by MPN cells or supporting stromal cells will predict response to pembrolizumab. Correlative studies within this trial will measure levels of expression of PD-1 and PD-L1 at baseline, after treatment with pembrolizumab at time or response assessment or at time of treatment discontinuation for any reason.

Currently, ruxolitnib is the only FDA-approved therapy for the treatment of MF and patients that have either primary or secondary resistance or intolerance to this agent have been shown to have a particularly poor outcome. No established standard of care exists for these patients. Additionally, patients with MPN-AP/BP have a dismal prognosis and there is no standard of care therapy for these patients. Outside of hematopoietic stem cell transplantation (HSCT), which is only a viable therapeutic option for a fraction of these patients, no other therapies have demonstrated clear disease course modification. Therefore, effective treatment options for these patients with advanced MPN are urgently needed. This is the subset of MPN patients that will be included in this phase II trial of pembrolizumab with a primary objective of disease response assessment after 6 cycles.

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, determined the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

4.2.3 Study Design and Statistical Methods:

This is an open-label phase II trial to assess the efficacy, safety and tolerability of pembrolizumab in patients with PMF, post-PV MF, and post-ET MF. We propose a Simon

Two-Stage design for this study; the first 9 MF patients will be enrolled to confirm a signal of activity and safety of pembrolizumab at the FDA approved dose (in head and neck cancer) of 200mg administered via intravenous infusion over 30 minutes given every 3 weeks for six cycles.

In stage 1, if at least one response (out of 9 subjects in the primary cohort) is observed after six cycles of treatment at week 18, 15 additional patients will be accrued in the second stage (total of 24 evaluable patients in the primary cohort). If however, fewer than 3 of 24 patients respond to therapy, pembrolizumab will be deemed not worthy of any further investigation in this patient population. If response is seen in greater than or equal to 3 of 24 patients, pembrolizumab will be considered to have activity in this patient population and will be recommended for further study. For this study we define inactivity as a response rate of 5%, and activity as a response rate of 25%, which is comparable to that seen with ruxolitinib in a mixed population. The probability of erroneously concluding that the drug has activity if the true but unknown response rate is 5% is 0.10. The power to detect a response rate of 25% with this design is 0.90.

In addition, a separate exploratory cohort of 10 patients with MPN in accelerated/blast phase diseases that are refractory or intolerant to conventional therapies will be eligible for the study as well.

See Section 11.3 for Safety Stopping Rules.

4.2.4 Rationale for Endpoints

4.2.4.1 Efficacy Endpoint

Primary endpoint:

1. The proportion of treated MF-CP patients (primary cohort) that achieve at least a clinical improvement (CI, PR, CR) by combined European Leukemia Net – International Working Group (ELN-IWG) criteria (Table 5) after 6 cycles of pembrolizumab therapy.

Secondary endpoint:

1. The proportion of treated MPN-AP/BP patients (exploratory cohort) that achieve at least a complete morphologic remission of the leukemic blasts (CR, Cri) by Acute Myeloid Leukemia Response Criteria (Table 6A) within 6 cycles of pembrolizumab therapy.
2. The proportion of treated MPN-AP/BP patients (exploratory cohort) that achieve at least an acute leukemia response-partial (ALR-P, ALR-C, CCR, CMR) using published consensus criteria by Mascarenhas et al. (Table 6B) within 6 cycles of pembrolizumab therapy.

4.2.4.2 Exploratory Endpoints:

1. Assess expression of PD-1/PD-L1 on peripheral blood PB-MNCs, T-cells and CD34+ cells by flow cytometry
2. Assess expression of PD-1/PD-L1 on bone marrow CD34+cells and supporting stromal cells by immunohistochemical staining
3. Assess PD-1/PD-L1 DNA methylation status by bisulfite-sequencing assay
4. Assess status and change in PD-1/PD-L1 expression with response to therapy.
5. Assess changes in driver mutation burden (*JAK2V617F, MPLW515L/K, CALR*) in granulocytes from treated patients at diagnosis, after 6 cycles and at 1 year of pembrolizumab therapy using the rapid heme panel for next generation sequencing at Brigham and Women's hospital and correlate with response to therapy.
6. Evaluate changes in immune response gene expression profile as well as tumor microenvironment in bone marrow biopsy specimens using nanostring technology after 6 cycles of treatment with pembrolizumab.

4.2.4.3 Safety Endpoints

1. Assess treatment emergent CTCAE v4.0 hematological and non-hematological adverse events
2. Clinical Laboratory test values including hematology, biochemistry and urinanlysis
3. Vital Signs

4.2.4.4 Biomarker Research

Immunotherapy as a target in MPN has not been extensively studied. As such, an important component of this study will be to gain better understanding of markers that predict outcome and response to PD-1 therapy.

1. Biomarker: Assess expression of PD-1/PD-L1 on peripheral blood PB-MNCs, T-cells and CD34+cells by flow cytometry:

Up-regulated PD-1 expression on peripheral blood MNCs and PD-L1 on MPN CD34+cells provides an inappropriate immune tolerance signal allowing for disease progression. Pre-clinical data suggests that PD-1 and PD-L1 are upregulated in patients with MDS and AML, especially in those who have received prior treatment. As part of this clinical trial we will test PD-1 and PD-L1 expression in circulating T-cells and tumor cells, respectively, to evaluate if expression of these molecules correlates with disease status and response to treatment.

Expected result: PD-1 expression is up-regulated on PB MNC and T-cells and PD-L1 is up-regulated on MPN HSC from patients enrolled on this trial at baseline.

Assay: Expression of PD-1 and PD-L1 will be measured by flow cytometry on cells from the peripheral blood of treated patients at baseline and at the start of cycle 3 (9 weeks).

2. Biomarker: Assess expression of PD-1/PD-L1 on bone marrow CD34+cells and supporting stromal cells by immunohistochemical staining

Up-regulated PD-1 expression on peripheral blood MNCs and PD-L1 on MPN CD34+cells provides an inappropriate immune tolerance signal allowing for disease progression. Pre-clinical data suggests that PD-1 and PD-L1 are upregulated in patients with MDS and AML, especially in those who have received prior treatment. As part of this clinical trial we will test PD-1 and PD-L1 expression in circulating T-cells and tumor cells, respectively, to evaluate if expression of these molecules correlates with disease status and response to treatment. Expression of CD45 surface marker will be evaluated in parallel with PD-1 and/or PD-L1 expression to differentiate between stromal (CD45 positive) and hematopoietic cells (CD45 positive) within the bone marrow.

Expected result: PD-1 expression is up-regulated on PB MNC and T-cells and PD-L1 is up-regulated on MPN HSC from patients enrolled on this trial at baseline.

Assay: Expression of PD-1 and PD-L1 will be measured by immunohistochemistry on bone marrow.

3. Biomarker: Assess PD-1/PD-L1 DNA methylation status by bisulfite-sequencing assay

Changes in gene methylation status correlate with protein expression. Little is known about the significance of methylation status and PD-1/PD-L1 expression in MPN. Differences in methylation status in MDS have been used to characterize different MDS risk groups as a result of epigenetic dysregulation.

Assay: We will measure PD-1/PD-L1 gene methylation status by bisulfite-sequencing assay.

Expected result: Differential methylation will correlate with expression levels of PD-1/PD-L1, which in turn will correlate with disease status and response to PD-1 antibody therapy. These results will be instrumental in determining treatment strategies after hypomethylating agent failure in MPN-AP/BP based on their PD-1 expression status and additionally may suggest a role for hypomethylating agent priming prior to PD-1 inhibition in MF-CP patients.

4. Biomarker: Assess status and change in PD-1/PD-L1 expression with response to therapy.

PD-1/PD-L1 expression has been investigated in a variety of malignancies as biomarkers of response to therapy, little is known about the significance of PD-1 and PD-L1 expression in MPN and if these markers are surrogates for disease response.

Expected result: Changes in PD-1 and PD-L1 expression, as measured in exploratory aims 1-3, will correlate with disease response assessed by conventional response criteria (IWG-ELN).

5. Biomarker: Assess changes in driver mutation burden (*JAK2V617F, MPLW515L/K, CALR*) in granulocytes from treated patients at diagnosis, after 6 cycles and at 1 year of pembrolizumab therapy and correlate with response to therapy.

Changes in mutational status allele burden may correlate with disease response. In addition, it is unknown if patients with different mutations will respond preferentially to PD-1 therapy. We will correlate mutational status as well as change in allele burden with disease status and response to therapy and with expression of PD-1 and PD-L1.

Expected Result: Driver mutation burden will decrease in response to pembrolizumab therapy.

Assay: In order to assess changes in driver mutation burden we will use the rapid heme panel for next generation sequencing at Brigham and Women's hospital.

6. Biomarker: **Evaluate changes in Immune response gene expression profile as well as tumor microenvironment in bone marrow biopsy specimens using nanostring technology after 6 cycles of treatment with pembrolizumab**

Immune dysregulation leads to upregulation of PD-L1 on tumor cells and contributes to tumor initiation and disease progression. These changes may also occur in the tumor microenvironment leading to inactivation of tumor infiltrating lymphocytes. Little is known about the changes in genes related to immune regulation in myeloproliferative neoplasms and how these change with treatment. To this end, we will correlate changes in immune gene expression before and after PD-1 antibody therapy.

We will applyenzyme-free nanostring technology, using the The nCounter® PanCancer Immune Profiling Panel system, for identification of immune cells and assesing immunological function in the local microienvironement of the bone marrow samples from patients with MPNs. We will use archival formalin-fixed, paraffin embedded (FFPE) samples. The nCounter® system uses enzyme-free nanostring technology and provides an excellent platform, to guide identification of microenvironment cell types and inform pathway activation patterns in these samples**.**

The nCounter Analysis System utilizes a novel digital technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (<1 copy per cell). The technology uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction. Each color-coded barcode is attached to a single target-specific probe corresponding to a gene or a fusion gene of interest. Mixed together with controls, they form a multiplexed CodeSet

Expected result: PD-1 blockade leads to changes in immune gene profile expression that promotes tumor tolerance and this is reversed with anti-PD-1 therapy

Assay: 500ng of RNA (at 100ng/ul) will be isolated from PMF bone marrow specimens for digital expression profilling that will be performed according to the standard nCounter Gene Expression Protocol as we recently reported (Liew et al 2015). Nanostring tecnilogy will be briefly dscribed in three simple steps as follows: i) *Hybridization:* NanoString's Technology employs two ~50 base probes per mRNA that hybridize in solution. The Reporter Probe carries the signal; the Capture Probe allows the complex to be immobilized for data collection. ii) *Purification and Immobilization:* After hybridization, the excess probes are removed and the probe/target complexes aligned and immobilized in the nCounter Cartridge. iii) *Count:*Sample Cartridges are placed in the Digital Analyzer for data collection. Color codes on the surface of the cartridge are counted and tabulated for each target molecule.

The immune profiling panel that we will apply, is designed to quantitate expression of enriched genes that fall into functional categories that allow identification of immune cell types, and measure gene expression associated biological process such as cytokines, complement and cytotoxicity etc. The panel will include also housekeeping genes that facilitate sample-to-sample normalization. A list of genes found in the PanCancer Immune Profiling Panel that we utilized in our preliminary data acquisition and their annotated functions is documented and available at www.nanostring.com. In addition to functional annotations, the HUGO Gene Nomenclature Committee (www.genenames.org) name and commonly used aliases are described.

The nanostring experiment and data analysis will be performed at ARUP by co-investigator Dr. Salama.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

This trial is targeting patients with advanced forms of myeloproliferative neoplasms (MPNs). This will include patients with myelofibrosis (MF) in chronic (CP), accelerated (AP), and blast phase (BP) disease. Patients with other MPNs such as essential thrombocythemia (ET) or polycythemia vera (PV) will be eligible if they have AP/BP disease. Patients with MF will include those with primary myelofibrosis (PMF) as well as post essential thrombocythemia (post-ET) or polycythemia vera (post-PV) myelofibrosis. MF-CP patients will be required to have survival risk scores associated with advanced disease (intermediate-2/high) by the established Dynamic International Prognostic Scoring System (DIPSS). Patients with MF-CP will have had to have been previously treated with ruxolitinib and either had a suboptimal response or been intolerant as determined by the investigator, or be ineligible for ruxolitinib treatment as determined by the treating investigator. Patients with MPN-AP/BP will have had to have been previously treated with a hypomethylating agent such as decitabine or azacytidine and have progressive or resistant disease as determined by the treating investigator. In all cases the subject must either not be eligible for hematopoietic stem cell transplant option or be unwilling to pursue this potentially curative treatment option.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Must have a diagnosis of chronic phase (CP) (defined as peripheral blood and bone marrow <10% blasts) primary myelofibrosis (PMF) or post essential thrombocythemia (post-ET) or polycythemia vera (post-PV) myelofibrosis by World Health Organization (WHO) criteria **OR** a diagnosis of a myeloproliferative neoplasm in accelerated/blast phase (MPN-AP/BP) defined as either a peripheral blood or bone marrow with ≥10% blasts

4. If the diagnosis is MF-CP, must have Dynamic International Prognostic Scoring System (DIPSS) intermediate-2/high risk disease and either be intolerant/resistant to ruxolitinib as determined by the treating investigator or ineligible for ruxolitinib therapy as determined by the treating investigator (see Appendix 12.3)

5. If the diagnosis is MPN-AP/BP, must have progressive/resistant disease after treatment with a DNMT1 inhibitor therapy (azacytidine, decitabine) as determined by the treating investigator

6. Either not eligible or unwilling to proceed with hematopoietic stem cell transplantation (HSCT)

7. Have a performance status of 0 or 1 on the ECOG Performance Scale.

8. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 28 days of treatment initiation.

Table 1. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥500 /mcL
Platelets	≥25,000 / mcL
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin[b]	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN

AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** **≤** 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a] Creatinine clearance should be calculated per institutional standard. [b] unless due to Gilbert's disease or hemolysis	

9. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

10. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

11. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

2. Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is investigational site or sponsor staff directly involved with this trial, unless prospective IRB approval (by chair or designee) is given allowing exception to this criterion for a specific subject

3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

4. Has a known history of active TB (Bacillus Tuberculosis)

5. Hypersensitivity to pembrolizumab or any of its excipients.

6. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

7. Has had prior chemotherapy (except hydroxyurea), targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has known history of, or any evidence of active, non-infectious pneumonitis.

11. Has an active infection requiring systemic therapy.

12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. "Anti-CD137 or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody

(including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways)

16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

18. Has received a live vaccine within 30 days of planned start of study therapy.

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2

Table 2 Trial Treatment

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental

Trial treatment should begin on the day of registration or as close as possible to the date on which treatment is available.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

5.2.1.2 Dose Modification

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3 below. See Section 5.6.1 and Events of Clinical Interest Guidance Document for supportive care guidelines,

including use of corticosteroids. Although leukopenia, anemia, and thrombocytopenia are expected as a natural consequence of the disease being treated and will be present at baseline for the majority of enrolled subjects, grade 4 treatment emergent hematologic toxicity will require drug treatment interruption regardless of attribution and can be re-started once the hematologic toxicity has resolved to baseline or at least grade 3. Treatment emergent grade 3/4 anemia and thrombocytopenia will be managed with red blood cell and platelet transfusional support as pre treating investigators discretion.

Table 3.Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 3 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Hyperthyroidism	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 3 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 3 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 3 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 3 weeks of last dose or inability to reduce

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Related Toxicity[2]			corticosteroid to 10 mg or less of prednisone or equivalent per day within 3 weeks.
	3-4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 3 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

Subjects should be monitored after each infusion for 30 minutes. Vitals should be done pre-infusin and post-infusion, and after the 30 minute monitoring period.

In addition, the investigator or designee should conduct a safety follow-up phone call the day after the first infusion of study drug and weekly during the first cycle. These telephone encounters should be documented in the MD or nursing note.

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

There is no randomization in this single arm trial.

5.4 Stratification

There is no formal stratification in this trial.

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications

or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

- For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid

taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
<u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing

In addition, the investigator or designee should conduct a safety follow-up phone call the day after the first infusion of study drug and weekly during the first cycle. These telephone encounters should be documented in the MD or nursing note.

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

• intrauterine device (IUD)

• vasectomy of a female subject's male partner

• contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

• diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)

• cervical cap with spermicide (nulliparous women only)

• contraceptive sponge (nulliparous women only)

• male condom or female condom (cannot be used together)

• hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, symptom-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation

of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study. The subjects who meet this criteria will be contacted every 30 days for the 120 day period and pregnancy status will be documented at these intervals by the investigator or designee.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Subject did not achieve at least a CI response per Table 5 for the MF-CP cohort or at least PR for the MPN-AP/BP cohort per Table 6A at response assessment.

- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1).

5.8.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment *may* be considered for subjects who have attained a confirmed CR by IWG/ELN criteria for chronic phase MF and by IWG-AML criteria for AP/BP patients that have been treated for at least 6 cycles with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Additional details are provided in Section 7.1.5.5. The investigator or designee will contact patients weekly to monitor for SAEs during the 60 day period following the 30 day adverse event monitoring period. These should be documented in an RN or MD note.

5.9 Subject Replacement Strategy

Patients who do not complete at least one cycle of therapy will be replaced.

5.10 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	Screening	Treatment Cycles[a] Core study period						Treatment cycles[b] Ext period	End of Treatment[h]	End of Study
Treatment Cycle/Title:	Main Study Screening	1	2	3	4	5	6	7+		30 days post discon
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	+/-5	+/-5	+/-5
Informed Consent	X									
Inclusion/Exclusion Criteria	X									
Demographics and Medical History	X									
Prior and Con Medication Review	X	X	X	X	X	X	X	X	X	X
Trial Treatment Administration		X	X	X	X	X	X	X		
Review Adverse Events		X	X	X	X	X	X	X	X	x
Full Physical Examination	X	X	X	X	X	X	X	X	X	X
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	x
ECOG Performance Status	X	X	X	X	X	X	X	X	X	x
Pregnancy Test – Urine or Serum □regn	X									
PT/INR and aPTT	X									
CBC with Differential	X	X	X	X	X	X	X	X	X	X

Trial Period:	Screening	Treatment Cycles[a] Core study period						Treatment cycles[b] Ext period	End of Treatment[h]	End of Study
Treatment Cycle/Title:	Main Study Screening	1	2	3	4	5	6	7+		30 days post discon
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	+/-5	+/-5	+/-5
Comprehensive Serum Chemistry Panel[f]	X	X	X	X	X	X	X	X	X	X
Urinalysis	X									
T3, FT4 and TSH	X			X				X		X
Chest X ray (PA and lateral)	X									
Electrocardiogram-12 lead	X									
Bone marrow (BM) evaluation[c]	X							X		
Flow cytometry [g]	X							X		
Cytogenentics[d,g]	X							X		
Correlative Studies BM Collection[e]	X							X		
Correlative Studies Blood Collection[e]	X			X				X		X

[a] core study period will be 6 cycles of therapy with pembrolizumab for primary response assessment

[b] ext= extension phase will include those that go on to receive additional cycles of therapy after cycle 6, this will include cycle 7 and each subsequent cycle until the patient is discontinued from treatment for reasons outlined in the previous section

[c] bone marrow evaluation consists of bone marrow biopsy and aspiration for pathology review done at screening and at C7D1 (after completion of 6 cycles of therapy). Outside bone marrow may be used if biopsy is done within 3 months prior to screening—peripheral blood should be collected at screening instead for correlative studies.

[d] conventional metaphase cytogenetics to be performed locally at study site

[e] Refer to biomarker table for blood and bone marrow collection in section 7.1.2.6 Table 8

[f] Comprehensive Serum Chemistry Panel should include the following: Albumin, Alkaline phosphatase, Glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH),(CO2 or bicarbonate),Uric Acid, Calcium, Chloride, Glucose, Phosphorus, Potassium, Sodium, Magnesium, Total Bilirubin, Direct Bilirubin (If total bilirubin is elevated above the upper limit of normal), Total protein, Blood Urea Nitrogen

g Flow cytometry for acute leukemia panel and cytogenetics will be sent from the bone marrow aspirate, if this is not obtainable then will be sent from the peripheral blood to be performed locally at study site

h If it is known, prior to C7D1, that the patient will not achieve the required response, CI, for continuation, the C7D1 and End of Treatment visit will be combined. All study activities for C7D1 will be done except dosing of the study medication.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

Not applicable.

7.1.1.6 Assignment of Screening Number

Not applicable.

7.1.1.7 Assignment of Randomization Number

Not applicable.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

Patients will be instructed to comply to study visits and concomitant medications by the research staff and compliance.

7.1.2 Clinical Procedures/Assessments

Response assessment will formally be conducted after 6 cycles of pembrolizumab therapy, This will include assessment of symptoms, hematologic profile, bone marrow histomorphologic abnormalities, spleen length by manual palpation on physical exam, cytogenetics, and changes in mutational burden from baseline measurement. These established consensus criteria (ELN-IWG) shown in TABLE 5 will allow for uniformity in response assessment and are standardized.

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs).

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening, and at each study visit as indicated in the trial flow chart.

7.1.2.2.1 MPN-SAF TSS

The investigator or qualified designee will use the MPN-SAF TSS measure at every study visit to assess constitutional symptoms as part of the interval history assessment.

7.1.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.5 Assessment of Disease Response

Clinical response for patients with MF-CP in the primary cohort will be evaluated using the Revised IWG-MRT and ELN response criteria for Myelofibrosis[12] as follows (patients must achieve at least CI to continue in the extension phase):

TABLE 5. International Working Group for Myelofibrosis Research and Treatment and European leukemiaNet (IWG-ELN) response criteria for myelofibrosis

Response categories	Required criteria (for all response categories, benefit must last for ≥12 weeks in order to qualify as a response)
Complete Remission (CR)	*Bone marrow:** Age-adjusted normocellularity; <5% blasts; ≤Grade 1 myelofibrosis**, AND *Peripheral blood:* Hemoglobin ≥100 g/L and <UNL; Neutrophil count ≥1 x 10^9/L and <UNL; Platelet count ≥100 x 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 ≥100 g/L and <UNL; Neutrophil count ≥1 x 10^9/L and <UNL; Platelet count ≥100 x 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 myelofibrosis**, AND *Peripheral blood:* Hemoglobin ≥85 but <100 g/L and <UNL; Neutrophil count ≥1 x 10^9/L and <UNL; Platelet count ≥50 but <100 x 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 ≥20 g/L 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*

	A spleen response requires confirmation by MRI or CT showing ≥35% spleen volume reduction
Symptoms response	A ≥50% reduction in the Myeloproliferative Neoplasm Symptom Assessment Form total symptom score (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 to 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 x 10(9)/L that lasts for at least two 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 one month, OR Loss of spleen response persisting for at least one month

Response assessment for patients with MPN-BP disease will follow the standard response assessment for AML defined by Cheson et al[13] as shown in Table 6A (patients must achieve at least a PR to continue to extension phase).

Table 6A. Acute Myeloid Leukemia Response Assessment Criteria

Complete Response (CR)
The subject must be free of all symptoms related to leukemia and have an absolute neutrophil count of greater than 1 x 10^9/L, no need for red blood cell transfusion, platelet count greater than 100x 10^9/L, and normal marrow differential (<5% blasts) in a normo- or hypercellular marrow
Cri
As per CR but incomplete count recovery
Partial Response
CR with 6-25% abnormal cells in the marrow or 50% decrease in bone marrow blasts

Additionally, response assessment for patients with accelerated or blast phase disease will be measured by published consensus criteria by Mascarenhas et al. specifically designed for MPN-AP/BP as shown in Table 6B. These criteria will also be utilized as there are no current standardized response criteria for MPN-AP or BP. These criteria are based on hematological, pathological, clinical, cytogenetic and molecular responses. These criteria incorporate established response criteria for AML, MDS and MPN.

TABLE 6B. Formal response assessment categories for MPN-AP/BP

Complete molecular response (CMR)	
Description	Complete remission of both leukemia and MPN without detectable molecular markers associated with either leukemia or MPN
Hematologic profile	ANC > 1000 Hemoglobin > 10 g/dL Platelets > 100×10^9/L Absence of leukoerythroblastosis[a]
Spleen	Non-palpable
Bone marrow	Cellularity appropriate for age Resolution of abnormal morphology Blasts ≤ 5%[b] ≤Grade 1 marrow fibrosis
Cytogenetics	Normal karyotype[c]
Molecular markers	Loss of any previously documented markers associated with either the leukemic or MPN clone[d]
Complete cytogenetic response (CCR)	
Description	Complete remission of both leukemia and MPN with detectable molecular markers associated with either leukemia or MPN
Hematologic profile	ANC > 1000 Hemoglobin > 10 g/dL Platelets > 100×10^9/L Absence of leukoerythroblastosis[a]
Spleen	Non-palpable
Bone marrow	Cellularity appropriate for age Resolution of abnormal morphology Blasts ≤ 5%[b] ≤Grade 1 marrow fibrosis
Cytogenetics	Normal karyotype[c]
Molecular markers	Residual expression of MPN/leukemia associated gene mutations (e.g. JAK2V617F, MPL515L/K)[d]
Acute leukemia response-complete (ALR-C)	
Description	Complete remission of leukemia with residual MPN features
Hematologic profile	Absence of blasts[a]
Spleen	<25% increase in spleen size by palpation or imaging if baseline spleen <10 cm or <50% if baseline spleen ≥10 cm
Bone marrow	Blasts ≤ 5%[b]
Cytogenetics	Loss of cytogenetic abnormality associated with leukemic clone, may have persistent abnormality associated with MPN
Molecular markers	Loss of any previously identified markers in leukemic clone, may have persistent molecular markers associated with MPN[d]

Acute leukemia response-partial (ALR-P)	
Description	Decrease in leukemic burden but without resolution of peripheral blood or bone marrow blasts and residual MPN features
Hematologic profile	>50% reduction in blasts
Spleen	<25% increase in spleen size by palpation or imaging if baseline spleen <10 cm or <50% if baseline spleen ≥10 cm
Bone marrow	>50% reduction in blasts
Cytogenetics	No new abnormalities
Molecular markers	No new abnormalities
Stable disease (SD)	
Description	Failure to achieve at least LR-P, but no evidence of progression for at least 8 weeks
Progressive disease (PD)	
Description	Progression of leukemia and/or background MPN
Hematologic profile	For patients with 10-20% blasts: ≥50% increase to >20% blasts
	For patients with >20% blasts: ≥50% increase to >30% blasts
Spleen	>25% increase in spleen size by palpation or imaging if baseline spleen <10 cm and >50% if baseline spleen ≥10 cm
Bone marrow	For patients with 5–10% blasts: ≥50% increase to >10% blasts For patients with 10–20% blasts: ≥50% increase to >20% blasts For patients with >20% blasts: ≥50% increase to >30% blasts
Cytogenetics	Does not apply
Molecular markers	Does not apply

a. Absence of peripheral blood blasts by morphologic review of the peripheral smear on two occasions separated by at least 2 weeks.
b. Blast percentage can be assessed by morphologic review of aspirate and in cases of inaspirate marrows, immunohistochemical staining of the marrow for CD34+, CD117+ is acceptable.
c. Normal karyotype by conventional cytogenetics in peripheral blood or bone marrow aspirate, if a cytogenetic abnormality is detected prior to treatment it must not be identified at time of assessment; if an abnormality is detected at baseline by FISH it must be absent by FISH at time of assessment.
d. Absence or loss of evidence of mRNA transcript by quantitative PCR assay performed in a validated laboratory, this will also include any exploratory biomarkers determined to be positive prior to therapy

7.1.2.6 Tumor Tissue Collection and Correlative Studies Blood and Bone Marrow Sampling

Both blood and bone marrow tissue will be collected and stored at time points indicated in Table 8 for the purposes of fulfilling the exploratory objectives of this trial. These samples will be collected as the protocol directs and then shipped to the central repository at Mount Sinai Under the supervision of Dr. Camelia Iancu Rubin.

Table 8. Correlative tissue banking of blood and bone marrow samples

Table 1. Tissue Collection ASSAY	Tissue collection	Tissue banking (not HMTB)	Tissue to be analyzed	Time of collection/banking/analysis Baseline (at screening)	Start of Cycle 3	End of Cycle 6	End of Cycle 12/EOS*	Assay employed	Performed at Site/Laboratory
1 Assess expression of PD-1/PD-L1 on peripheral blood PB-MNCs, T-cells and CD34+cells	PB - 1 green top tube	Yes - 1 green top tubes	PB-MNCs	X	X	X	X*	Flow cytometry	ISMMS/Dr. Iancu-Rubin
2 Assess expression of PD-1/PD-L1 on bone marrow CD34+cells and supporting stromal cells	BM aspirate -1 green top tube	Yes - remaining tissue	BM-MNCs	X	—	X	X	Flow cytometry	ISMMS/Dr. Iancu-Rubin
	BM (biopsy)	N/A Recover from Pathology	Paraffin embeded BM biopsy core	X	—	X	X	Immunohistochemistry	ISMMS & Mayo Clinic/ Dr. Salama
3 Assess PD-1/PD-L1 DNA methylation status	PB 1-green top or 1 PaxGene	PB	PB-MNCs	X	X	X	X	Bisulfite-sequencing assay (methylation)	TBD
4 Assess status and change in PD-1/PD-L1 expression with response to therapy.	PB - 1 green top tube	PB - 1 green top tube	PB-MNCs	X	X	X	X*	Flow Cytometry	ISMMS/Iancu-Rubin
	BM (biopsy)	N/A Recover from Pathology	Paraffin embeded BM biopsy core	X	—	X	X	Immunochesmisty	ISMMS & Mayo Clinic/ Dr. Salama
5 Assess changes in driver mutation burden (*JAK2V617F, MPLW515L/K, CALR*) in granulocytes from treated patients at diagnosis, after 6 cycles and at 1 year of pembrolizumab therapy and correlate with response to therapy.	PB - 1 PaxGene DNA tube	PB - 1 PaxGene DNA tube	Whole blood	X	X	X		Heme Panel NSG	MGH/Dr. Borger
6 Evaluate changes in Immune response gene expression profile as well as tumor microenvironment in bone marrow biopsy specimens	BM (biopsy)	N/A Recover from Pathology	Paraffin embeded BM biopsy core	X	—	X	X	Nanostring Technology	Mayo Clinic/Dr. Salama

*At EOS or study discontinuation for any reason, collect peripheral blood for biomarkers (2 green top tubes). Biopsy is not required.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 9.

Table 9. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡ (CO2 or biocarbonate)	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count∓	Creatinine	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
MCV	Uric Acid		Blood for correlative studies
NRBC	Calcium		
	Chloride		
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		

Hematology	Chemistry	Urinalysis	Other
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

∓ This value may be manually calculated.

Laboratory tests for screening or entry into the Core Treatment Phase should be performed within 28 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.3.1 Pharmacodynamic Evaluations

The pharmacodynamic studies performed in this trial are incorporated in the exploratory objectives and listed in TABLE 8. These include assessing PD-1/L1 expression levels in different cellular compartments in the blood and bone marrow as discussed above as well as determining treatment effect on the malignant clone by assessment of changes in driver mutation levels and histomorphologic changes in the bone marrow of treated patients.

7.1.3.1.1 Blood Collection for Serum Pembrolizumab

Sample collection, storage and shipment instructions for serum samples will be provided in the Laboratory Manual.

This will not be assayed in this trial.

7.1.3.1.2 Blood Collection for Anti-Pembrolizumab Antibodies

This will not be assayed in this trial.

7.1.4 Other Procedures

No other intended procedures.

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who do not attain at least CI (MF-CP) or PR (MPN AP/BP). Subjects who attain a CR by IWG-ELN criteria after 6 cycles of therapy with pembrolizumab may discontinue treatment. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit 30 days post last dose. (described in Section 7.1.5.3.1).

7.1.4.2 Blinding/Unblinding

Not applicable.

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening

Subjects who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria have been satisfied and that the subject is eligible for participation in this clinical study. The Study Chairs will confirm eligibility for all subjects prior to receipt of the first dose of pembrolizumab.

7.1.5.1.1 Screening Period

The screening period will begin after signed consent is obtained and will last for 28 days (Day -28 to day -1). During this period Inclusion and exclusion criteria will be reviewed by the PI and this will include the following tests and procedures to be completed: demographics and medical history, prior con med review, physical exam with attention to spleen length in craniocaudal direction measured by a ruler at the mid clavicular line from the costal margin, vitals, ECOG, CBC and comprehensive serum chemistry, PT/PTT, serum or urine HCG, urinalysis, thyroid function tests, Chest X ray (PA and lateral), bone marrow biopsy and aspiration, flow cytometry and cytogenetics (if aspirate is not obtained then will be done from the peripheral blood). Biomarkers from the peripheral blood and aspirate will also be collected as outlined in the biomarker section of this protocol and delineated in the Biomarker table.

7.1.5.2 Treatment Period

The treatment period will consist of 3 week cycles in which the study drug will be given on day 1 of each cycle and the following tests and procedures will be obtained: prior con med review, adverse event review, physical exam with attention to spleen length in craniocaudal direction measured by a ruler at the mid clavicular line from the costal margin, vitals, ECOG, CBC and comprehensive serum chemistry, and thyroid function tests (on C3D1).

The core study period is a total of 6 cycles (study visits and drug administration will take place on day 1 of each cycle and the window is +/- days). Response assessment visit will take place on cycle 7 day 1 which will include the addition of a bone marrow biopsy and aspiration and biomarker evaluation from the aspirate and peripheral blood. The PI will decide if the subject will continue receiving therapy in the extension phase (Cycle 7+) of the trial as long the following is true: at least a clinical improvement by IWG-ELN criteria (Table 5) for chronic phase patients and at least a partial response by Table 6A and 6B (secondary objective) for accelerated and blast phase patients; the absence of unacceptable toxicity; both patient and treating physician are in favor of continued treatment on protocol. Patients can continue receiving study drug in the extension phase as long as they do not meet criteria for discontinuation of treatment defined in Section 5.8 and to a maximum duration of 2 years

7.1.5.3 Post-Treatment Visits

There will be a protocol driven 30 day follow up visit (see section 7.1.5.3.1) after the last dose of pembrolizumab. Visits to manage toxicity or disease related issues during this period will be scheduled as per the treating physician. The 30 day follow up visit (+/- 5 days) will include prior con med review, adverse event review, exam, vitals, ECOG, CBC and comprehensive serum chemistry, and thyroid function tests.

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first.

If it is known, prior to C7D1, that the patient will not achieve the required response, CI, for continuation, the C7D1 and End of Treatment visit will be combined. All study activities for C7D1 will be done except dosing of the study medication. The patient should return 30 days later for the safety follow-up visit.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 10 for additional details regarding each of the above criteria.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 30 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and the Sponsor will report to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 7.1.6 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

> *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 10. Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid 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 or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are	

	intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors
Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		

Record one of the following	Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

Data analyses will be performed after the study is completed and the database is released. All statistical programming and analyses will be performed using SAS Version 9.4. Data from participating centers in this protocol will be combined, so that an adequate number of patients will be available for analysis.

For all analyses, data from patients with MPN-AP-BP (exploratory cohort) will be summarized separately, unless otherwise stated.

8.1.1 Analysis Populations

8.1.1.1 Definition of Populations

Full Analysis

The Full Analysis (FA) population includes all patients who received at least one cycle of pembrolizumab and had at least one valid post-baseline efficacy assessment. The FA population will be used for all listings of raw data. Unless otherwise specified, the FA population will be the default analysis set used for all analyses.

Safety

The safety population includes all patients (primary and exploratory cohorts) who received at least one cycle of pembrolizumab and have at least one valid post-baseline safety assessment.

Per Protocol

The Per-Protocol (PP) population will consist of a subset of patients in the FA population who completed 6 treatment cycles and did not have major protocol violations or deviations. This population will be the secondary population for the analysis of the primary efficacy endpoint.

8.1.1.2 Applicability of Population Efficacy analyses

- The primary efficacy analysis of clinical improvement (CI) or better (partial response (PR) or complete response (CR)) as shown in TABLE 5, will be based on the FA population. A secondary analysis of CI or better (PR or CR) will be performed on the PP population.

- All secondary efficacy analyses will be performed for the FA population.

Safety analyses

All safety analyses will be based on the safety population. In addition, specific assessments of late toxicity will be performed for patients in the safety population who are followed for safety for at least one year from treatment initiation.

Exploratory analyses

Biomarker analyses will be performed in patients for the FA population.

8.2 Statistical Analysis Methods

Description of Data

Data will be presented in patient data listings by patient and according to visit. Continuous variables will be summarized using standard summary statistics such as number of observations (n), mean value, standard deviation (SD), minimum and maximum values, median value, and 1st and 3rd quartiles. Categorical variables will be summarized in frequency tables as counts and percentages. All individual data collected in the CRFs will be presented in data listings. Patients screened but not included in the study (i.e. screen failures) will not be presented in any tables or listings.

8.2.1 Patient demographics and other baseline characteristics

Demographic data and other baseline characteristics (including medical and disease history) will be summarized using descriptive statistics.

8.2.2 Analysis of Efficacy Endpoints

8.2.2.1 8.2.3.1 Primary Endpoint

The primary efficacy endpoint, CI or better (PR,CR) after 6 cycles of treatment as defined by the combined European Leukemia Net –International Working Group (ELN-IWG)) (Table 5) in patients with MF-CP (primary cohort) will be reported as a percentage of the total number of subjects enrolled for both FA and PP populations. A two-sided 90% exact confidence interval for the percentage will be computed using the Clopper and Pearson method.

8.2.2.2 8.2.3.2 Secondary Endpoints

The secondary efficacy endpoint, CRi or better (CR), after 6 cycles of treatment as defined by Acute Myeloid Leukemia Response criteria (Table 6A) in patients with MPN-AP/BP) (exploratory cohort) will be reported as a percentage of the total number of subjects enrolled for the FA population.

The secondary endpoint, ALR-P or better (ALR-C, CCR, CMR), after 6 cycles of treatment as defined using published consensus criteria by Mascarenhas et al. (Table 6B) in patients with MPN-AP/BP (exploratory cohort) will be reported as a percentage of the total number of subjects enrolled for the FA population.

8.2.3 Analysis of Safety Endpoints

All safety summaries will be presented for the safety population. No formal statistical analysis of safety endpoints will be performed. Safety data to be evaluated includes AEs, physical examination and clinical laboratory results.

The overall observation period will be divided into three mutually exclusive segments:

1. Pre-treatment period: from day of patient's informed consent to the day before first dose of study medication.
2. On-treatment period: from day of first dose of study medication to 30 days after last dose of study medication.
3. Post-treatment period: starting at day 31 after last dose of study medication.

If dates are incomplete in a way that clear assignment to pre-, on-, post-treatment period cannot be made, then the respective data will be assigned to the on-treatment period.

8.2.3.1 Adverse Events (AEs)

Summary tables for adverse events (AEs) have to include only AEs that started or worsened during the on-treatment period, the treatment-emergent AEs. However, all safety data (including those from the pre and post-treatment periods) will be listed and those collected during the pre-treatment and post-treatment period are to be flagged.

The incidence of treatment-emergent adverse events (new or worsening from baseline) will be summarized by system organ class and or preferred term, severity (based on CTCAE grades), type of adverse event, relationship to study treatment.

Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by type of adverse event.

Specific safety event categories (SEC) will be considered. Such categories consist of one or more well-defined safety events which are similar in nature and for which there is a specific clinical interest in connection with the study treatment(s).

For each specified SEC, number and percentage of patients with at least one event within the SEC will be reported.

8.2.3.2 Laboratory abnormalities

Descriptive statistics (number of observations, mean, standard deviation, minimum, median

and maximum values) will be presented for clinical laboratory tests (hematology, biochemistry, urinalysis), and their changes from baseline, at applicable visits.

For laboratory data, summary statistics will be presented for each parameter, together with their changes from baseline, by visit. In addition, changes from baseline will be summarized in shift tables according to severity during treatment and baseline grade. Graphical presentations of the mean value (± 2 SD) at each visit will be generated for each laboratory parameter.

8.2.3.3 Other safety data

Data from other tests (e.g., vital signs) will be listed, notable values will be flagged, and any other information collected will be listed as appropriate. Any statistical tests performed to explore the data will be used only to highlight any interesting comparisons that may warrant further consideration. Additionally, the following outputs will be produced:

Vital signs
• Shift table baseline to worst on-treatment result
• Table with descriptive statistics at baseline, one or several post-baseline time points and change from baseline to this/these post-baseline time points.

8.2.4 Analysis of Exploratory Endpoints

8.2.4.1 Biomarker: Assess expression of PD-1/PD-L1 on peripheral blood PB-MNCs, T-cells and CD34+cells by flow cytometry:

To assess expression of PD-1/PD-L1 on peripheral blood PB-MNCs, T-cells and CD34+cells, median fluorescence intensity (MFI) and the percentage of cells expressing each antigen within a gate will be estimated for each sample by flow cytometry. If there are no expression levels equal to 0 among the samples, geometric means of MFIs and percentage of cells will be reported at baseline and at the start of cycle 6. The change in geometric mean MFIs/percentage of cells from baseline to end of cycle 6 will be reported as the ratio of geometric means and tested for difference from one using a paired t-test on log transformed data. If, however, there are expression levels equal to 0, median and interquartile range of MFIs/percentage of cells will be reported at baseline and end of cycle 6 treatment. Difference between median MFIs/percentage of cells from baseline to end of cycle 6 will be tested using a Wilcoxon Signed Rank test.

8.2.4.2 Biomarker: Assess expression of PD-1/PD-L1 on bone marrow CD34+cells and supporting stromal cells by immunohistochemical staining:

To assess expression of PD-1/PD-L1 on bone marrow CD34+ cells and supporting stromal cells by immunohistochemical staining, percentages of PD-L1 and PD-1 positive tumor cells and staining intensity will be evaluated for each sample. Staining intensity will be scored

considering 0 as negative or trace, 1 as weak, 2 as moderate and 3 as high. A semi-quantitative approach will be used to generate an H-score for each tissue core defined as the percentage of stained cells (0–100%) multiplied by the dominant intensity pattern of staining ranging from 0 to 3. The overall semi-quantitative H-score will range from 0 to 300. If there are no H-scores of 0 among the samples, geometric means of H-scores will be reported at baseline and at the end of cycle 6 treatment. The change in geometric mean H-scores from baseline to end of cycle 6 will be reported as the ratio of geometric means and tested for difference from one using a paired t-test on log transformed data. If, however, there are H-scores of 0, median and interquartile range of H-scores will be reported at baseline and following cycle 2 treatment. Difference between median H-scores from baseline to end of cycle 6 will be tested using a Wilcoxon Signed Rank test. In addition to the semi-quantitative H-score approach, a dichotomous variable indicating expression positivity will be created. In the absence of any standardized scoring system, all cases with staining intensity ≥ 2 in more than 5% of tumor cells will be considered as positive, similarly to previous studies (Antonia *et al*, 2013; Grosso *et al*, 2013; Soria *et al*, 2013). McNemar's test will be used to test for a change in the percentage of patients with positive PD-1/PD-L1 expression from baseline to the start of cycle 6 follow-up.

8.2.4.3 Biomarker: Assess PD-1/PD-L1 DNA methylation status by bisulfite-sequencing assay

To assess PD-1/PD0L1 DNA methylation status geometric means of PD-1/PD-L1 methylation levels (expressed as percentage) will be reported at baseline and at the start of cycle 3. The change in geometric mean methylation levels from baseline to start of cycle 6 will be reported as the ratio of geometric means and tested for difference from one using a paired t-test on log transformed data.

8.2.4.4 Biomarker: Assess status and change in PD-1/PD-L1 expression with response to therapy.

To assess status and change in PD-1/PD-L1 expression with response to therapy, the proportion of patients responsive to treatment following their 6th cycle will be computed and compared between PD-1/PD-L1 positive and negative patient groups, with positivity based on 3rd cycle expression levels, using a Fisher's exact test of association.

8.2.4.5 Biomarker: Assess changes in driver mutation burden (*JAK2V617F, MPLW515L/K, CALR*) in granulocytes from treated patients at diagnosis, after 6 cycles and at 1 year of pembrolizumab therapy and correlate with response to therapy.

To assess the correlation between changes in driver mutation burden from diagnosis to 1 year of pembrolizumab therapy and response to therapy, the median JAK2V617F, MPLW515L/K and CALR allele burden (expressed as a percentage) will be computed at baseline, after 6 cycles of treatment and at 1 year, with significant changes from baseline evaluated using the Wilcoxon signed rank test at each of the two follow-ups. Among patients positive for each mutation, a linear mixed-effects model will be fit to log-transformed allele burden data with

patient as the random effect and time points as the fixed effect. The mean changes from baseline in PD-1 and PD-L1 and their standard errors will be derived at each time point from the model.

8.2.4.6 Biomarker: Evaluate changes in Immune response gene expression profile as well as tumor microenvironment in bone marrow biopsy specimens using nanostring technology after 6 cycles of treatment with pembrolizumab.

To evaluate changes in immune response, we will identify and characterize the gene expression signatures of both the global immune status as well as for each of the functional biological processes before therapy as well as at 6 cycles and 1 year post therapy. The regulation of these complex immunological processes involves hundreds of genes, many of which function in multiple biological processes. We anticipate that cases with different molecular make-up or outcome may have different immune response / microenvironment gene expression signature either in the global immune response level or at the level of selective functional biological process such as T-cell functions, cytokines or possible exctracellular matrix constituents, thereby could propose relevant immune response and potential resistance mechanisms to therapy.

8.3 Interim Analysis

In addition to periodic safety monitoring, a formal interim analysis will be performed after 9 patients (from the primary cohort) have been assessed at the post cycle six assessments or at the time of withdrawal for those patients not completing six cycles of treatment. If at least one response is observed after six cycles of treatment, 15 additional patients will be accrued in the second stage. If no responses are observed the trial will be terminated.

8.4 Sample Size Calculation

Refer to Section 4.2.3: Study Design and Statistical Methods.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized in Table 7.

Table 7. Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

Icahn School of Medicine at Mount Sinai in New York, NY will serve as the Central Office for the management of this trial. Under the guidance of Principal Investigator, Dr. John Mascarenhas, a Clinical Trials, will provide oversight and guidance on regulatory issues and trial management. This trial will be monitored closely by a Clinical Research Coordinator who

will also be located at Icahn School of Medicine at Mount Sinai. Biweekly meetings will be held to discuss this trial with the participating Sites andMerck.

Lonette Sandy

Clinical Trials Manager

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10.1 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.2 Quality Management System

10.3 Data Management Plan

10.4 ELECTRONIC Data CAPTURE (edc) SYSTEM

All computerized data entry will be performed by the Sponsor's representative. All CRFs will be logged electronically and the data entered into a web-based electronic research application portal known as eRAP. eRAP's role-based access control security and audit capability ensures that the research data is protected from unauthorized access, modification, and exposure. Key features of this web-based database system include allowing access and data entry from multiple sites, with each site having a separate pool of data as necessary. All data stored in the eRAP system is backed up daily. Detailed audit services include any data field level changes, who made the changes, when the changes were made, the old value and the new value. Data from eRAP can easily be extracted to excel or flat text file formats for easy import into SAS or other statistical software packages.

11.0 DATA AND SAFETY MONITORING PLAN

The site principal investigator (PI) will be responsible for ensuring participants' safety on a daily basis. The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to monitor participant safety, evaluate the progress of the study, to review procedures for

maintaining the confidentiality of data, the quality of data collection, and data management and analyses for all subjects.

11.1 Frequency of Data and Safety Monitoring

The Sponsor will be informed of serious adverse events as soon as they occur and will notify Merck within 24 hours of notification. The frequency of data review for this study differs according to the type of data and can be summarized in the following table.

Data Type	Frequency of Review	Reviewer
Stopping Rules (Treatment Response)	Biweekly Quarterly	Conference Call among sites PI, Sponsor,DSMB
Subject accrual (including compliance with protocol enrollment criteria)	Quarterly	,Sponsor, DSMB
Status of all enrolled subjects, as of date of reporting	Quarterly	Sponsor, DSMB
Adherence data regarding visits and intervention	Quarterly	Sponsor, DSMB
AEs and rates (including out-of-range lab values)	Quarterly	Sponsor, DSMB
SAEs	Per occurrence	PI, Merck

11.2 Contents of Data and Safety Monitory Report

The content of the data and safety monitoring report will include study status, participant descriptive information, safety information and measures of study quality. See DSMR document for table templates.

11.3 Safety Stopping Rules

Sponsor and DBMB will meet quarterly to review the patient data for safety as related to the stopping rules as defined in this section.

If a patient experiences grade 3 or higher toxicities (excluding grade 3/4 hematologic toxicity) deemed at least possibly related to the study drug (and not clearly disease related), or if grade 2 laboratory (excluding grade 2 hematologic toxicity) or clinical toxicities deemed at least possibly related to the study drug (and not clearly disease related) do not resolve within 3 weeks in spite of treatment, we will stop study drug administration.

If, among the MF-CP (primary cohort) patients, 3 or more of the first 5, 4 or more of the first 10, 6 or more of the first 15, or 7 or more of the first 24 patients stop study drug within the first 3 cycles due to toxicity as defined above in the previous paragraph, we will stop

enrolling patients to the primary cohort. The probability of stopping early if the true toxicity rate is 20%, 30%, 40% or 45% is 0.18, 0.51, 0.81, 0.91, respectively.

If, among the MPN-AP/BP (exploratory cohort) patients, 2 or more of the first 5 or 3 or more of the first 10 patients, stop study drug within the first 3 cycles due to toxicity as defined above in the previous paragraph, we will stop enrolling patients to the exploratory cohort. The probability of stopping early if the true toxicity rate is 20%, 30%, 40% or 50% is 0.26, 0.48, 0.66, 0.81, respectively.

11.4 Efficacy Stopping Rules

Sponsor and DBMB will meet quarterly to review data for efficacy as related to the stopping rules as defined in this section.

Based on Simon's optimal two stage design, 9 patients (among primary cohort patients only) will be enrolled at stage one. If no patient shows response, the study will be terminated.

12.0 APPENDICES

12.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

12.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

12.3 Dynamic International Prognostic Scoring System (DIPSS) for primary myelofibrosis

Values from anytime after diagnosis are acceptable to achieve score.

DIPSS score

The DIPSS score assigns points for the following five variables:

- Age >65 years: 1 point
- Leukocyte count >25,000/microL (>25 x 10^9/L): 1 point
- Hemoglobin <10 g/dL (<100 g/L): 2 points

- Circulating blast cells ≥1%: 1 point
- Constitutional symptoms*: 1 point

The resulting score is interpreted as follows:

- 0 points – low risk
- 1 to 2 points – intermediate-1 risk
- 3 to 4 points – intermediate-2 risk
- 5 to 6 points – high risk

PMF: primary myelofibrosis; DIPSS: Dynamic International Prognostic Scoring System. * Constitutional symptoms include: Weight loss >10% of the baseline value in the year preceding PMF diagnosis, and/or unexplained fever or excessive sweats persisting for more than one month.

Source: Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol 2011; 29:392

12.3 MPN-SAF TSS

Medical History	
Modified Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS)	
Fatigue (weariness, tiredness) in the past **24 hours**	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Circle the one number that describes how, during the past **week** how much difficulty you have had with each of the following symptoms:	
Filling up quickly when you eat (Early satiety)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal discomfort	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Inactivity	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with concentration - Compared to prior to my MPD	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Night sweats	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Itching (pruritus)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Bone pain (diffuse not joint pain or arthritis)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Pain under ribs on the left side	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Fever (>100 F)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Unintentional weight loss last 6 months	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)

References

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