SUMMARY OF CHANGES

HCC #: 15-101 Version 5.0

Previous Protocol Date:
08/07/2017
Current Protocol Date:
06/03/2019

#	Section	Page	Change
1.	Title	1	Updated Principal Investigator information and version date
2.	4.2.2 Timing of Dose Administration	25	Updated vital signs to be current with the SOC procedures for vitals on patients being treated with pembrolizumab
3.	6.1.2.4 Vital Signs	39	Updated vital signs to be current with the SOC procedures for vitals on patients being treated with pembrolizumab
4.	Footer	All	Added version # and date
5.	Throughout	All	Ensured section numbers are consistent; spelling and grammar corrections that don't affect content.

A Phase II study of Pembrolizumab as Post-remission Treatment of Patients ≥ 60 with AML Who Are Not Transplantation Candidates

HCC# 15-101; Version 5.0 dated 06/03/2019

IND SPONSOR AND PRINCIPAL INVESTIGATOR

Michael Boyiadzis, M.D., M.H.Sc
Associate Professor of Medicine
Division of Hematology-Oncology
UPMC Hillman Cancer Center
UPMC Cancer Pavilion
5150 Centre Avenue, Suite 572
Pittsburgh, PA 15232
Phone: 412-623-0040
Email: boyiadzism@upmc.edu

BIOSTATISTICIAN

Daniel P. Normolle, PhD
Biostatistics Facility
UPMC Hillman Cancer Center
Suite 325 Sterling Plaza
201 North Craig St.
Pittsburgh, PA 15213
Phone: 412-383-1581
Email: dpn7@pitt.edu

CLINICAL PHARMACY SPECIALIST

Shrina Duggal, PharmD
UPMC Cancer Pavilion, room 456
5150 Centre Ave
Pittsburgh, PA 15232
Tel: 412-623-4745
E-mail: patelsh@upmc.edu

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TRIAL SUMMARY

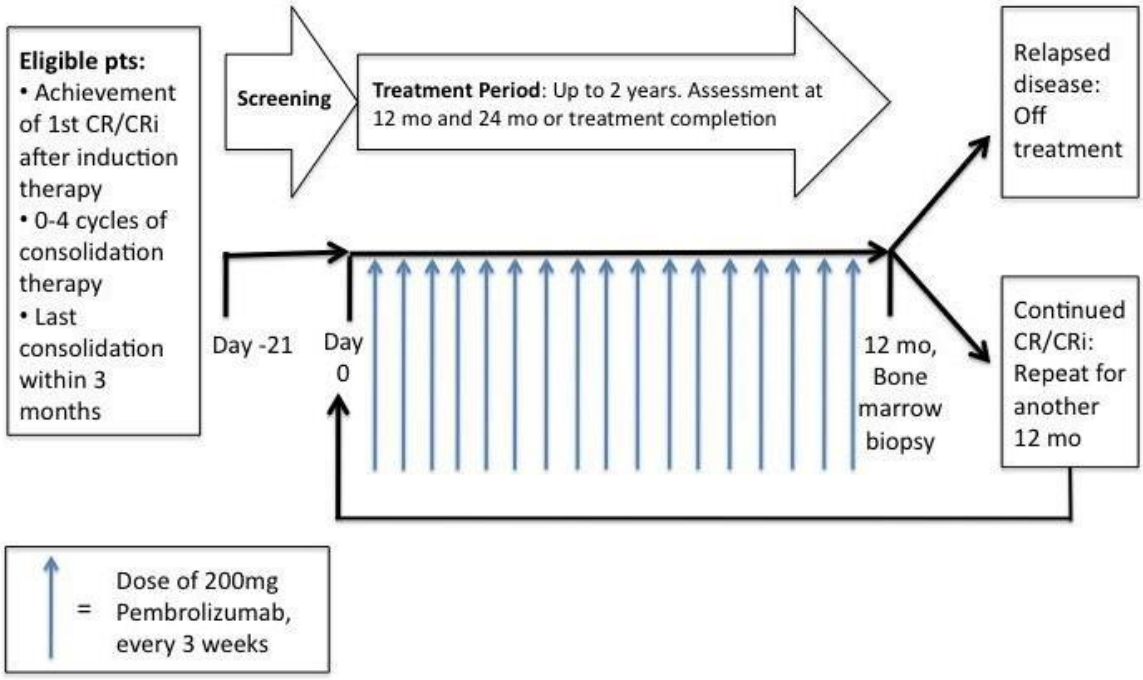
Title	A Phase II study of Pembrolizumab as Post-remission Treatment of Patients ≥ 60 with AML Who Are Not Transplantation Candidates
Short Title	Pembrolizumab as Maintenance Therapy for AML in CR1
Protocol Number	
Phase	Phase II
Methodology	Open label
Study Center(s)	Single-center
Primary Objective	To evaluate the time to relapse (TTR) in patients > 60 years with AML in complete remission undergoing post-remission therapy with pembrolizumab.
Number of Subjects	40 subjects
Diagnosis and Main Inclusion Criteria	Patients with AML in first complete remission/complete remission with incomplete count recovery who are not unable or unwilling to undergo allogeneic stem cell transplant or who allogeneic stem cell transplant is not indicated.
Study Product, Dose, Route, Regimen	Pembrolizumab, intravenous, 200mg, every 3 weeks with dosing adjustments as needed for toxicity
Duration of administration	Total duration of pembrolizumab is up to 2 years if patients tolerate the therapy and remain in complete remission
Reference therapy	Patients who achieve a complete response to induction chemotherapy followed by chemotherapy consolidation therapy who do not undergo allogeneic transplant are observed. Despite the high relapse rates in this group, there is not therapy that has been proven to be effective to prevent relapse.
Statistical Methodology	The relapse function will be estimated by using the Kaplan-Meier method. A 26% two-year TTR under the standard induction therapy is associated with a median of 12.3 months. The null hypothesis that the treatment with pembrolizumab offers no increase in TTR will be tested by determining if 12.3 months is within an 80% confidence interval around the median, a one-sided α=0.10 test.
Estimated Enrollment Period	18-24 months
Estimated duration of trial	4 years, but with planned analysis at 18 months after last patient enrolled
Duration of Participation	2 years/patient

1.0 TRIAL DESIGN

1.1 Trial Design

This is a phase II, single-center, single-arm trial to evaluate the efficacy and safety of pembrolizumab in post-remission treatment of patients ≥ 60 years with AML who are not candidates for HCT. Patients must have achieved CR or CRi with induction chemotherapy and have an ECOG performance status of 0-1. Patients can receive no consolidation or up to 4 cycles of consolidation per treating physicians' preference. Patients are eligible if their last dose of chemotherapy was received within 3 months of trial enrollment. Remaining in CR or CRi is required prior to enrollment. The enrollment target for this study is 40 patients evaluable for efficacy. Planned analysis will occur 18 months after the initiation of treatment of the last patient on the trial.

1.2 Trial Diagram



2.0 OBJECTIVE(S) & HYPOTHESIS(ES)

2.1 Primary Objective(s) & Hypothesis(es)

(1) **Efficacy Objective:** To evaluate the time to relapse in patients > 60 years with AML in complete remission undergoing post-remission therapy with pembrolizumab

Hypothesis: Post-remission treatment with pembrolizumab will improve time to relapse compared to historical controls in patients > 60 years with AML who are not candidates for HCT.

(2) **Safety Objective:** To evaluate the safety and tolerability of pembrolizumab in patients > 60 years with AML

Hypothesis: Post-remission treatment with pembrolizumab will be safe and tolerable in patients ≥ 60 years with AML who are not transplantation candidates

2.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To evaluate overall survival with post-remission pembrolizumab treatment in patients > 60 years with AML

Hypothesis: Post-remission treatment with pembrolizumab will improve overall survival compared to historical controls (where the median OS is 17.2 months) in patients ≥ 60 years with AML who are not candidates for HCT.

2.3 Exploratory Objective

(1) Objective: To evaluate the effect of pembrolizumab on AML blast-reactive T-cells by quantification of activated T and NK cells, which are predicted to increase, and regulatory T cells (Treg), which are predicted to decrease

(2) Objective: To evaluate the effect of pembrolizumab on AML blast-reactive T-cells through functional analysis, through assessment of cytokine and Granzyme B/perforin expression in response to blast antigens.

(3) Objective: To evaluate the effect of pembrolizumab on the immunosuppressive activity of exosomes, measured by their effects on NK-cell activity and T-cell expansion.

3.0 BACKGROUND & RATIONALE

3.1 Background

3.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [1]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [2; 3; 4; 5; 6]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells (Treg) 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 and has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [7; 8]. The structure of murine PD-1 has been resolved [9]. 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 [7; 10; 11; 12]. 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 [13; 14].

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 [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. 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 [18; 19; 20; 13]. 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 [13]. 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) [21]. 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.

3.1.2 Clinical Trial Data

Pembrolizumab is currently being investigated in a number of clinical trials in many solid tumors and hematologic malignancies. In the first open-label Phase I trial (PN001) of pembrolizumab in progressive or locally advanced metastatic carcinomas, enriched for melanoma and non-small cell lung cancer (NSCLC). The overall response rate across all dose

levels (2mg/kg every 3 weeks, 10mg/kg every 3 weeks, and 10mg/kg every 2 weeks) ranged from 26% for the melanoma patients previously exposed to ipilumimab [22] and 38.7% for the melanoma patients not previously exposed to ipilumimab (Investigator's Brochure). The median PFS and PFS at 24 weeks in the patients exposed to ipilumimab was 17.5 weeks and 41.7% and 28.4 weeks and 52.2% in the patients not exposed to ipilumimab. These overall response rate data demonstrate that pembrolizumab is active in the treatment of advanced melanoma. Similar promising response rates in NSCLC were seen, with an objective response rate of 21%, with a median progression free survival not yet reached with a minimum of 62 weeks, with most responders remaining on treatment.

Pembrolizumab has been generally well-tolerated with manageable side effects in most patients. Safety data from PN001 is available on 479 patients, all of whom received the last dose of medication by July 26, 2013. In the dose-escalation portion of the study, no DLTs were reported and the MTD was not identified. In the full patient cohort, nearly all patients (97.3%) experienced adverse events (AEs), of which 76.8% were considered drug related. Serious adverse events (SAEs) were reported in 30.1% of patients, but were considered potentially drug-related in 6.7% of patients. Furthermore, there were no patient deaths attributable to study medication.

Adverse events were similar between dose schedules and disease groups. In melanoma patients, the most common drug-related adverse events of any grade were fatigue (34%), pruritus (22%), rash (18%), diarrhea (15%), arthralgia (13%) and nausea (11%). Grade 3-5 drug-related AEs were reported in 11% of melanoma patients, with fatigue and diarrhea the most commonly reported, experienced in 1.7% and 0.7% of patients, respectively. Immune-related adverse events also routinely occurred in patients on pembrolizumab. In melanoma patients, the most commonly reported immune-related AEs include rash (3.2%), pruritis (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2.%), and pneumonitis (1.9%). NSCLC patients experienced similar drug-related adverse events: rash (21%), pruritis (18%), fatigue (16%), diarrhea (13%), and arthralgia (11%). The only Grade 3-5 AE experienced in the NSCLC group was pulmonary edema occurring in 1 patient (2.6%). Immune-related AEs in NSCLC patients occurred in 3 of 38 patients (7.9%), and included hypothyroidism, hyperthyroidism, pruritis, and rash in one patient each, and diarrhea in 2 patients. Most patients enrolled on PN001 did not discontinue study medication to due AEs. 9.4% discontinued due to an AE, but only 4.2% of patients discontinued due to an AE that was considered related to study treatment.

3.2 Rationale

3.2.1 Rationale for the Trial and Selected Subject Population

Acute myeloid leukemia (AML) is a malignant hematopoietic disorder characterized by the unregulated growth of myeloid precursors (blasts) and associated marked reduction in normal hematopoiesis. It is the most common acute leukemia in adults, with an estimated 18,860 new cases of AML diagnosed per year in the United State and 10,460 deaths. [23] Disease related morbidity and mortality arise from cytopenias, leading to complications such as bleeding and/or infection, as well as end organ damage from the circulating blasts. If left untreated, AML can lead to death within weeks. [24]

The standard treatment for newly diagnosed AML consists primarily of an anthracycline combined with a nucleoside analogue, cytarabine. The goal of remission induction chemotherapy is the rapid restoration of normal bone marrow function. [25] Once a complete remission is attained, relapse invariably occurs without additional consolidation therapy. Consolidation approaches are based on prognosis and age, but may include 1-4 cycles of high dose cytarabine chemotherapy or allogeneic stem cell transplant. While consolidation therapy reduces relapse, up to 50% of younger patients still relapse. [26]

Although standard therapeutic approaches are tailored to younger patients, the median age of diagnosis of acute myelogenous leukemia (AML) is 67 years. [27] Unfortunately, the outcomes for patients older than 60 years are quite poor. Population studies in AML report 5-year survival rates of 3% to 8% compared to up to 50% for younger patients. [28] Intensive induction therapy, as described above, remains the standard of care for those elderly patients who are able to tolerate it, but rates of complete remission (CR) range from 20% to 60% dependent on the number of adverse prognostic conditions present. [29] The 2-year DFS of patients $\geq$60 yrs of age with AML who undergo induction therapy has been consistently at 24-26% in two large studies conducted in 2001 and 2010. [30; 31] Unfortunately, there have been no important advances in induction therapy since then.

Although patients $\geq$60 years old can obtain a morphologic CR, defined as hematopoietic recovery with $\leq$5% blasts present, relapse still occurs in up to 80% of these patients. [32] These high relapse rates demonstrates that the majority of patients $\geq$60 years old have submicroscopic residual disease (MRD), leading to subsequent relapse. Reduced intensity conditioned (RIC) allogeneic stem cell transplant (HCT) offers a survival benefit when performed after induction chemotherapy for a proportion of patients with high-risk AML. [33; 34; 35] Unfortunately, transplant-related mortality remains high in elderly patients and those with multiple comorbidities, limiting the availability of this consolidation approach for many patients over the age of 60. [35] In a prospective study aimed to offer RIC transplant to all eligible patients with high-risk AML over age 50, only 15% of patients in CR proceeded with allogeneic stem cell transplant. [36] New strategies to prevent relapse in older patients who are not eligible or willing to undergo an allogeneic transplant are desperately needed.

Acute myeloid leukemia (AML) has historically been sensitive to immunotherapy. The graft-versus-leukemia (GVL) effect of allogeneic hematopoietic stem cell transplant (HCT) is a well-established, successful form of immunotherapy. AML is the most common indication for HCT in North America. [37] Donor lymphocyte infusions performed for relapsed AML, which rely solely on the GVL effect, lead to complete remission (CR) rates of 15-29%. [38; 39] The CRs attained through DLI are frequently durable. [40]

Despite the sensitivity of AML to immune attack, the microenvironment in AML is immunosuppressive, facilitating immune tolerance of leukemia cells. In vitro studies have demonstrated that factors secreted by primary AML cells, particularly arginase II, can prevent T-cell activation and proliferation. [41; 42] Tregs are also critical mediators of immunosuppression in AML. [43] Increased Tregs are present in patients with AML at various stages of diagnosis and treatment. Tregs are increased at diagnosis in patients with AML compared to healthy controls [44; 45; 46] and higher frequencies at diagnosis are associated with a poor prognosis. [44; 45] Early recovery from both cytotoxic chemotherapy and

maintenance therapy for AML also led to increased frequencies of functional immunosuppressive Tregs. [47; 48]

PD-1 and its ligands have emerged as an important contributor to the immunosuppressive microenvironment in AML. Many preclinical studies have demonstrated upregulation of the PD-1 pathway and the negative impact that it has on disease control in AML. In mice injected with a strain of AML, the percentage of CD8 T-cells expressing PD-1 dramatically increased in the liver, a major site of disease for AML dissemination. [49] Similarly, when AML cells are injected into mice and allowed to grow in the mouse, PD-L1 expression increases compared to baseline. [50] Furthermore, PD-1 knock-out mice with AML have slower progression of AML burden than wild-type (WT) mice and significantly longer survival. [49; 50] When a PD-1 blocking antibody is administered to WT mice with AML, those mice have a lower AML burden, more CD8+ cells infiltrating the liver, and longer survival. [50]

PD-1 and its ligands are also upregulated on immune cells and leukemic cells in humans with AML. One study of 124 patients with myeloid malignancies, including 69 with myelodysplastic syndrome (MDS) and 9 with AML sampled at various stages of treatment found that PD-L1 mRNA expression level was upregulated by $\geq$2 fold in 36% and 25% of CD34+ cells of those with MDS and AML respectively compared to CD34+ normal control cells. [51] Another cohort of 154 patients with AML demonstrated no significant increase in surface PD-L1 expression on AML blasts at initial diagnosis compared to myeloid precursors cells from healthy controls. However, stimulation with IFN-γ significantly increased PD-L1 expression in AML blasts but not in myeloid precursors from normal controls. [52] Interestingly, PD-L1 expression increased more dramatically with IFN-γ stimulation from samples of myeloid precursors cells from patients in complete remission or at relapse than newly diagnosed patients. [52] These findings demonstrate that PD-L1 expression on AML blasts definitely occurs in a substantial portion of patients with AML. Understanding of the timing of expression requires more study larger cohorts where PD-1 and its ligands are measured longitudinally. However, existing data suggests the PD-1 pathway, like the Treg population in AML, [48] may be particularly increased upon recovery from cytotoxic chemotherapy. [52]

PD-L1 expression on AML blasts pre-treatment does not appear to have prognostic significance in small cohorts of patients with AML. In 72 patients with MDS and AML tested prior to any treatment, PD-L1 expression in bone marrow samples was not associated with worse survival. [51] However, in a smaller cohort of those 72 patients treated on a clinical trial with hypomethylating agents and vorinostat, development of PD-L1 or PD-L2 upregulation on peripheral blood mononuclear cells during therapy was associated with a significantly worse median survival, 6.6 months compared to 11.7 months in those with without overexpression of PD-1 ligands. [51] Similarly, Norde et al. found that in patients who relapsed late after allogeneic transplant despite the presence of circulating alloreactive T-cells to hematopoietic cell-restricted minor histocompatibility antigens, PD-L1 was highly expressed on the leukemic cells at baseline or upon stimulating with IFN-γ. [53] Furthermore, stimulation of allogeneic CD3+ T-cells with those PD-L1-expressing AML cells led to significantly enhanced T-cell proliferation and cytokine production when performed in presence of PD-1 blockade compared to isotype controls. [53] These findings suggest that the development of functionally impaired

T-cells during therapy through manipulation of the PD-1 checkpoint leads to impaired control of leukemia, and that PD-1 blockade may offer therapeutic advantages.

In summary, patients ≥60 years old with AML have a dismal prognosis. Even though many of these patients are able to obtain a CR, relapse occurs in the vast majority of patients. RIC transplant may reduce relapse rates in this population, but is only feasible in a minority of patients. AML's immunosuppressive microenvironment in general and PD-1/PD-L1 upregulation in particular appears to increase the risk of relapse. Importantly, PD-1 and its ligands are particularly increased after therapy compared to initial diagnosis. As such, PD-1 inhibition with pembrolizumab offers to limit leukemic cell immune escape, thereby allowing the patient's immune system to eradicate the submicroscopic residual disease and reducing relapse rates.

3.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being 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, was 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 Investigator Brochure). 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 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 was 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.

3.2.3 Rationale for Endpoints

3.2.3.1 Efficacy Endpoints

3.2.3.1.1 Primary Endpoint: Time to relapse (TTR)

The primary endpoint for this study is time to relapse (TTR). Patients with AML ≥60 years old in CR have a high relapse rate, which is the primary cause of death. The goal of PD-1 inhibition in AML is to harness the patient's immune system to eradicate submicroscopic disease and prevent relapse. As such, TTR was selected as the primary efficacy endpoint. TTR has been used as the endpoint in many similar clinical trials, and using this endpoint allow for valid comparison to historical controls. TTR is defined as survival in the absence of relapse, as defined by the Cheson criteria (see Section 12.3).

3.2.3.1.2 Second Efficacy Endpoints: Overall Survival

Overall survival is limited in AML, and the goal of all new therapy options in AML is to enhance overall survival. However, overall survival may be altered by subsequent therapy choices after relapse. As such, it will be measured as a secondary but not the primary endpoint.

3.2.3.2 Biomarker Research

Under normal physiologic conditions, check-point inhibition is a protective mechanism which prevents expansion of dangerous T cells capable of harming normal tissues. Tumors, including

PD-L1+ AML blasts, have corrupted this protective pathway by "bombarding" PD-1+ T cells, including anti-leukemia T cells, with the ligand overexpressed on AML blasts, thus inducing functional T-cell paralysis. The so called "exhaustion" of anti-leukemic T cells is, in fact, tumor-driven, chronically silencing activated T cells which are unable to mediate anti-leukemia activity in the tumor environment.

One objective of pembrolizumab therapy in AML is to rescue anti-leukemia T cells from blast-mediated chronic check-point inhibition by targeting PD-1 and allowing T cells to exercise anti-AML blast activity. Another objective is to decrease immune suppression mediated by regulatory T cells (Treg) which are increased in the frequency and activity in AML. [44] Treg, especially activated Treg present in patients with cancer, express PD-1 and it is likely that they are targeted by pembrolizumab via up-regulated antibody-mediated cellular cytotoxicity (ADCC). It is possible that this mechanism is responsible for, or contributes to, recovery of anti-leukemia T cell activity by removing Treg. As ADCC is largely mediated by Ab-armed activated NK cells, their functional recovery from check-point inhibition is an important consequence of pembrolizumab therapy. Exosomes produced by tumor cells or leukemic blasts are known to carry immunosuppressive cargo and to contribute to immune dysfunction of T cells and NK cells. We have recently observed that plasma-derived exosomes obtained from patients with AML carry PD-L1. We, therefore, expect that pembrolizumab would neutralize immunosuppressive exosomes.

In this study, we will measure recovery in the frequency/absolute numbers of activated immune cells and anti-leukemic functions of T cells after pembrolizumab therapy. We will measure anti-AML reactivity of T cells using autologous AML blast-derived antigens for ex vivo activation of T cells. We will monitor changes in the frequency and absolute numbers of Treg in the patients' circulation. In addition, we will study levels and cargo of exosomes in the plasma. The hypothesis is that these immune parameters will be altered following pembrolizumab therapy and that change in the immune cell numbers and functions will provide insights into cellular mechanisms responsible for potential clinical responses observed in the proposed study.

Blood/tissue specimens will be saved at University of Pittsburgh Health Sciences Tissue Bank in the Department of Health Sciences Core Research where specimens will be coded using an Honest Broker**.** Correlative studies will be conducted in the laboratory of Dr. Theresa Whiteside at the University of Pittsburgh. Blood/tissue specimens will be labeled with study number not patient identifiers. Information linking these study numbers with the subject's identity will be kept separate from the research records. Any breech in confidentiality will be reported to the IRB.

3.2.3.2.1 Quantitative study of T-cell subsets

Assay Description: 30ml in non-citrate tubes (heparinized) and 10ml in citrate tube(s) of peripheral blood will be obtained at the following time points: at diagnosis, prior to pembrolizumab initiation, prior to Cycles 2, 4, 8, 17 (± 1 treatment cycle) , and at disease progression or the end of 2 years of treatment, whichever comes first. We will perform immunofluorescence staining and multiparameter flow cytometry to evaluate expression of PD-L1 and PD-1 on blasts and effector cells and changes in the patients' lymphocyte subsets and the expression of their surface receptors at these specific time points. The conjugated monoclonal antibodies CD 45, 3, 4, 8, 25, 39, 69, 56, FOXP3 and NKp46 will be used in

combinations to evaluate Total CD8+ Tcells and activated T-cells, total and activated CD4+ Tcells, total and activated NK cells and Treg.

Assay Performance: The frequency of the aforementioned T-cell and NK cell subsets in healthy donors is well documented. On the other hand, the frequency of these T and NK cells subset in AML patients is incompletely documented. PBMCs from 15 age-matched healthy donors will be studied as control group. A sample that is positive for above markers will be used as the positive control for the staining as well. Isotype controls for all Abs will be used. Flow cytometry is an established methodology with well-defined accuracy and precision.

Data interpretation and considerations: The proportions and absolute numbers of total and activated (CD69+/ CD25+) CD8+ T-cells, total and activated CD4+ Tcells, and CD3-CD56+ NK cells in AML patients at diagnosis and prior to pembrolizumab will be compared to values obtained with PBMC of healthy donors. These values in AML patients are expected to be lower than the established normal, suggesting the presence of T-cell suppression and/or apoptosis. Following pembrolizumab therapy, these values are expected to increase. Tregs (CD4+CD39+FOXP3+ and CD4+FOXP3+CD25+PD-1+) are expected to be elevated in frequency and absolute numbers in AML patients prior to pembrolizumab therapy. We expect Treg percentages/absolute numbers to decrease after pembrolizumab therapy based on the hypothesis that this Ab targets PD-1+ Treg and eliminates them. Expression of PD-1 is expected to decrease after therapy. This would suggest that pembrolizumab restores anti-leukemia immune responses not only by removing the checkpoint blockade and allowing for immune cell activation but also by removing Treg-mediated suppression.

3.2.3.2.2 T-cell expression of IFN- γ and Granzyme B/Perforin in T-cells stimulated ex vivo with autologous AML blast-derived antigens

Assay Description: Cryopreserved PBMCs will be thawed and washed. Autologous AML blasts will be separated from PBMCs at diagnosis, cryopreserved and stored. Just prior to the assay, AML blasts will be repeatedly frozen/thawed to solubilize the potentially relevant antigens. The protein content of the lysates will be determined. Lysates of autologous AML blasts will be used for in vitro stimulation of lymphocytes in PBMC. Antigen-presenting cells (APC) in PBMCs will be able to present antigens in the lysate to immune cells. Stimulation may be antigen-specific via the Tcell receptor (TCR) on cognate T cells or non-specific via TLRs or other receptors on immune cells responding to antigens in the lysate. IFN- γ expression by CD8+ T-cells and granzyme B (GrB) and perforin expression by CD8 T-cells and NK cells will be measured as previously described. [54] Briefly, lysates of autologous blasts will be added to PBMC at 3 different concentrations and incubated for 18 h. Cells harvested from these cultures will be incubated with mAbs specific for surface markers (CD3, CD4, CD8, CD56), fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 20 min at room temperature, washed once with PBS containing 0.5% bovine serum albumen (BSE) and 2 nM ethylene diamine triacetic acid, permeabilized with PBS containing 0.5% BSA and 0.1% saponin, washed and stained with pre-titrated anti-GrB-PE, anti-perforin-FITC, and IFN-γ-PE. Flow cytometry will be performed immediately on a Beckman Coulter equipped with Expo32 software. Percentages of IFN-γ-positive cells among CD8+ T cells and GrB/Perforin-positive CD8+ T cells and CD3-CD56+NK cells will be determined.

Assay Performance: This is a two-step assay selected for monitoring of immune cell (T and NK cell) responses to a mix of blast-derived antigens. [54] This is an autologous system, with PBMC serving as APC. The assay can be readily adapted to serial monitoring. PBMC and autologous lysates will be cryopreserved and thawed for culture. Serially-collected specimens of each patient (as described in Section 4.2.3.2.1) will be batched and tested in one assay to avoid inter-assay variability. Culture controls set in parallel with patients' samples will be PBMCs of a normal donor (cryopreserved in bulk to supply the same control for all patients' cultures) stimulated with anti-CD3/CD28 Abs. Also, PBMCs of each evaluated AML patient will be stimulated with anti-CD3/CD28 Abs to serve as control for the ability to respond to TCR-mediated signals. 18h activation was previously shown to be a sufficient time period for induction of intra-cytoplasmic cytokines or GrB/perforin expression. [54] Multicolor flow cytometry assessments after cell permeabilization and staining with labeled antibodies to T and NK cell surface markers and the relevant cytokines are routinely performed on an instrument which is calibrated daily. The SOP for the assay is on file.

Data interpretation and considerations: This assay should be able to inform us about the ability of AML patients' immune cells to respond by cytokine or GrB/perforin expression to external stimulatory signals. [55] We expect from 10 to 15% of CD8+T cells of a normal donor to respond to activation with anti-CD3/CD28 Abs by up-regulating expression of these mediators. We expect to see a much lower frequency of responsive T-cells in AML patients. Only a small percentage of immune cells in AML patients (2-4%) are expected to respond to AML blast-derived lysates. Cells of normal donors (used as controls) are likely to respond but this will reflect alloantigen- rather than AML antigen-driven response. In patients receiving pembrolizumab, we expect to see recovery of T- cell responses to anti-CD3/CD28 Abs and of T and NK cell responses to antigens in AML blast-derived lysates by up-regulated expression of either IFN-g or GrB/perforin or both.

The proportions/ absolute numbers of CD8+ Tells and NK cells expressing IFN-γ as well as CD8+ T-cells and NK cells expressing GrB and Perforin will be evaluated at diagnosis and just prior to starting pembrolizumab therapy and will be compared to the proportions/absolute numbers of positive cells at pre-determined intervals after pembrolizumab treatment. The numbers of CD8+ T cells and NK cells expressing IFN- γ, GrB and/or perforins are expected to increase from values at diagnosis and those prior to pembrolizumab treatment. This increase would suggest recovery of normal immune responses to general stimulatory signals and to AML blast-derived signals, providing evidence for the effect of pembrolizumab on AML blast-reactive T-cells and NK cells through elimination of the checkpoint blockade and/or potentially also through ADCC directed at Treg.

3.2.3.2.3 : Effects of pembrolizumab on functions and levels of exosomes

Exosomes are small (30-150mm) vesicles secreted by all cell types and present in all body fluids. [56] While exosome secretion occurs under physiologic conditions, tumor cells are avid exosome producers. In AML, blast-derived exosomes carry an immunosuppressive cargo. Furthermore, plasma exosomes in patients with newly diagnosed with AML prior to any therapy had higher levels of exosomes (in µg protein/mL plasma) compared to normal donors [57]. *Ex vivo*, exosomes decrease NK-cell cytotoxicity and down-regulate NKG2D expression in normal NK cells. More recently, we have shown elevated levels of exosomes in AML patients that are in complete remission and that these exosomes contain PD1 and PD-L1. [58]

We hypothesize that the use of pembrolizumab will affect the immunosuppressive activity of exosomes.

Assay Description: Exosomes will be isolated from the plasma of AML patients using differential centrifugation, ultrafiltration and size-exclusion chromatography on mini-Sepharose 2a columns. Using Western blotting, we will identify the exosomal cargo, including PD1, PD-L1, TGF-β1 and CD39 and CD73. To study immune suppression mediated by exosomes, AutoMACs-isolated normal NK or T cells will be co-incubated with exosomes isolated at the time of enrollment prior to pembrolizumab therapy. The levels of exosomes and the effects on the function of the immune cells will be monitored serially at diagnosis, prior to pembrolizumab initiation, at 1, 3, 6, 12, and 24 months post initiation of pembrolizumab .

Data interpretation and considerations*:* The hypothesis is that suppression of normal NK or T cells by AML exosomes and concomitant activation of Treg reverse with the use of pembrolizumab. Silencing of the negative signals should diminish or ablate exosome-mediated immune suppression and restore immune cell functions.

4.0 METHODOLOGY

4.1 Entry Criteria

4.1.1 Diagnosis/Condition for Entry into the Trial

Subject eligible for entry into the trial must have a diagnosis of non-M3 AML and currently be in first CR/CRi (see Appendix I for response criteria). Patients should receive 2-4 cycles of consolidation, provided that the last dose of either induction chemotherapy or consolidation chemotherapy falls within 3 months prior to enrollment in this trial. A patient receiving less than 2 of consolidation therapy is eligible only if they are deemed intolerant to consolidation therapy by the treating physician.

4.1.2 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 for the trial.

2. Be ≥ 60 years of age on day of signing informed consent.

3. Have a newly diagnosed AML, based on World Health Organization criteria [59], currently in 1st CR/CRi (see 12.3 for definition of CR/CRi) on a bone marrow biopsy performed within 4 weeks of study enrollment.

4. Have received the last dose of induction or consolidation chemotherapy within 3 months of enrollment.

5. Have a performance status of ≤1 on the ECOG Performance Scale.

6. Demonstrate adequate organ function as defined in Table 1, with all screening labs performed within 10 days of treatment initiation.

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,000 /mcL with no growth factor support in the previous 3 weeks
Platelets	≥50,000 / mcL (transfusion independent)
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
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	≤ 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
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.	

7. Transfusion independent (no red blood cell or platelet transfusions in the preceding 2 weeks of screening).

8. Female subject of childbearing potential require a negative pregnancy test 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.

9. Female subjects of childbearing potential must 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.

10. Male subjects must 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.

4.1.3 Subject Exclusion Criteria

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

1. Has a diagnosis of Acute Promyelocytic Leukemia (APL) as defined by the World Health Organization. [59]

2. Eligible and willing to proceed with an allogeneic stem cell transplant with an acceptable stem cell donor

3. Has favorable risk AML as defined by the presence of isolated t(8;21) or inv(16) or t(16;16)(p13.1;q22) on a standard karyotype or mutated NPM1 with concurrent wild-type FLT3 on molecular testing.

4. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.

5. 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.

6. Has had a prior monoclonal antibody 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, 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 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, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

9. Has known active central nervous system (CNS) involvement. Subjects with previously treated CNS disease may participate provided they are stable (without evidence of CNS leukemia at the time of screening and any neurologic symptoms have returned to baseline) and are not using steroids for at least 7 days prior to trial treatment.

10. Has an active automimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy are an exception to this rule, and are eligible. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study.

11. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

12. Has an uncontrolled, life-threatening active infection.

13. 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.

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

15. 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.

16. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, 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).

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

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

19. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

4.2 Trial Treatments

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

Table 2: Trial Treatment

Drug	Dose	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
pembrolizumab	200 mg	Every 3 weeks	IV infusion	Up to 2 years	Experimental
The pembrolizumab dosing interval may be increased due to toxicity as described in Section 4.2.1.2.					

Trial treatment should begin on the day of treatment assignment, or as close as possible to the day at which treatment is assignment. Treatment must occur within 10 days of official enrollment, or all screening tests must be repeated.

4.2.1 Dose Selection/Modification

4.2.1.1 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 are provided in the Pharmacy Manual.

4.2.1.2 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. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening drug-related AEs as per Table 3 below. See Section 4.6.1 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

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 12 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 12 weeks of last dose.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
	3-4	Permanently discontinue	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	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 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
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 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
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 12 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
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 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
All Other Drug-Related Toxicity[1]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 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

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

[1] 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 12 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 Investigator. The reason for interruption should be documented in the patient's study record.

Subjects with a history of AML who undergo induction therapy may have intermittent thrombocytopenia or neutropenia. This may be due to relapse of AML but could also be due to viral infection, a medication effect, or an unexplained by transient etiology. Thrombocytopenia and neutropenia are not commonly (>10%) experienced adverse event with pembrolizumab in other clinical studies in solid tumors (Investigator's Brochure). As such, pembrolizumab treatment should not be withheld for a platelet count of $\geq$30,000/mcL or an absolute neutrophil count of $\geq$500/mcL unless the investigator feels that disease assessment is warranted. However, if platelet count is <30,000/mcL or ANC is <500/mcL, pembrolizumab therapy should be held and the etiology of the thrombocytopenia evaluated. If platelets increase to $\geq$30,000/mcL and ANC to $\geq$500/mcL within 3 weeks, pembrolizumab may be restarted at the current dose. If a subject develops febrile or prolonged neutropenia which requires growth

factor support at the discretion of the investigator, pembrolizumab should be permanently discontinued.

The dose may be held based on assessment of the patient at the time of the scheduled dose. If the dose is held, the patient will be reassessed weekly (or more frequently if clinically indicated) and dosing will be restarted at the discretion of the principal investigator.

4.2.2 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 5.0). Trial treatment may be administered within a window of +/-3 days from the scheduled Day 1 of each cycle.

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). Vital signs should be monitored within 60 minutes prior to infusion and if reaction occurs every 15-30 minutes until symptoms resolve.

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

4.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the investigators, research staff, and subject will know the treatment administered.

4.3 Randomization or Treatment Allocation

This is an open-label, single arm, phase II trial in which all enrolled subjects will receive the study drug.

4.4 Stratification

As a single arm study, there is no need for stratification of patients between arms.

4.5 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. Treating sub-investigators should discuss any questions regarding this with the Principal Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

4.5.1 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 up to 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 6.2.3.

4.5.2 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:

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

- 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.

4.6 Rescue Medications & Supportive Care

4.6.1 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 and in greater detail in the ECI guidance document. 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 is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 4.2.1 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. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **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 3-4** 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.

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 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**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of Pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	permanently discontinued from further trial treatment administration.	
Grades 3 or 4 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.		

4.7 Diet/Activity/Other Considerations

4.7.1 Diet

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

4.7.2 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. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

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 they must adhere to the contraception requirement (described above) for the duration

of the study and during the follow-up period defined in section 6.2.2 Reporting of Pregnancy and Lactation to the Investigator and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

4.7.3 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 Investigator and to Merck without delay and within 24 hours 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 Investigator. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Investigator and to Merck and followed as described above .

4.7.4 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.

4.8 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 treating sub-investigator or the Principal Investigator 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 6.1.4 – 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.
- Confirmed pathologic disease progression
- Unacceptable adverse experiences as described in Section 6.2.1.2
- 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
- Completed 24 months of treatment with pembrolizumab and required follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 5 (Trial Flow Chart) and Section 6.1.5 (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 6.2.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

4.9 Subject Replacement Strategy

Participants who are not evaluable for efficacy because they have not received at least one dose of drug will not be included in analyses for either efficacy or toxicity and will be replaced. Any participant who receives at least one dose of pembrolizumab will be included in both efficacy and safety analyses, and will not be replaced.

4.10 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.

5.0 TRIAL FLOW CHART

5.1 Study Flow Chart

Trial Period:	**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
						To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Main Study Screening	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Pre-screening Consent													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Subject Identification Card		X	X	X	X	X	X	X	X	X	X	X	
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X				
Trial Treatment Administration		X	X	X	X	X	X	X	X				
Post-study anticancer therapy status												X	X
Survival Status													X
Review Adverse Events		X	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X				X				X	X	X		
Directed Physical Examination		X	X	X		X	X	X					
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X		
ECOG Performance Status	X	X	X	X		X		X		X	X		
Pregnancy Test – Urine or Serum β-HCG	X	X[c]											
PT/INR and aPTT	X												
CBC with Differential	X[d]	X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Serum Chemistry Panel	X[d]	X	X	X	X	X	X	X	X	X	X		
Urinalysis	X[d]		X		X		X		X	X	X		

Trial Period:	**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Main Study Screening	1	2	3	4	To be repeated beyond 8 cycles 5	6	7	8	Discon	Safety Follow-up	Follow Up Visits[b]	Survival Follow-Up
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
T3, FT4 and TSH	X[d]		X		X		X		X	X	X		
Chest radiograph	X												
Bone marrow biopsy	X[e]								X[f]	X[f]			
Archival or Newly Obtained Tissue Collection	X[g]												
Correlative Studies Blood Collection[h]		X	X		X				X[h]	X			

a: Treatment cycles occur every 3 weeks, but may be given +/- 3 days of the scheduled dose. See Section 6.1.5.2

b: Follow-up CBC with differential should occur every 12 weeks +/- 7 days after discontinuation of therapy until disease progression, initiation of new anti-neoplastic therapy, death, end of study, re-treatment with pembrolizumab., or a total of 5 years from the subject's enrollment.

c: Pregnancy tests must occur within 72 hours of the first dose of pembrolizumab

d: All screening laboratory assessment must occur within 10 days prior to the first treatment

e: The screening bone marrow biopsy must be performed and read by a UPMC hematopathologist within 4 weeks prior to the 1st treatment cycle, and include flow cytometry and cytogenetic assessment. If possible, the screening bone marrow biopsy should be sent to the Fred Hutchingson Cancer Center for multiparameter flow cytometric minimal residual disease testing.

f: Bone marrow biopsy for efficacy assessment should occur at one year (between treatment cycle 16-18), and at treatment discontinuation (due to completion of 2 years, toxicity, or relapse) and include flow cytometry and cytogenetic assessment.

g: Subject should be assessed for achieved marrow or peripheral leukemic blasts from diagnosis of AML at screening visit

h: Correlative blood draws may be collected up to two (2) days prior to dosing. Blood collection for correlative studies should occur prior to cycle 1, 2, 4, 8, 17 (+/-1 treatment cycle), and at disease progression or the end of 2 years of treatment, whichever comes first.

6.0 TRIAL PROCEDURES

6.1 Trial Procedures

The Trial Flow Chart - Section 5.0 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 Investigator 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.

6.1.1 Administrative Procedures

6.1.1.1 Informed Consent

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

6.1.1.1.1 General Informed Consent

Consent must be documented by the subject'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 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.

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 local IRB/ERC requirements and applicable laws and regulations.

6.1.1.2 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 trial *as defined in Sections 4.1.2 and 4.1.3.*

6.1.1.3 Medical History

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

6.1.1.4 Prior and Concomitant Medications Review

6.1.1.4.1 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.

6.1.1.4.2 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 6.2.

6.1.1.5 Disease Details and Treatments

6.1.1.5.1 Disease Details

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

6.1.1.5.2 Prior Treatment Details

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

6.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

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

During treatment, subjects must not use a prohibited concomitant medication, as detailed in 4.5.2. These include other anti-cancer systemic chemotherapy or biologic therapy, other investigation agents, radiation therapy, live vaccines, or glucocorticoids except as required to modulate symptoms from an adverse event of suspected immunologic etiology, as determined by the study sub-investigators or physiologic doses of steroids that are approved by the Principal Investigator. Subjects requiring prohibited concomitant medications must withdraw from the study. No diet or activity modification is required when enrolling on this study. Patients must have a negative urine or serum pregnancy test within 72 hours of the first dose of pembrolizumab, and agree to appropriate contraception during the duration of the study for 120 days after the last dose of medication, as described in Section 4.7.2.

6.1.2 Clinical Procedures/Assessments

6.1.2.1 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); see the separate ECI guidance document regarding the identification, evaluation and management of potential irAEs.

Please refer to section 6.2 for detailed information regarding the assessment and recording of AEs.

6.1.2.2 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,

6.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

6.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, within 60 minutes prior to infusion and if reaction occurs every 15-30 minutes until symptoms resolve 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.

6.1.2.5 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. After Cycle 8, assessment of ECOG status will be performed every other cycle in conjunction with the directed or full physical exam.

6.1.2.6 Assessment of Disease

The subject will have a bone marrow biopsy and aspirate with flow cytometric studies and cytogenetics performed and officially reviewed by a UPMC hematopathologist within 4 weeks prior to the first treatment as part of the screening period. Subsequent disease assessments are performed by bone marrow biopsy and aspirate with flow cytometric studies and cytogenetics after 1 year (+/- 4 weeks) and after 2 years (+/- 4 weeks) or at disease relapse or treatment discontinuation.

6.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

As part of the functional correlative studies, it is recommended that patients enrolled on the study have cyropreserved autologous AML blasts within the UPMC Tissue Bank. Correlative studies blood sampling includes 30ml of peripheral blood in non-citrate tubes (heparinized) and 10ml in citrate tube(s), collected prior to cycle 1, 2, 4, 8, 17, and 34. Correlative studies are described in full detail in Section 3.2.3.2.

6.1.3 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	Bicarbonate	results are noted	Free tyroxine (T4)
	Uric Acid	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Calcium		
	Chloride		
	Glucose		Blood for correlative studies
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		
	Creatinine		
† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.			

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 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.

6.1.4 Other Procedures

6.1.4.1 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. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 6.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 6.1.5.4).

6.1.4.2 Blinding/Unblinding

N/A

6.1.5 Visit Requirements

Visit requirements are outlined in Section 5.0 Trial Flow Chart. Specific procedure-related details are provided above in Section 6.1 - Trial Procedures.

6.1.5.1 Screening

6.1.5.1.1 Screening Period

Potential subjects who enter the screening period must undergo a full medical history, complete physical exam, chest radiograph, laboratory assessment as detailed in Section 6.0, a bone marrow biopsy, and meet full inclusion/exclusion criteria. All screening assessments must be completed within 4 weeks of obtaining full informed consent for the study. A negative serum pregnancy test must be documented within 72 hours of the first treatment. Full laboratory assessment must be completed no more than 10 days before the first treatment.

6.1.5.2 Treatment Period

Subjects enrolled on the study should receive treatment as close as possible to the data when enrollment occurred, and must start treatment within 10 days of official enrollment or full screening assessment must be repeated. Those who enter the treatment period are expected to receive treatment with pembrolizumab every 3 weeks $\pm$ 3 days in the absence of adverse events requiring treatment delays. Treatment delays and dose modifications are discussed in full detail in Section 4.2. Physical exam, laboratory assessment, efficacy assessments, and correlative blood sampling is required during treatment as detailed in Section 6.0.

6.1.5.3 Post-Treatment Visits

6.1.5.3.1 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, as described in Section 6.0. 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. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

6.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (84 ± 7 days) by complete blood count to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, or end of the study or up to 5 years from enrollment. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

6.1.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

6.2 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 investigational 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.

Adverse events may occur during the course of the use of the investigational 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.

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 6.2.3.1.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

6.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Investigator and to Merck

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and 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 the investigational product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of the investigational 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 Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

6.2.2 Reporting of Pregnancy and Lactation to the Investigator 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 Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

6.2.3 Immediate Reporting of Adverse Events to the Investigator and to Merck

6.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of the investigational 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

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

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 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to the investigational product, must be reported within 24 hours to the Investigator 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 the investigational 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 Investigator and 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

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

6.2.3.2 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 Investigator 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 the investigational product, as defined in Section 6.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Investigator, 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).

1. Additional adverse events:

   A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document").

   ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

   Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

6.2.4 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 investigational 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 investigational product to be discontinued?	
Relationship to test drug	Did the investigational product cause the adverse event? The determination of the likelihood that the investigational 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 investigational product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the investigational product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the investigational 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 investigational 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 investigational product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the investigational 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 investigational product; or (3) the trial is a single-dose drug trial); or (4) investigational product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the investigational 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) investigational 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 INVESTIGATIONAL 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 investigational 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 investigational product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the investigational product. The temporal sequence of the AE onset relative to the administration of the investigational product is reasonable. The AE is more likely explained by the investigational product than by another cause.	
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the investigational product OR temporal sequence of the AE onset relative to administration of the investigational product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

6.2.5 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.

The principal investigator / sponsor must promptly review all information relevant to the safety of the drug obtained or otherwise received from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States. The study principal investigator / sponsor must notify all participating investigators of potential serious risks, from clinical trials or any other source, as soon as possible.

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Event Description of the MedWatch 3500 form:

- CTCAE term(s) and grade(s)
- current status of study drug
- all interventions to address the AE (testing and result, treatment and response)
- hospitalization and/or discharge dates
- event relationship to study drug

Follow-up reports:

Additional information may be added to a previously submitted report by adding to the original MedWatch 3500 report and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form.

7.0 STATISTICAL ANALYSIS PLAN

7.1 Analysis Samples

For both the primary safety and efficacy endpoints, the analysis sample will be eligible patients who have taken at least one dose of pembrolizumab. Patients who are eligible but withdraw prior to taking the first dose will not be included in these analyses and will be replaced, so that, barring early stopping, the analysis sample will be 40 patients who have taken at least one dose of drug.

7.2 Statistical Analysis Plan Summary

7.2.1 Primary Efficacy Objective: *To evaluate the time to relapse (TTR) in patients > 60 years with AML in complete remission undergoing post-remission therapy with pembrolizumab.* The time to relapse function will be estimated by the product-limit (Kaplan-Meier) method, with an appropriate 80% confidence interval. Death is considered a censoring event in determining TTR.

The median TTR will also be estimated, along with an appropriate confidence interval. Assuming an exponential time to failure distribution, 26% two-year TTR under the standard induction therapy is associated with a median of 12.3 months. The null hypothesis that the treatment with pembrolizumab offers no increase in TTR will be tested by determining if 12.3 months is within an 80% confidence interval around the median, a one-sided α=0.10 test. The sensitivity of the treatment of death as a censoring event will be assessed by repeating the above analysis on relapse-free survival, where death and relapse are both considered events. Patients who drop out of the study will be treated as censored at the last observation time.

Potential prognostic factors for TTR in this population will also be explored. These include the use of consolidation therapy (0 vs 1-4), cytogenetic risk category (high-risk, intermediate risk, or low risk), FLT3 status (FLT3-ITD mutation present or absent), NPM1 status (NPM1 mutation present or absent), age >70 years, minimal residual disease, and ECOG performance status. The survival distributions for TTR will be computed using the Kaplan-Meier method and the log-rank test used to test for statistical differences in the distributions for the aforementioned exposure groups. Multivariate analysis using Cox regression will then be used to adjust for all significant covariates. Patients who drop out of the study will be treated as censored at the last observation time. Patients with missing covariate data will be excluded from this exploratory analysis.

7.2.2 Primary Safety Objective: *To evaluate the safety and tolerability of pembrolizumab in patients > 60 years with AML.* The NCI CTCAE type and grade of adverse events and their association with treatment will be tabulated. Section 8.3 contains a stopping rule for excess toxicity.

7.2.3 Secondary Efficacy Objective: *To evaluate overall survival with post-remission pembrolizumab treatment in patients ≥ 60 years with AML.* The analysis plan is similar to that of TTR. Assuming an 8% five-year survival rate with induction therapy alone, median overall survival is 17.2 months.

7.2.4 Secondary Biomarker Objectives

7.2.4.1 Second Biomarker Objective

To evaluate the effect of pembrolizumab on AML blast-reactive T-cells by quantification of activated T and NK cells, which are predicted to increase, and Tregs, which are predicted to decrease. The changes in these markers from baseline after treatment will be assessed by paired comparison t-test or Wilcoxon rank sum test, as appropriate. All point estimates will be accompanied by appropriate 95% confidence intervals. In all secondary biomarker analyses, observations with missing data will be excluded.

7.2.4.2 Secondary Biomarker Objective

To evaluate the effect of pembrolizumab on AML blast-reactive T-cells through functional analysis, through assessment of cytokine and Granzyme B/perforin expression in response to blast antigens. The analysis plan is similar to 7.2.4.1.

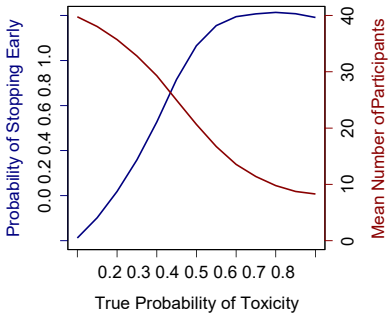
7.2.4.3 Secondary Biomarker Objective: *To evaluate the effect of pembrolizumab on the immunosuppressive activity of exosomes, measured by their effects on NK-cell activity and T-cell expansion.* The analysis plan is similar to 7.2.4.1.

7.3 Stopping Rule for Excess Toxicity

The table displays the stopping rule for excessive toxicity. Excessive toxicity is defined as any drug-related toxicity that results in withholding or discontinuation of pembrolizumab. Additionally, any unexpected grade 3-4 AEs will be defined as excessive toxicity."

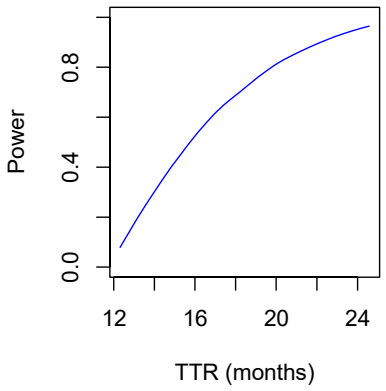
# Patients	#Toxicities
5	3
10	4
15	5
20	7
25	8
30	9
35	10

The trial is suspended for toxicity if the greater number of toxicities is instance, patients than or equal to the toxicity, in the second column. For if 5 out of the first 15 experience severe the trial is suspended.



7.4 Justification of Design

The plot presents the power of the hypothesis test of the primary efficacy endpoint against the median TTR after *pembrolizumab* treatment. It is seen that 80% power is achieved (at α=0.1 in a one-sided test) if the true median TTR is 19.5 months or greater. The stopping rule is based on a beta-binomial probability model that stops the trial if PP(P(Toxicity)>0.2)>0.8, where PP is the posterior probability function for P(Toxicity). The posterior model includes a binomial data model and a Beta(2,8) prior for the P(Toxicity). The plot displays the operating characteristics of the rule as a function of the true (unobserved) probability of toxicity. It is seen that, for instance, if the true probability of toxicity is 0.4, the probability the trial will stop early is 0.5, and the expected number of patients accrued is 28.



8.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

8.1 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 11.

Table 11 Product Descriptions

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

8.2 Packaging and Labeling Information

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

8.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Investigator 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.

8.4 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.

8.5 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 a r r a n g e f o r 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.

9.0 ADMINISTRATIVE AND REGULATORY DETAILS

9.1 Confidentiality

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records. All study data reviewed and discussed will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission per reporting guidelines.

9.2 Compliance with Law, Audit and Debarment

The Sponsor-Investigator will maintain records in accordance with Good Clinical Practice guidelines.

9.3 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.

9.4 Quality Management System

Independent monitoring of the clinical study for protocol and GCP compliance can be conducted upon PI request by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

9.5 Data Management

Investigator/Sub-investigators, regulatory, site management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet regularly in disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events
- subject safety issues
- recruitment issues
- accrual
- protocol deviations
- unanticipated problems

- breaches of confidentiality

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4.0. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB per reporting guidelines. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed semi-annually.

The summaries of these meetings are forwarded to the UPCI DSMC which meets monthly following a designated format. Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

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11.0 APPENDICES

11.1 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.

11.2 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 Response Criteria for Acute Leukemia

Morphologic leukemia-free state
Bone marrow < 5% blasts in an aspirate with spicules
No blasts with Auer rods or persistence of extramedullary disease

Complete remission
Patient achieves a morphologic leukemia-free state and
Absolute neutrophil count > 1000/mcL
Platelets ≥100,000/mcL
No residual evidence of extramedullary disease
Morphologic CR - patient independent of transfusions
Cytogenetic CR - cytogenetics normal (in those with previously abnormal cytogenetics)
Molecular CR - molecular studies negative

Complete remission with incomplete count recovery
Patient fulfill all of the criteria for CR except for residual neutropenia (< 1,000/mcL) or thrombocytopenia (< 100,000/mcL).

Partial remission
Decrease of at least 50% in the percentage of blasts to 5 to 25% in the bone marrow
Patients failing to achieve a complete response are considered treatment failures

Relapse
Relapse following complete response is defined as reappearance of leukemic blasts in the peripheral blood or the finding of more than 5% blasts in the bone marrow, not attributable to another cause (eg, bone marrow regeneration after consolidation therapy)

Cheson BD, Bennett JM, Kopecky KJ et al. J Clin Oncol 2003;21 (24):4642-4649