Protocol Amendment #8 (October 14, 2020)

J1651: A Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

Amendment #8 Incorporates:

X Administrative Protocol Revisions:

- Telemedicine options have been added to the protocol in the study calendar section (Section 6.1, page 56)

X Investigator's Brochure:

- Pembrolizumab IB edition 19
- The updated IB did not result in any changes to the study ICF.

Protocol Amendment #7 (January 14, 2020)

J1651: A Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

Amendment #7 Incorporates:

X Administrative Protocol Revisions:

- List of JH Co-Investigators updated
- Statement added to Section 5.1.1 reflecting study enrollment closure on 1/8/2020. The relapsed/refractory cohort has been completed, and new enrollment to the newly diagnosed AML cohort has been closed based on FDA guidance on the risk benefit analysis.
- Unit for albumin changed from mg/dL to g/dL in Section 5.1.2 Table 1

X Changes to the Informed Consent Form:

- Information added to section 2 to notify patients of enrollment closure and updated risk benefit analysis information from the FDA
- (Note: Risk language was previously updated with ICF template version 8-15-19 in accordance with Pembrolizumab IB v17 and aggregate safety report for Pembrolizumab Primary Adrenal Insufficiency. JHMIRB approved on 9-4-19.)

Protocol Amendment #6 (July 18, 2018)

J1651: A Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

Amendment #6 Incorporates:

X Changes to the Protocol:

- Correction of the spelling of pembrolizumab throughout the protocol
- An echocardiogram exception for screening has been added to section 7.1.5.1.1 and Section 6.1, footnote k. If an echocardiogram was conducted within 3 months prior to the screening date and the patient has not received any chemotherapy in the time between and has not experienced any cardiac symptoms or events (heart attack, chest pain, arrhythmia, etc), then the screening echocardiogram does not need to be repeated, unless it is clinically indicated per investigator's discretion.
- Vital signs will no longer be obtained post pembrolizumab infusion. This revision has been made to Section 6.1, footnote g.
- For pembrolizumab related toxicities, additional information about resumption of dosing has been added to Table 4 in Section 5.2.1.4.
- A window of 4 days has been added to the 30 day follow up visit in Section 6.1 so that the patients can be seen on their physicians' clinic days.
- The D-Dimer laboratory test has been removed from section 7.1.5.1.1.

X Changes to the Informed Consent Form to update the risk language as per new Pembrolizumab IBv16 and Merck recommendations, and include:

- The risk language in section 4 has been updated.
    - Statement added to indicate number of patients treated with pembrolizumab

- Statement under pembrolizumab risks was revised to note that immune-mediated adverse reactions may be fatal and may occur after discontinuation of pembrolizumab.
- Solid organ transplant risk information has been added.
- A new paragraph was included to note that “arthritis” was added after the Rare side effects section as an adverse reaction from the post-marketing environment.

Protocol Amendment #5 (January 23, 2018)

J1651: A Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

Amendment #5 Incorporates:

- Administrative changes to the protocol
    - Correcting name spelling of non-JHU co-investigator Robert K. Stuart, MD, Medical University of South Carolina, Hollings Cancer Center

- Changes to do the dose modification table for pembrolizumab-related immune adverse events in Section 5.2.1.4 according to Merck recommendations (letter dated 11/28/2017).

- Correction to the study calendar Section 6.1. to be consistent with footnotes and the intended collection schedule

PROTOCOL AMENDMENT #4 (10/24/2017)

J1651: Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years AML Patients)

AMENDMENT #4 INCORPORATES:
X Administrative Changes to the Protocol

- Non-JHU co-investigator change from Juan Varela, MD, PhD to Robert Stewart, MD
- Clarification of cohort #2 exclusion criteria in Section 5.1.3

X Changes to the Informed Consent Form to update the risk language as per new Pembrolizumab IBv15 and Merck recommendations, and include:

- Addition of allogeneic stem cell transplant risk language
- Administrative changes

PROTOCOL AMENDMENT #3 (08/17/2017)

J1651: Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

This protocol is amended to update definition of disease response based on revisions included in AML European Leukemia Net criteria 2017 and provide clarification on the assessment of disease progression in accordance with AML ELN 2017, and expectations for response and follow up in AML patients undergoing low dose chemotherapy in combination with immunotherapy. The informed consent form is revised to update risk language for pembrolizumab according to a new version of the MK-3475 Risk Wording document issued on 06.09.2017 by Merck and Co.

AMENDMENT #3 INCORPORATES:
X Administrative, Editorial Changes to the Protocol

- Section 11.3 and 12 (Reference #63) revised to include online reference to revised AML criteria in 2017.
- Section 5.2.1.3 - corrected grammatical error, and section 5.2.1.4 –removed reference to Appendix, section 11.4 as it was incorrect.

X Scientific Changes to the Protocol

- Section 5.2.1.5.3- Added requirement that dose reductions/delays during cycle 1, 2 or subsequent cycles should be discussed with the Protocol Chair before being made.
- Section 7.1.2.6 – Response criteria were updated to reflect changes incorporated in new AML ELN criteria 2017. In particular, new categories for response assessment including complete remission without minimal residual disease (CR_{MRD-}), morphologic leukemia-free state (MLFS), stable disease and molecular relapse were added. Also, definitions for response assessment (CR, CRi, PR, CD) were updated according to AML ELN 2017. In particular, progressive disease criteria were updated, with detailed clarification provided for follow up and subsequent treatment, as well as when patient should be removed from study for progressive disease before completion of 6 cycles of therapy.
- In accordance with updated response criteria (Section 7.1.2.6), changes to the section 1.0, section 2.1, section 3.3 (3. and 4.), section 4.2.3.1.2, 5.2.1.5.4, 7.1.5.5, 8.2.4, include adding morphologic leukemia-free state (MLFS) as a

- category of response; and minor changes to the section 1.0 (Duration of participation), section 2.1, section 5.2.1.3 to be consistent throughout the protocol, we changed wording to the bone marrow assessment.

X Eligibility Changes

- Inclusion criterion (Section 5.1.2, Cohort #1 and #2, criterion 6.) changed to ECOG PS < 2 (previously ≤ 2)

X Changes to the ICF to update the risk language for pembrolizumab according to a new version of the MK-3475 Risk Wording document issued on 06.09.2017 by Merck and Co. High level summary of changes:
1. General
a. Wording of Rarely this condition can lead to death has been changed to Sometimes this condition can lead to death
b. Changed the wording of some terms to align better with the other terms with regards to grammar and/or addition of symptoms upon Medical Director review.
c. Removed KEYTRUDA® from document
2. What side effects could the study drug(s) cause?
a. Section streamlined to remove specific reference to the approved indications by name as well as specific clinical trial exposure numbers, etc. as information changes continually.
b. Added pembrolizumab information from patient medication guides (USPI medication guide and/or SmPC patient leaflet) to explain how pembrolizumab works and how it may cause side effects.
c. Used the format of 4 categories (Very Common, Common, Uncommon, and Rare) which simplifies and eliminates need for repetition of terms by overall and serious frequencies, etc. vs percentages.
i. Removed duplicate terms, ie, that were in the previous version's Common and Serious side effect percentage categories
d. Removed reference to "immune-mediated" serious events <1%, as not helpful or informative for patient and has created numerous site/IRB/EC and Health Authority questions.
e. Added simplified language for 4 new terms: thyroiditis, myasthenic syndrome, sarcoidosis, and encephalitis.
3. Hematologic Malignancy trials language
a. Changed paragraph order and text for readability

PROTOCOL AMENDMENT #2 (03/07/2017)

J1651: Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

AMENDMENT #2 INCORPORATES:

X Administrative Changes to the Protocol

- Clarified which type of tubes can be used for the specimen collection in the Section 6.1, 7.1.3.1.3.5., and 11.4.
- Provided additional clarification in the text for the Table 6.1 Study Flow Chart that the bone marrow should be obtained at the treatment discontinuation as well as PB and BM research specimens collected.
- Clarified that correlative studies will be done in collaboration with UNC (a participating site for this study), Section 7.1.3.1.3.

X Changes to the Informed Consent Form to update the risk language as per new Pembrolizumab IBv13 (02/17/2017) and Merck recommendations, and include:

- Administrative changes
- Update on the risks:

i. Additional language added to Severe Skin Reactions (first bullet) to reflect symptoms associated with Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis.

ii. Diabetes language updated to ensure subjects know that they will likely need regular insulin

iii. New side effect language added for myocarditis (last bullet)

iv. New text for the potential side effect, myasthenic syndrome, has been added.

PROTOCOL AMENDMENT #1 (12/28/2016)

J1651: Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients

AMENDMENT #1 INCORPORATES:

X Administrative Changes

X Scientific Changes

X Eligibility Changes

A summary of revisions is provided as follows:

1. Corrected language in Section 2.1 (page 8) to specify that patients will be assessed during cycle 1-3 for DLT to be consistent with the rest of the protocol.
2. Section 4.2.1. updated clinical results on use of anti-PD1 and azacitidine in AML.
3. Revised eligibility criteria (Section 5.2.1. Subject Inclusion Criteria) for Cohort #1 (4.), for Cohort #2 (4.) to allow prior treatment with hypomethylating agents.
4. Revised eligibility criteria (Section 5.2.2 Subject Exclusion Criteria) for Cohort #2 – Removed exclusion criteria (1.) to allow prior treatment with hypomethylating agents.
5. Revised Section 5.2.1.5 to be consistent with Section 5.2.1.4. specifying when delay in treatment between cycles will be allowed.
6. Revised Section 5.2.1.5.1 to better define circumstances when azacitidine treatment can be interrupted and restarted within a cycle.
7. Revised Section 6.1. Table footnote “a” to reflect changes listed under 5-6 of this amendment.
8. Revised Section 5.6.2 for hydroxyurea dosing during study.

TITLE: **Phase 2 Study of Azacitidine in Combination with Pembrolizumab in Relapsed/Refractory Acute Myeloid Leukemia (AML) Patients and in Newly Diagnosed Older (≥65 Years) AML Patients**

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1.0 TRIAL SUMMARY

Abbreviated Title	Azacitidine and Pembrolizumab for the treatment of relapsed/refractory AML patients and newly diagnosed older (≥ 65 years) AML patients who are not candidates for intensive induction chemotherapy
Trial Phase	*Phase II*
Clinical Indication	**Safety Run In Phase (only Cohort 1):** The treatment of relapsed and refractory AML patients with no prior history of allogeneic hematopoietic stem cell transplant (HSCT). Phase 2: **Cohort 1:** The treatment of relapsed and refractory AML patients. **Cohort 2:** The treatment of newly diagnosed AML patients (≥ 65 years) who are not candidates for intensive induction chemotherapy.
Trial Type	Interventional
Type of control	No control arm
Route of administration	Intravenous pembrolizumab; intravenous or subcutaneous azacitidine
Trial Blinding	Open Label
Treatment Groups	**Cohort 1:** Relapsed/refractory AML patients **Cohort 2:** Newly diagnosed older patients (≥65 years) who are not candidates for intensive induction chemotherapy
Number of trial subjects	Cohort 1: 10-40 Cohort 2: 10-40
Estimated enrollment period	*24 months*
Estimated duration of trial	*48 months*
Duration of Participation	Each subject will participate in the trial from the time the subject signs the Informed Consent Form through the final protocol-specified contact. Each treatment cycle will last 28 days. Bone marrow will be examined after 2 and 6 cycles and every 3-6 months thereafter. Patients who achieved complete remission (CR/CRi) or patients without remission but benefiting from therapy (partial remission [PR], morphologic leukemia-free state [MLFS], hematologic improvement [HI], stable disease [SD]) can continue on study receiving both azacitidine and pembrolizumab as long as they have ongoing clinical benefit without evidence of significant toxicity or disease progression and up to 2 years.
Estimated average length of treatment per patient	Up to 2 years or as long as subjects have ongoing clinical benefit without evidence of disease progression or significant toxicity.

2.0 TRIAL DESIGN

2.1 Trial Design

This is a multicenter, nonrandomized, open-label phase 2 study (with a safety run-in phase) of azacitidine (AZA) 75 mg/m^2 given IV or SQ on days 1-7 every 28 days in combination with pembrolizumab 200 mg given IV every 3 weeks (starting on day 8 of cycle 1). The dose/schedule of AZA selected for this study is FDA approved for patients with MDS/AML. The rationale for pembrolizumab dose/schedule- Section 4.2.2.

We plan to examine two AML patient cohorts:

Cohort 1: relapsed/refractory AML.

Cohort 2: newly diagnosed AML in older patients (≥65 years) not candidates for induction chemotherapy.

Each cohort will be enrolled separately with distinct null hypotheses and statistical plans.

Azacitidine and pembrolizumab have distinct mechanisms of action with no common overlapping toxicities; however, as azacitidine and pembrolizumab have not been tested previously in combination in AML patients, we plan to start enrollment with **Cohort 1** (**a safety run-in phase**) and include only relapsed/refractory AML patients excluding those relapsing after allogeneic hematopoietic stem cell transplant (alloHSCT).

Cohort 1 (a safety run-in phase, 3 + 3 design): The first three patients will be enrolled and receive planned azacitidine and pembrolizumab in cycle 1 (Section 2.2). If 0 or 1 patient of the first three patients have a DLT (dose limiting toxicity) in cycle 1, we will enroll additional 3 patients to better assess for toxicities. If less than 2 among first 6 patients experiences DLT in the cycle 1-3, we will open enrollment in Cohort 1 for all relapsed/refractory patients including those with prior history of alloHSCT and will also start to enroll patients in Cohort 2. We plan to monitor for DLTs up to the end of cycle 3 as some of immunological side effects associated with pembrolizumab may occur with the delay.

If ≥2 of the first 3 patients or ≥2 of 6 patients have DLTs during cycle 1, we will perform safety (detailed toxicity) review to assess for disease versus treatment-related toxicities. Given that pembrolizumab is given only once in cycle 1 (day 8), we will first dose de-escalate azacitidine to 75 mg/m^2 x 5 days. However, if the safety data analysis suggests that the toxicities are more likely pembrolizumab related, an alternative approach we may take is to exclude pembrolizumab in cycle 1 and start pembrolizumab dosing in cycle 2 when AML is better controlled but maintain azacitidine dose/schedule. If ≤1 of the 6 patients experience DLTs during cycle 1 but ≥ 2 of 6 patients experience DLTs during cycle 2 and cycle 3 or cycle 1, 2 and 3, we will first dose de-escalate pembrolizumab to 200 mg given every 4 weeks starting on Day 1 of Cycle 2 and maintain azacitidine dose/schedule. When each dose de-escalation occurs, additional 6 patients will be enrolled at that dose level. For the details of dose de-

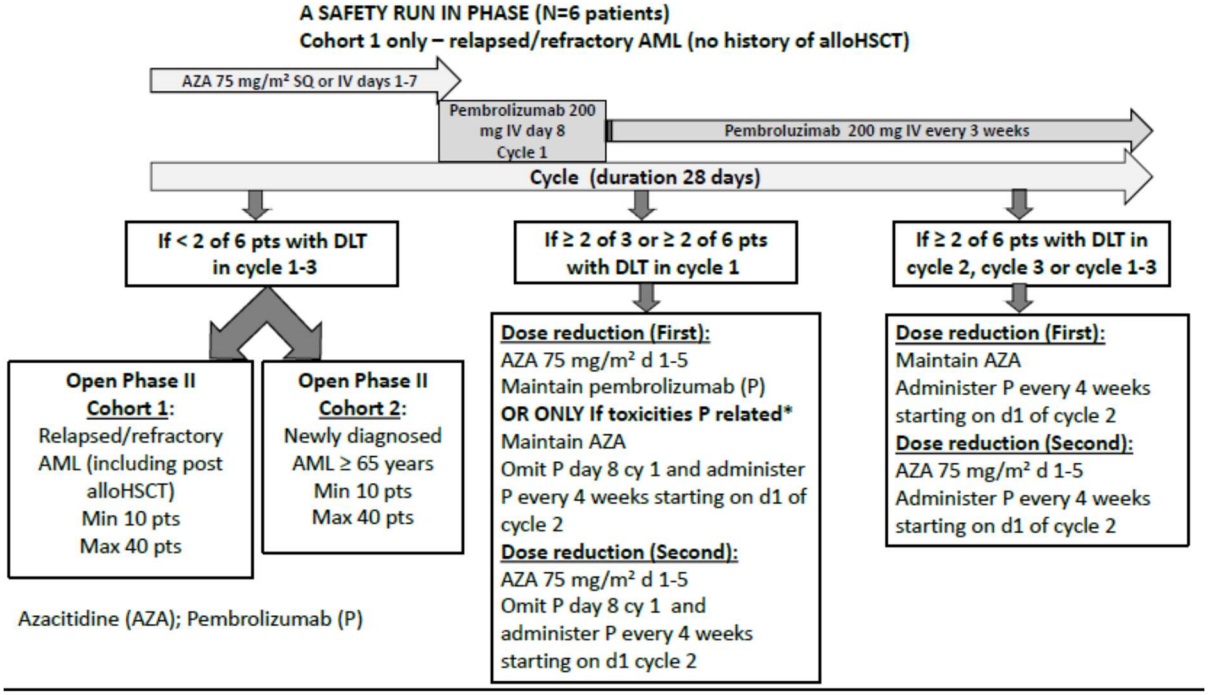
escalation, please see Section 2.2 Trial Diagram.

Only after safe dose/schedule is defined (≤ 1 of 6 patients having DLT in cycle 1-3) we will start enrollment in the phase 2 cohorts. A full safety assessment of the combination of AZA and pembrolizumab will be performed in all patients after 1, 2, and 3 cycles and in subsequent cycles according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. For efficacy analysis, those patients who were treated at dose/schedule selected for Phase 2 will be included.

Bone marrow biopsy/aspirate will be performed after 2 and 6 cycles of therapy, and every 3-6 months thereafter as described in Section 6.0 Trial Flow Chart. Treatment with azacitidine/pembrolizumab will continue until confirmed disease progression, unacceptable toxicity, or if patient is withdrawn from study for other reasons (Section 5.9). Patients having a clinical benefit from this treatment combination including CR/CRi but also those who achieve response less than CR/CRi (ie., PR/MLFS/HI/SD) will be able to continue on treatment on clinical study (AZA and pembrolizumab) until they meet criteria specified in section 5.9. After the end of treatment, each subject will be followed for 30 days for AEs monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.3.2.). Subjects who discontinue treatment for reasons other than disease progression will have post-treatment follow up for disease status until disease progression, initiating non-study cancer treatment, withdrawing consent or becoming lost for follow up. All subjects will be followed for overall survival until death, withdrawal of consent or the end of the study, whichever comes first.

The primary objective of the run in safety phase would be to determine the safe and tolerable dose/schedule of azacitidine and pembrolizumab in patients with AML. We will perform safety run in phase only in Cohort 1 as Cohort 2 patients would be expected to have a same or better tolerability given that they are not heavily pretreated. Patients who relapse post alloHSCT will be enrolled on the study only if they are at least 3 month after alloHSCT and off all immune suppression for at least 3 weeks and have no evidence of active graft versus host disease (GVHD). These patients are expected to tolerate treatment as well as all other relapsed/refractory patients but will be monitored for any GVHD exacerbation in the course of treatment. The primary objective of this Phase 2 study would be to estimate the CR/CRi rates in two separate cohorts of patients: Cohort 1: relapsed or refractory AML patients. Cohort 2: Newly diagnosed older AML patients (≥65 years) who are not candidates for intensive induction chemotherapy. Secondary objectives of the trial are to determine safety, tolerability, and analysis of various efficacy parameters such as overall response rate, disease-free survival, progression-free survival, time to progression, overall survival, and exploration of association between potential biomarkers and clinical activity. These analyses will be performed separately for each patient cohort.

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es) (safety run in-phase)

(1) **Objective:** Determine the safe and tolerable dose of AZA followed by pembrolizumab in relapsed and refractory AML patients.

Hypothesis: AZA followed by pembrolizumab will be safe and tolerable in relapsed and refractory AML.

3.2 Primary Objective(s) & Hypothesis(es) (Phase 2)

(1) **Objective (Cohort 1):** Estimate the complete remission (CR/CRi) rate of AZA followed by pembrolizumab in relapsed and refractory AML patients.

Hypothesis: AZA followed by pembrolizumab will be effective (CR/CRi) in relapsed and refractory AML.

(2) **Objective (Cohort 2):** Estimate the complete remission (CR/CRi) rate of AZA followed by pembrolizumab in older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

Hypothesis: AZA followed by pembrolizumab will be effective (CR/CRi) in older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

3.3 Secondary Objective(s) & Hypothesis(es)

(1) **Objective (Cohort 1)**: Characterize the toxicity of AZA followed by pembrolizumab in relapsed and refractory AML.

Hypothesis: AZA followed by pembrolizumab will be safe and tolerable when administered to relapsed and refractory AML patients.

(2) **Objective (Cohort 2)**: Characterize the toxicity of AZA followed by pembrolizumab in older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

Hypothesis: AZA followed by pembrolizumab will be safe and tolerable when administered to older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

(3) **Objective (Cohort 1):** Assess the overall response rates [ie.CR/CRi/partial response (PR)/morphologic leukemia-free state (MLFS)/hematologic improvement (HI)] of AZA followed by pembrolizumab in relapsed/refractory AML patients.

Hypothesis: AZA followed by pembrolizumab will lead to a promising overall response rate in relapsed and refractory AML patients.

(4) **Objective (Cohort 2):** Assess the overall response rates [ie.CR/CRi/partial response (PR)/ morphologic leukemia-free state (MLFS)/hematologic improvement (HI)] of AZA followed by pembrolizumab in newly diagnosed AML patients not candidates for intensive induction chemotherapy.

Hypothesis: AZA followed by pembrolizumab will lead to a promising overall response rate in older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

(5) **Objective (Cohort 1 and Cohort 2)**: Estimate disease-free survival (DFS) and overall survival (OS) of patients who achieve a CR/CRi for each cohort separately: relapsed/refractory (Cohort 1) and newly diagnosed older AML patients (Cohort 2).

Hypothesis: Patients who achieve a CR/CRi will have durable responses.

(6) **Objective (Cohort 1 and Cohort 2):** Estimate progression-free survival (PFS), time to progression (TTP), and OS for all patients. These survival parameters will be estimated separately for each Cohort.

Hypothesis: AZA followed by pembrolizumab will lead to a promising PFS, TTP and OS in both relapsed/refractory and older newly diagnosed AML patients not candidates for intensive induction chemotherapy.

3.4 Exploratory Objective

(1) **Objective:** Explore association between potential biomarkers and clinical activity of the combination of AZA and pembrolizumab in patients with relapsed and refractory AML and in older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Pharmaceutical and Therapeutic Background

4.1.1.1 Pembrolizumab

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[2–6]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2)[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[7,10–12]. 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[13,18,19]. 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)[20]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

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

4.1.1.1.1 Pembrolizumab Preclinical and Clinical Trial Data

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

4.1.1.1.1.1 Preclinical Findings

In preclinical studies, T-lymphocyte responses in cells from healthy human subjects, patients with cancer, as well as in primates were strongly enhanced by pembrolizumab. The EC50 (i.e., concentration in which 50% of the maximum effect is seen) in human donor blood cells exposed to pembrolizumab has been reported to be between ~0.1 and 0.3 nM based on T-cell activation assays, and levels of cytokines, including but not limited to interleukin-2 (IL-2), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) were changed by this antibody. Of note, pembrolizumab does not impact immune responses unless antigen is present. In syngeneic murine tumor models, an anti-murine PD-1 analog significantly inhibited tumor growth, and was synergistic with chemotherapy agents resulting in increased complete tumor regression rates.

Safety pharmacology/toxicology studies of pembrolizumab were conducted in cynomolgus monkeys and included both a 1-month and 6-month repeat dosing period study. The maximum dose administered in each was 200mg/kg, administered weekly in the 1-month study and every other week in the 6-month study. The antibody was well tolerated in both studies, with an increased incidence of inguinal swelling and increased splenic weights at the 200mg/kg dose the only notable findings in the 1-month study, neither of which was considered adverse. Anti-pembroliuzmab antibodies were detected in both studies, but not at the 200mg/kg dose.

Further, based on the level of target engagement, the antibodies do not seem to impact pembrolizumab pharmacodynamics. The no observable adverse effect level (NOAEL) based on both studies is ≥200mg/kg/dose. See the most recent pembrolizumab Investigator's Brochure for additional details.

4.1.1.1.1.2 Clinical Findings

As of the April 2015 Investigator's Brochure (IB: data cut-off of October 2013), 1,000 patients across 7 studies have been treated with pembrolizumab at various dose-schedules, including 10 mg/kg administered IV over 30 minutes every 2 weeks. The drug has been well tolerated to date, with no serious infusion reactions and no DLT reported. The most commonly reported treatment emergent adverse events (TEAEs) experienced are fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhea (22.3%) and rash (21.5%).

An immune-related adverse event (irAE) is defined as a clinically significant AE of any organ that is associated with study drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism. The most commonly reported irAEs across the dose-schedules in pembrolizumab studies are rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%) and pneumonitis (1.9%). The organ most frequently affected by irAEs with pembrolizumab is the skin. Less frequently affected tissues include thyroid gland, colon, lung, kidney, and liver.

There have been no serious infusion reactions reported with pembrolizumab. Less than 1% of patients (one report) have had confirmed positive treatment emergent anti-drug antibodies and no clear impact on exposure to pembrolizumab has been observed.

4.1.1.2 Azacitidine

Azacitidine (AZA), a hypomethylating agent, is widely used in the treatment of high-risk myelodysplastic syndrome (MDS) and AML in older patients. In the phase III study, AZA improved survival over conventional care regimens not only in high-risk MDS patients but also AML patients having 20-30% blasts (24.5 vs 16 months)[21,22]. Besides a CR/CRi rate of 18%, an additional 20-30% of AML patients benefited from AZA treatment in terms of transfusion-independence[22]. Subsequent, mainly retrospective studies have shown that AZA is effective in AML patients irrespective of blast percentage, with overall response rate (ORR) and CR/CRi rate of 40-50% (10%-31%) in newly diagnosed and 35-55% (0%-27%) in relapsed/refractory (including post alloHSCT) AML patients[23–27].

4.1.1.2.1 AZA: Indication and Usage

Subcutaneous or intravenous azacitidine (75 mg/m^2 for 7 days every 28 days) is approved for treatment of patients with all subtypes of MDS (AML up to 30% blasts) including chronic myelomonocytic leukemia (CMMoL). The pivotal study leading to approval of the agent in MDS showed a median OS of 25 months vs the control arm showing 15 months in a randomized phase III study[28].

4.1.1.2.3 Adverse Events

For the SC or IV formulation, the most common adverse reactions (>30%) as detailed in the package insert for the agent are; nausea, anemia, thrombocytopenia, vomiting, pyrexia, leukopenia, diarrhea, injection site erythema, constipation, neutropenia, ecchymosis and injection site reactions.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) remains a therapeutic challenge. Although 60-80% of newly diagnosed patients with AML respond to induction chemotherapy, the relapse rate remains high (40%-50%) even after allogeneic stem cell transplant (alloHSCT)[29–31]. In particular, two groups of AML patients have an immediate and unmet need for novel and improved therapies: 1) Relapsed/refractory AML. Different salvage regimens yield 20-30% response rate with a very few long-term survivors[32,33]. The outcomes are even worse for those patients relapsing after alloHSCT who also have a heightened risk of toxicity from aggressive chemotherapy [30,31]; 2) Newly diagnosed AML in older patients. The long-term survival of this patient population, which represents the majority of patients with AML, has been consistently <10%[29,34]. Furthermore, even when an older patient is a candidate for intensive induction chemotherapy, the AML is frequently chemoresistant and often progresses to the relapsed/refractory state. Novel paradigms of treatment are desperately needed to target AML, such as exploiting the immune system to exert an anti-leukemia effect[35]. The efficacy of alloHSCT and immunotherapy with donor lymphocyte infusion (DLI) underscores the fact that AML is sensitive to immunotherapeutic approaches[36,37] and there is also evidence for autologous anti-leukemia reactivity[38,39]. Thus, strategies to unleash endogenous anti-leukemia immunity applied in the context of a regimen that is tolerable and effective, are needed to effectively combat AML in these two populations.

Azacitidine (AZA) effect on immune system

There is growing evidence that AZA may induce immune reaction alterations ranging from increased expression of cancer testis antigens (CTA) and MHC class I co-stimulatory molecules to upregulation of co-inhibitory ligands such as PD-L1 on tumor cells, including myeloid malignancies[40–43]. Given intriguing responses to PD-1 blockade in patients with NSCLC pretreated with AZA/vorinostat, further in vitro studies were undertaken by the Baylin group[44]. Using the genome wide expression and DNA methylation analyses of solid tumor cell lines, they found that AZA upregulates genes and pathways related to both innate and adaptive immunity, including immune evasion[41,42]. Upregulation of PD-L1 was noted, suggesting that AZA may prime NSCLC cells to PD-1/PD-L1 blockade, and thus subverting this axis with antibody inhibition may be a useful therapeutic strategy[42]. The importance of epigenetic regulation in T cell lineage commitment and function has been increasingly recognized. DNA

methylation at the promoter/enhancer region can influence the transcription of PD-1 and several key cytokines (IL-2, IFN-y) after TCR stimulation, thus controlling T cell responses and differentiation. In particular, upregulation of PD-1 on exhausted effector memory T cells is noted in chronic viral infections and is associated with maintained demethylation of the PD-1 locus[45,46]. By contrast, IL-2 promoter methylation is increased in T cells from patients with HIV suggesting a crucial link between changes in DNA methylation and downregulation of IL-2 expression[47]. Interestingly IL-2 co-administration with blockade of PD-1 synergistically enhanced virus-specific T cell responses[48]. Thus, **we hypothesize that epigenetic re-programing of IL-2 and PD-1 promoter by AZA will result in increased IL-2 production and consequent activation of PD-1-expressing T cells that will be further augmented by subsequent blockade of PD-1/PD-L1, thus unleashing anti-leukemia immunity and enhancing efficacy of this approach in AML patients.**

PD-1 and AML

Preclinical studies using murine models of AML have shown that signaling through the PD-1/PD-L1 axis impairs anti-leukemic immunity. Firstly, long-lived murine leukemia cells (minimal residual disease) have upregulated PD-L1 that mediate resistance to cytotoxic lymphocyte (CTL)-mediated killing; upregulation of PD-L1 has also been observed on murine leukemia cells (C1498) *in vivo*[49,50]. Secondly, progressive tumor burden increases expression of the co-inhibitory molecule PD-1 on T cells[51]. Consequently, the *in vivo* blockade of this pathway improved outcomes in murine models of AML[49–51]. Across different human studies, the frequency of PD-L1-expressing AMLs varies from 18% to more than 50%. Furthermore, PD-L1 expression on AML cells increases after *ex vivo* stimulation by IFN-γ[52–55]. Higher PD-L1 expression on AML cells also is observed after chemotherapy or at relapse[52–54], providing a unique opportunity to augment the efficacy of chemotherapy by inhibiting immune inhibitory pathways after chemotherapy administration.

PD-1 Post-alloHSCT

In clinically relevant murine models of alloHSCT, the recipient antigen expression by non-hematopoietic cells promotes T cell exhaustion and abrogates the graft vs leukemia (GVL) reactivity through upregulation of PD-1 on donor T cells[56,57]. Donor T cell dysfunction could partially be restored by blockade of PD-L1 and delayed blockade of PD-L1 after transplant improved the beneficial GVL effect without inducing graft vs host disease (GVHD)[56–58]. In patients relapsing after alloHSCT, PD-L1 was found to be upregulated on leukemic progenitors[59]. Furthermore, high PD-1 expression was noted on allo-reactive minor histocompatibility antigen (MiHA)-specific effector memory CD8+ T cells (T_{EM}s). *Ex viv*o treatment with monoclonal antibodies (mAbs) against PD-1 or PD-L1 restored the proliferation and IFN-γ production of MiHA-specific T_{EM}s, more potently in relapsed patients than in those in remission[59]. Similarly, silencing of PD-L1 and PD-L2 on MiHA-loaded dendritic cells augmented the expansion of functional MiHA-specific T_{EM}s from leukemia patients early after DLI and later during relapse[60]. Safety of immune checkpoint blockade after alloHSCT has been demonstrated for ipilimumab, a mAb targeting CTLA-4[61]. Thus, it is reasonable to hypothesize that PD-1 blockade will be feasible, may bolster donor specific T cell responses,

and consequently augment a GVL effect in patients who relapse post-alloHSCT as long as it is administered to patients having no evidence of active GVHD and not requiring ongoing immune suppression.

All together, these data suggest that epigenetic therapy with azacitidine combined with blockade of PD-1/PD-L1 pathway is a rational therapeutic approach both in newly diagnosed older patients and those with relapsed and refractory AML. Furthermore, recent data suggest that the addition of PD-1 blockade (nivolumab) to azacitidine in patients with AML who have previously failed hypomethylating agents may induce responses in up to 20% patients (Daver N; ASH abstract #763, ASH 2016), which is comparable to the responses obtained with intensive salvage therapies (for which many of AML patients may not be eligible due to age and co-morbidities).

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001/NCT01295827) was conducted to evaluate the safety and clinical activity of single agent MK-3475. 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 MK-3475 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, is the dose and schedule utilized in a Phase IB trial (Protocol 013/ NCT01953692) in patients with hematologic malignancies (including MDS) to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 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. MK-3475 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 MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and

exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

Thus, based on these pharmacokinetic data, the dose/schedule of pembrolizumab selected for this study is a flat dose of Pembrolizumab 200 mg every 3 weeks starting with Day 8 of Cycle 1. Azacitidine will be used at the dose/schedule FDA approved for MDS/AML (≤30% blasts) and used in the clinic in AML patients. Recent study demonstrated that azacitidine induces demethylation of PD-1 locus in T cell *in vivo* in MDS/AML patients which correlated with poorer overall response rate, suggesting that this may represent potential resistance mechanism to the activity of AZA in this patient population and calling for an intervention such as pembrolizumab administration to inhibit PD-1/PD-L1 pathway[62]. Furthermore, in some patients the PD-1 locus in T cells becomes re-methylated close to the beginning of the next cycle; thus, administration of pembrolizumab on every 3 week schedule appears to be the most rationale approach to promote synergism of these two agents with no overlapping toxicities.

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

4.2.3.1.1 Primary Endpoint

The rate of CR+CRi (separately for each Cohort) as defined by the International European LeukemiaNet Guidelines in AML[63].

4.2.3.1.2 Secondary Endpoints

- Characterize the toxicity of AZA followed by pembrolizumab in each Cohort (summary statistics). The toxicity will be classified and graded according to National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE, version 4.0)

- The overall response rate CR+CRi+partial response (PR)+ morphologic leukemia-free state (MLFS)+hematologic improvement (HI) (separately for each Cohort) as defined by the International European LeukemiaNet Guidelines in AML[63] and IWG Modified Response Criteria (2006) for MDS (for definition of HI)[64].

- Disease-free survival and overall survival of patients who achieve a CR/CRi for each Cohort separately. DFS will be defined as the time from CR/CRi to either relapse, death or last follow-up date (censored). OS will be defined as the time from study initiation to either death or last follow-up date (censored).

- Estimate progression free survival, time to progression, and overall survival for all patients (separately for each Cohort). PFS will be defined as the time from study initiation to progression or death or last follow-up date (censored).

4.2.3.2 Biomarker Research

We will be conducting an extensive set of correlative studies to evaluate the effect of azacitidine (epigenetic priming) and pembrolizumab on the leukemia cells and host immune responses. The overarching goals of our correlative studies are to i) identify and characterize mechanisms involved in T cell dysfunction in patients with AML; ii) identify potential predictive biomarker candidates to allow for better selection of AML patients who may benefit from AZA/pembrolizumab therapy; and iii) identify new pathways that could be targeted to enhance the efficacy of combined epigenetic/immunomodulation strategy in patients with AML. To accomplish these goals, we will perform multi-color flow cytometric analyses of immune markers pre- and post-treatment on both leukemia and immune cells (T cells, NK cells, MDSCs), T cell methylation analysis (DNA methylation of the IL-2 and PD-1 promoter), high-throughput DNA sequencing of rearranged TCRβ CDR3 regions from T cell genomic DNA, cytokine measurements, and immunohistochemistry on the bone marrow biopsies. In addition, we may perform exploratory gene expression analysis of immune biomarkers in the bone marrow aspirates and whole or targeted exome sequencing of the leukemia cells.

We hypothesize that treatment with azacitidine will increase expression of co-signaling molecules on both leukemia cells and T cells, making them susceptible to subsequent therapeutic modulation of PD-1/PD-L1 pathway with pembrolizumab. In particular, we expect that epigenetic re-programing of IL-2 and PD-1 promoter by azacitidine will result in increased IL-2 production and consequent activation of PD-1-expressing T cells that will be further augmented by subsequent blockade of PD-1/PD-L1 pathway, thus unleashing anti-leukemia immunity and enhancing efficacy of this approach in AML patients. We also hypothesize that

a primary action of azacitidine/pembrolizumab will be to modulate the TCR diversity resulting in the emergence of unique TCR clonotypes that may be relevant in the anti-leukemia response.

All patients will have pre- and post-treatment bone marrow aspirates (tumor tissues) as well as serial blood samples submitted for correlative studies to determine the dynamic nature of changes in leukemia cells/T cells.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

1. Relapsed and refractory acute myeloid leukemia **(Cohort 1)** – This cohort has enrolled the expected 40 patients and is now completed.

2. Newly diagnosed acute myeloid leukemia in older patients (≥65 years) not candidates for intensive induction chemotherapy **(Cohort 2)** – This cohort is closed to new accrual as of 1/8/2020 based on risk benefit analysis information received from the FDA on 1/3/2020.

5.1.2 Subject Inclusion Criteria

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

Cohort #1

1. Have histologically or cytologically confirmed relapsed or refractory AML (i.e. ≥5% blasts by manual differential on bone marrow aspirate/biopsy/flow cytometry), excluding acute promyelocytic leukemia (APL; FAB M3; t(15;17)).

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

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

4. Not be appropriate candidate for intensive salvage chemotherapy due to co-morbidities or other disease- or treatment-related factors.

NOTE: Subjects who received prior treatment with hypomethylating agents either for Myelodysplastic Syndrome (MDS), Myeloproliferative Neoplasm (MPN), or AML will be eligible.

NOTE: Subjects who had prior allogeneic stem cell transplant (alloHSCT) will be eligible as long as they have been at least 3 months after allogeneic HSCT and are off of all immune suppression for at least 3 weeks (>21 days) and have no evidence of active graft versus

host disease (GVHD). **Subjects with prior alloHSCT will NOT be eligible for enrollment during the safety run in phase.**

5. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of treatment initiation.

6. ECOG performance status < 2.

7. A projected life expectancy of at least 12 weeks.

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

9. Female subjects of childbearing potential (Section 5.8.2) must be willing to use an adequate method of contraception as outlined in Section 5.8.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

   Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

10. Male subjects of childbearing potential (Section 5.8.2) must agree to use an adequate method of contraception as outlined in Section 5.8.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

11. As determined by the enrolling physician or protocol designee, ability of the patient to understand and comply with study procedures for the entire length of the study.

Cohort #2

1. Have histologically and cytologically confirmed newly diagnosed AML (i.e. ≥ 20% blasts by manual differential on bone marrow aspirate/biopsy and/or in peripheral blood), excluding acute promyelocytic leukemia (APL; FAB M3, t (15;17)).

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

3. Be ≥65 years of age on day of signing informed consent.

4. Have received NO prior treatment for AML with the exception of hydroxyurea/leukapheresis.

NOTE: Subjects may have been treated for pre-existent myeloid disorder such as Myelodysplastic Syndrome or Myeloproliferative Neoplasm including hypomethylating agents.

5. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of treatment initiation.

6. ECOG performance status < 2.

7. A projected life expectancy of at least 12 weeks.

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

9. Female subjects of childbearing potential (Section 5.8.2) must be willing to use an adequate method of contraception as outlined in Section 5.8.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

   Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

10. Male subjects of childbearing potential (Section 5.8.2) must agree to use an adequate method of contraception as outlined in Section 5.8.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

11. As determined by the enrolling physician or protocol designee, ability of the patient to understand and comply with study procedures for the entire length of the study.

Table 1. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
White blood cell (WBC) count	≤30,000 /mcL NOTE: Hydroxyurea/leukapheresis use is allowed to meet this criterion.
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*

	*unless considered due to leukemic organ involvement, Gilbert's or hemolysis, **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 3 X ULN
Albumin	>2.5 g/dL
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.	

5.1.3 Subject Exclusion Criteria

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

Cohort #1

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

   NOTE: Subjects who were treated on a clinical study of allogeneic stem cell transplant (alloHSCT) will be eligible if they are at least 3 months after allogeneic HCT and are at least 3 weeks (>21 days) off of all immune suppression and have no evidence of active GVHD (physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency is allowed). **Subjects with prior alloHSCT will not be eligible for enrollment during the safety run in phase.**

2. 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.

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

4. Hypersensitivity to pembrolizumab or any of its excipients.

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

6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks or growth factors within 1 week prior to study Day 1 or who has not

recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

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

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

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

8. Has known active central nervous system (CNS) leukemia. Subjects with previously treated CNS leukemia may participate provided that they have documented clearance of CNS leukemia and are not actively treated with intrathecal chemotherapy.

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

10. Has known history of non-infectious pneumonitis that required steroids or current pneumonitis.

11. Has an active uncontrolled infection requiring systemic therapy (viral, bacterial or fungal). Patients with infection under active treatment and controlled with antibiotics are eligible.

12. Has a white blood cell count > 30 x 10^9/L.

NOTE: Leukapheresis and Hydroxyurea is permitted to meet this criterion and should be stopped ≥12 hours before starting treatment on the study.

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, or anti-PD-L2 agent.

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 of planned start of study therapy.

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

Cohort #2

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment for pre-existent myeloid disorder such as MDS or MPN.

2. Is eligible for treatment with a standard cytarabine and anthracycline or similar intensive induction chemotherapy, or is willing to receive intensive induction therapy. If subject is not considered eligible for treatment with standard or similar intensive induction chemotherapy due to comorbidities or other factors, or is unwilling to receive intensive induction therapy will be allowed to participate in this study.

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

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

5. Hypersensitivity to pembrolizumab or any of its excipients.

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

7. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks or growth factors within 1 week prior to study Day 1 or who has not

recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

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

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

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

9. Has known active central nervous system (CNS) leukemia. Subjects with previously treated CNS leukemia may participate provided that they have documented clearance of CNS leukemia and are not actively treated with intrathecal chemotherapy.

10. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment. Subjects that require intermittent use of bronchodilators or local steroid injections will not be excluded from the study.

11. Has known history of non-infectious pneumonitis that required steroids or current pneumonitis .

12. Has an active uncontrolled infection requiring systemic therapy (viral, bacterial or fungal). Patients with infection under active treatment and controlled with antibiotics are eligible.

13. Has a white blood cell count > 30 x 10^9/L.

    NOTE: Leukapheresis and hydroxyurea is permitted to meet this criterion and should be stopped ≥12 hours before starting treatment on the study.

14. 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.

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

16. 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.

17. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

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

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

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

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

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 1

Table 1. Trial Treatment

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Azacitidine	75 mg/m^2	Daily x 7 days	Subcutaneous (SQ) or IV infusion	Day 1 to 7 of each 28 day cycle	Standard
Pembrolizumab	200 mg	Q 3W	IV infusion	*# Starting on Day 8 of Cycle 1	Experimental

- *Pembrolizumab administration will start on Day 8 of Cycle 1.
- # On days when both agents are administered, azacitidine should be administered before the pembrolizumab. An anti-emetic, preferably a serotonin antagonist, should be administered prior to azacitidine; the individual anti-emetic selected is at the discretion of the investigator.

Trial treatment should begin as close as possible to the date on which treatment is allocated/assigned.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

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

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

Azacitidine will be administered at the dose and schedule approved by the FDA for treatment of MDS/AML. The azacitidine IV/SQ will be administered before the dose of pembrolizumab on days that both agents are administered.

5.2.1.2 Dose Modification (Escalation/Titration/Other)

- Dose modifications will be made based on Common Terminology Criteria for Adverse Events (CTCAE) grading (version 4.0)

- Patients may remain on study and therapy even if one of the agents are discontinued due to toxicity. No more than two dose reductions of any agent is allowed.

5.2.1.3 Dose Limiting Criteria

- Dose limiting toxicities during **safety run in phase** will be determined during Cycle 1, Cycle 2, and Cycle 3 of study treatment. Adverse events that occur after the first three cycles will also be evaluated by investigator and may be considered as dose limiting.

- Subjects with AML have frequently ≥ grade 3 cytopenias; thus, will be un-evaluable for hematologic DLT with the exception listed below.

- For hematologic toxicity, the only adverse event that will be considered dose limiting is:

    - grade ≥ 4 neutropenia and thrombocytopenia with a hypocellular bone marrow and no evidence of residual leukemia lasting for 42 days

      NOTE: only patients evaluable for hematologic toxicity as defined in Section 5.2.1.5.2.1, 5.2.1.5.2.3 and Section 5.2.1.5.3 may need to have a bone marrow assessment after cycle 1 if no count recovery to baseline. Otherwise, all patients will have bone marrow after cycle 2);

- For non-hematologic toxicities, the only adverse events that will be considered dose-limiting are:

- Any grade 4 toxicity considered at least possibly related to study drugs excluding asymptomatic electrolyte aberrations that can be corrected to ≤ grade 2 and excluding specific toxicities that are frequent in AML population such as fever, infections, fatigue, bleeding, bone pain.

- Any grade 3 toxicity considered at least possibly related to study drugs that does not resolve to ≤ grade 2 within 48 hours with the exception of Grade 3 anorexia, nausea, vomiting, diarrhea, or mucositis if it resolves to ≤ grade 2 within 72 hours of maximal supportive care; asymptomatic electrolyte abnormalities that can be corrected to ≤ grade 2 and excluding specific toxicities that are frequent in AML population such as fever, infections, fatigue, bleeding, bone pain. Grade 3 neurotoxicity and nephrotoxicity of any duration will be considered dose-limiting if at least possibly drug-related.

Table 3. Safety Run In Phase- Dose De-escalation Rules

Number of patients with DLT during run in phase (Planned 6 patients)	Decision rule
0 or 1 out of 3 in Cycle 1	Enter 3 more patients
≥2 of 3 in Cycle 1 OR ≥2 of 6 in Cycle 1	Patients will be assessed for safety. Based on the complete assessment of AEs, the dose reduction as proposed in the Section 2.1 and 2.2.
≥2 of 6 in Cycle 2 or/and Cycle 3 OR ≥2 of 6 in Cycle 1+2+3	Patients will be assessed for safety. Based on the complete assessment of AEs, the dose reduction as proposed in the Section 2.1 and 2.2.
≤ 1 of 6 in Cycle 1 + 2 + 3	Open Phase II Cohort 1 and Cohort 2.

5.2.1.4 Immune Adverse Events Associated with Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below. See Section 5.7.5 for supportive care guidelines, including use of corticosteroids.

Table 4. **Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab**

General instructions:
1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.

2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. For Grade 3 (excluding alopecia, fatigue) toxicities which are eligible for re-dosing: Increase dose interval to every 4 weeks, and to every 6 weeks with second occurrence. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.

3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis.
	Grade 4	Permanently discontinue		

				• Participants with 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.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		

Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		
1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. **NOTE:** For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).				

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 4 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.2.1.5 General dose modifications

Every effort should be made to administer the study drug treatment according to the planned dose and schedule.

In the event of significant toxicity, pembrolizumab dosing should be interrupted, delayed and/or reduced as outlined above in the Table 4. In the event of multiple toxicities, dose modification should be based on the worst toxicity observed.

Dose modifications may occur in three ways:

•Within a cycle: Dosing interruption until adequate recovery followed by dose reduction (if required) during a given treatment cycle.

Azacitidine dosing interruption is not allowed within a cycle unless there is an unexpected and unacceptable toxicity or as defined in Section 5.2.1.5.1.

•Between cycles: The next treatment cycle may be delayed if toxicity from the preceding cycle persists or if specific non-treatment related circumstances arise as defined in Section 5.2.1.4 (other non-treatment related medical/surgical events or logistical reasons).

•In the next cycle: Dose reduction may be required based on toxicities experienced in the previous cycle.

5.2.1.5.1 Dose interruptions/modification for non-hematologic toxicities

Complications of myelosuppression include infections and bleeding, these may be exacerbated with the use of azacitidine and pembrolizumab treatment. Azacitidine and pembrolizumab treatment may be delayed at the discretion of the investigator, if the subject experiences myelosuppression-associated complications, such as those described below:

● Febrile neutropenia (temperature ≥ 38.5°C and absolute neutrophil count < 1,000/μL)

● Active uncontrolled viral, bacterial or fungal infection (i.e., requiring intravenous anti-infectives or extensive supportive care)

● Hemorrhage (gastrointestinal, genito-urinary, pulmonary with platelets < 25,000/μL or any central nervous system hemorrhage)

Treatment may be resumed once the above conditions have improved or have been stabilized with adequate treatment (anti-infective therapy, transfusions).

Azacitidine dose reductions are not recommended other than specified below. If a dose reduction is believed to be necessary for different reasons, a discussion with the Sponsor is required.

Azacitidine dose interruption is not recommended within a cycle unless an unacceptable or unexpected study drug combination related toxicity occurs. Azacitidine and pembrolizumab may have some overlapping toxicities. Dose modifications/interruptions recommendations for non-hematologic toxicities:

- For any grade 3 or higher diarrhea/colitis, hold azacitidine and pembrolizumab. <u>If not immune-related colitis</u>, and if symptoms resolve within 5 days with maximum therapy to ≤ grade 1, may restart azacitidine and pembrolizumab as per schedule. The azacitidine doses can be resumed in the same cycle if no more than a 5 day gap occurred and/or the patient is still likely to have clinical benefit. Pembrolizumab dose will be omitted if delayed for more than 7 days. <u>If not immune-related colitis</u> and but if it requires more than 5 days for toxicities to resolve to ≤ grade 1 then dose reduce azacitidine to 75 mg/m^2 x 5 day in the next cycle and pembrolizumab to every 4 weeks. For recurrent grade 3 colitis, azacitidine dose can be reduced to 75 mg/m^2 x 5 days (if

receiving 75 mg/m^2 x 7 days) or to 50 mg/m^2 x 5 days (if receiving 75 mg/m^2 x 5 days). Even if not considered immune-related colitis, unless there is an objective reason (i.e., *C diff* infection, viral infection) for recurrent grade 3 colitis, the pembrolizumab will be discontinued for second occurrence of grade 3 colitis. If there is an objective reason, pembrolizumab may be reduced to every 6 weeks (if given every 4 weeks) or to every 4 weeks (if given every 3 weeks).

- For grade 2 diarrhea/colitis that persists more than 3 days hold azacitidine. Pembrolizumab should be held immediately for grade 2 diarrhea/colitis. If not immune-related colitis, and if symptoms resolve within 7 days with maximum therapy to ≤ grade 1, may restart azacitidine and pembrolizumab as per schedule. The azacitidine doses can be resumed in the same cycle if no more than a 5 day gap occurred and/or the patient is still likely to have clinical benefit. Pembrolizumab dose will be omitted if delayed for more than 7 days. If not immune related colitis but if it requires more than 7 days for toxicities to resolve to ≤ grade 1 then dose reduce azacitidine to 75 mg/m^2 x 5 days in the next cycle, and reduce pembrolizumab to every 4 weeks. For any recurrent grade 2 diarrhea/colitis lasting more than 7 days, the dose of azacitidine should be reduced to 75 mg/m^2 x 5 days (if pt is on azacitidine 75 mg/m^2 x 7 days) or to 50 mg/m^2 x 5 days (if pt is on azacitidine 75 mg/m^2 x 5 days). If not immune related recurrent grade 2 diarrhea/colitis but if it requires more than 7 days for toxicities to resolve to ≤ grade 1, dose-reduce pembrolizumab to every 4 weeks (if on every 3 weeks schedule) or every 6 weeks (if on every 4 weeks schedule).

- If unexplained reductions in serum bicarbonate levels to < 20 mEq/L occur, azacitidine dosage should be reduced by 50% on the next course (50 mg/m^2 x 5 days). Similarly, if unexplained elevations of BUN or serum creatinine occur, the next cycle should be delayed until values return to normal or baseline and the dose of the azacitidine should be reduced by 50% on the next treatment course.

Azacitidine administration should not be interrupted if pembrolizumab dosing is interrupted for toxicity except as defined above or at the discretion of the investigator for any clinical toxicities where ongoing administration of azacitidine is felt to be unsafe (grade 3 or 4 pembrolizumab-related toxicities). The azacitidine doses can be resumed in the same cycle if no more than a 5 day gap occurred, the patient is still likely to have clinical benefit, and the investigator feels that it is safe to restart azacitidine therapy. No dose reduction of azacitidine will be required.

Patients experiencing Grade 3 or 4 toxicities potentially attributable to pembrolizumab should have their pembrolizumab treatment interrupted regardless of when it occurs in the cycle until the toxicity resolves or returns to baseline.

Cycle duration maybe extended beyond 28 days to allow resolution of toxicities related to study treatments (see Table 4). In general, the next cycle of treatment should be restarted when drug-related toxicities resolve to ≤ 1; even if pembrolizumab cannot be administered (ongoing steroid taper), azacitidine should be restarted. The pembrolizumab can be restarted on any day

of the cycle when the criteria are met for its administration (steroids tapered to the required level) and according to the dose reduction recommendations.

If for unforeseen circumstances (for reasons other than toxicity, eg, emergency surgery, unrelated medical events, or logistical reasons not related to study therapy such as patient vacation, and/or holidays) the patient misses a doses of azacitidine within a cycle, the doses can be resumed in the same cycle if no more than a 5 day gap occurred and the patient is still likely to have clinical benefit. The dose of pembrolizumab within 7 days of surgery will be omitted.

If a treatment delay continues beyond Day 28 of the current cycle, then the day when azacitidine treatment is restarted will be counted as Day 1 of the next cycle.

5.2.1.5.2 Definitions of hematologic toxicity

5.2.1.5.2.1 For patients with ANC ≥ 500/μL at baseline:
Persistent neutropenia (ANC < 500/μL) for greater than 42 days without evidence of leukemia in peripheral blood or on bone marrow examination.

5.2.1.5.2.2 For patients with baseline ANC < 500/μL:
Patients with baseline neutropenia (ANC < 500/μL) which is persistent for greater than 42 days without evidence of leukemia in peripheral blood or on bone marrow examination. All patients will be evaluated for time course and severity of neutropenia to determine whether any trend can be identified related to study drug administration. Patients with baseline neutropenia (ANC < 500/μL) can proceed to cycle 2 with persistent neutropenia (ANC < 500/μL) without having repeat bone marrow assessment. However, bone marrow assessment is required after cycle 2.

5.2.1.5.2.3 For patients with non-transfused platelet count of ≥ 20,000/μL at baseline:
Persistent thrombocytopenia (platelet count of < 20,000/μL or necessitating platelet transfusion for more than 42 days) without evidence of leukemia in peripheral blood or on bone marrow evaluation.

5.2.1.5.2.4 For patients with baseline thrombocytopenia (platelet count < 20,000/μL or platelet transfusion dependent)
Patients with baseline thrombocytopenia (platelet count < 20,000/μL) which is persistent for greater than 42 days without evidence of leukemia in peripheral blood or on bone marrow examination. The time course and severity of thrombocytopenia will be evaluated on all patients to determine whether any trends can be identified related to study drug administration. Patients with baseline thrombocytopenia (platelet count < 20,000/μL) can proceed to cycle 2 with persistent thrombocytopenia (platelet count < 20,000/μL) without having repeat bone marrow assessment. However, bone marrow assessment is required after cycle 2.

5.2.1.5.3 Dose delays/modifications for hematologic toxicity

There will not be any dose delays for hematological toxicities given that these patients have acute myeloid leukemia. Delays in dosing would not be helpful in the context of active leukemia. We do not expect that acute leukemia will be cured with only one or two cycles of this therapy and this underscores the importance of staying on schedule. If a patient is evaluable for drug induced neutropenia or thrombocytopenia as discussed in section 5.2.1.5.2 and develops critical neutropenia or thrombocytopenia (as defined in sections 5.2.1.5.2.1-5.2.1.5.2.4) lasting more than 42 days, azacitidine and pembrolizumab will be held for up to 28 days, only if bone marrow or peripheral blood does not show evidence of active leukemia. [Exception are patients with baseline neutropenia (< 500/μL) and thrombocytopenia (< 20,000/μL who can proceed to cycle 2 with persistently low count without need for bone marrow assessment]. If this happens, dose reduction to the next dose level AZA 75 mg/m^2 x 5 days (or AZA 50 mg/m^2 x 5 days if pt receiving AZA 75 mg/m^2 x 5 days), will be required in the next cycle. Subsequent cycles of azacitidine will be administered only once the ANC is ≥ 500/μL (cycle 2 and 3) and platelets ≥20,000/μL (cycle 2 and 3) in the absence of residual leukemia in the bone marrow. In patients with baseline neutropenia (< 500/μL) and thrombocytopenia (< 20,000/μL, and bone marrow assessment after cycle 2 shows no evidence of leukemia, cycle 3 may proceed after ANC ≥ 500/μL and platelets ≥20,000/μL. After the first 3 cycles of therapy, subsequent cycles of azacitidine will be administered only if ANC is ≥ 1,000/μL and platelets ≥30,000/μL in the absence of residual leukemia in the bone marrow. Also, if there is increasing blasts in peripheral blood or the investigator has a suspicion that there is progressive disease, then a bone marrow biopsy can be performed at any time. Any dose reductions/delays during cycle 1, 2 or subsequent cycles should be discussed with the Protocol Chair before being made.

First occurrence: If the counts do not return to baseline prior to the next cycle, a bone marrow aspirate/biopsy should be performed and patients evaluated to determine if they have evidence of persistent disease or bone marrow suppression. Patients may resume next cycle when counts have recovered or if there is evidence of persistent leukemia.

5.2.1.5.4 Dose Reductions for Azacitidine and Pembrolizumab

Following dosing interruption or cycle delay due to toxicity, the azacitidine and/or pembrolizumab dose may need to be reduced when treatment is resumed.

Dose reduction of azacitidine and/or pembrolizumab-if necessary, 2 dose levels will be allowed depending on the type and severity of toxicity encountered.

Table 5. Dose Modifications for Azacitidine and Pembrolizumab

Dose modification	Azacitidine	Pembrolizumab
1st dose reduction	75 mg/m^2 x 5 days	200 mg every 4 weeks
2nd dose reduction	50 mg/m^2 x 5 days	200 mg every 6 weeks

Patients requiring more than 2 dose reductions of azacitidine should permanently discontinue both azacitidine and pembrolizumab, unless patient has achieved response (CR, PR, MLFS, HI, SD) in which case patients may be allowed to continue pembrolizumab only as long as they have ongoing clinical benefit without evidence of significant toxicity or disease progression and up to 2 years.

All dose modifications/adjustments must be clearly documented in the patient's notes and CRF. Once dose has been reduced, all subsequent cycles should be administered at that dose level, unless further dose reduction is required. Dose re-escalation is not allowed.

Once the azacitidine dose has been reduced for a given patient, subsequent cycles should be administered at that dose level. If a patient undergoes two cycles at a reduced dose without recurrent hematologic toxicity, dose re-escalation to the next higher dose level is allowed if the dose was initially reduced for hematologic toxicity. Azacitidine dose re-escalation must be discussed and agreed with the Sponsor. If hematologic toxicity recurs, the patient will have the prior dose reduction re-instituted and will not be eligible for a second round of dose re-escalation.

5.2.2 Timing of Dose Administration

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

All trial treatments will be administered on an outpatient basis unless the patient is already hospitalized or requires monitoring during initial therapy for AML as per standard of care.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks (treatment cycle intervals may be increased due to toxicity as described in Section 5.2.1.4 and 5.2.1.5). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

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

Azacitidine 75 mg/m^2 will be administered subcutaneously or as an IV infusion (10-40 minutes) on scheduled days 1-7 as per the Trial Flow Chart (treatment duration and/or dose may be decreased due to toxicity as described in Section 5.2.1.5).

On the days of therapy with both pembrolizumab and azacitidine, the azacitidine should be administered first.

5.2.3 Trial Blinding/Masking

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

5.3 Randomization or Treatment Allocation

Patients will be allocated to two different Cohorts, Cohort 1- relapsed/refractory AML and Cohort 2- newly diagnosed AML in patients ≥65 years old and not considered candidates for intensive induction chemotherapy. Each Cohort has a specific Inclusion/ Exclusion criteria and statistics/monitoring rules.

5.4 Stratification

There are no stratification factors.

5.5 Drug Information

5.5.1 Pembrolizumab

5.5.1.1 Mechanism of Action

Pembrolizumab (MK-3475) is a potent and highly selective IV humanized monoclonal antibody (mAb) of the immunoglobulin (Ig) G4/kappa isotype that directly blocks the interaction between Programmed Death-1 (PD-1) and its ligands, PD-L1 and PDL-2. KeytrudaTM (Pembrolizumab) has recently been approved (at a dose of 2 mg/kg IV every 3 weeks) 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.

5.5.1.2 Drug Supply

Pembrolizumab will be provided at no cost to the study patient by Merck, the manufacturer of the drug. Information on clinical supply, storage and handling requirements are provided in Section 9.0 and in the Pharmacy Manual provided as a document separate from this protocol.

5.5.1.3 Adverse Events Associated with Pembrolizumab

The most common adverse reactions (reported in ≥20% of patients in clinical trials of pembrolizumab) included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea.

The following warnings are associated with the use of pembrolizumab:

Immune-Mediated Pneumonitis

Pneumonitis occurred in ~3% of melanoma patients treated in clinical trials of pembrolizumab. The median time to development of pneumonitis was 5 months with a median duration of 4.9 months. The one patient with Grade 3 pneumonitis required initial treatment with high-dose systemic corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. Pneumonitis completely resolved in seven of the nine patients with Grade 2-3 pneumonitis.

Immune-Mediated Colitis
Colitis (including microscopic colitis) occurred in 1% of melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of was 6.5 months with a median duration of 2.6 months. All three patients with Grade 2 or 3 colitis were treated with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day).

Immune-Mediated Hepatitis
Hepatitis (including autoimmune hepatitis) occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 22 days for the case of Grade 4 hepatitis which lasted 1.1 months. The patient with Grade 4 hepatitis permanently discontinued pembrolizumab and was treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) systemic corticosteroids followed by a corticosteroid taper. Both patients with hepatitis experienced complete resolution of the event.

Immune-Mediated Hypophysitis
Hypophysitis occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 1.7 months for the patient with Grade 4 hypophysitis and 1.3 months for the patient with Grade 2 hypophysitis. Both patients were treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) corticosteroids followed by a corticosteroid taper and remained on a physiologic replacement dose.

Renal Failure and Immune-Mediated Nephritis
Nephritis occurred in 3 (0.7%) patients of melanoma patients treated in clinical trials of pembrolizumab, consisting of one case of Grade 2 autoimmune nephritis (0.2%) and two cases of interstitial nephritis with renal failure (0.5%), one Grade 3 and one Grade 4. The time to onset of autoimmune nephritis was 11.6 months after the first dose of pembrolizumab (5 months after the last dose) and lasted 3.2 months; this patient did not have a biopsy. Acute interstitial nephritis was confirmed by renal biopsy in two patients with Grades 3-4 renal failure. All three patients fully recovered renal function with treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper.

Immune-Mediated Hyperthyroidism
Hyperthyroidism occurred in 5 (1.2%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset was1.5 months and the median duration was 2.8 months (range 0.9 to 6.1). One of two patients with Grade 2 and the one patient with Grade 3 hyperthyroidism required initial treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. One

patient (0.2%) required permanent discontinuation of pembrolizumab due to hyperthyroidism. All five patients with hyperthyroidism experienced complete resolution of the event.

Immune-Mediated Hypothyroidism
Hypothyroidism occurred in 34 (8.3%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of hypothyroidism was 3.5 months. All but two of the patients with hypothyroidism were treated with long-term thyroid hormone replacement therapy. The other two patients only required short-term thyroid hormone replacement therapy. No patient received corticosteroids or discontinued pembrolizumab for management of hypothyroidism. Thyroid disorders can occur at any time during treatment.

Other Immune-Mediated Adverse Reactions
Other clinically important immune-mediated adverse reactions can occur. The following clinically significant, immune-mediated adverse reactions occurred in less than 1% of patients treated with pembrolizumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures arising in a patient with inflammatory foci in brain parenchyma, and adrenal insufficiency.

Across clinical studies with pembrolizumab in approximately 2000 patients, the following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients: myasthenic syndrome, optic neuritis, and rhabdomyolysis.

Embryofetal Toxicity
Based on its mechanism of action, pembrolizumab may cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PDL-1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue.

5.5.2 Azacitidine

5.5.2.1 Mechanism of Action

Azacitidine is an analogue of the naturally occurring pyrimidine nucleoside cytidine. Two main mechanisms behind antitumor activity of azacitidine include cytotoxicity, resulting from incorporation into RNA and DNA, and DNA hypomethylation, restoring normal growth control and differentiation in hematopoietic cells. Induction of DNA hypomethylation appears to require lower azacitidine doses than does cytotoxicity, as the concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not suppress DNA synthesis. Upon uptake by cells, azacitidine is converted to its active compounds, 5-Azacitidine triphosphate, which is incorporated into RNA, and 5-azadeoxycitidine triphosphate, which is incorporated into DNA. Azacitidine inhibits methylation of replicating DNA by stoichiometric binding with DNA methyltransferase 1, resulting in DNA hypomethylation. DNA hypermethylation at the CpG islands has been described in MDS, AML, and other malignancies.

5.5.2.2 Drug Supply and Preparation

Azacitidine is commercially available. Azacitidine is supplied as 100 mg of white, lyophilized powder with 100 mg of mannitol, USP in 30 ml flint vials. For subcutaneous injection: The contents of each vial should be dissolved in 4mL of sterile water or 0.9% sodium chloride to provide a 25 mg/ml slurry. Azacitidine does not go into solution but forms a loose slurry when reconstituted in this fashion. Do not inject the slurry intravenously. Single injections should not exceed 2ml. Doses requiring larger volumes may be split into multiple injection sites if volume to be administered is too large. Injection sites should be rotated on a daily basis. For intravenous injection: Reconstitute the appropriate number of azacitidine vials to achieve desired dose. Reconstitute each vial with 10 milliliters (mL) of sterile water for injection for a resulting concentration of 10 milligrams/mL. Shake vigorously or roll the vial until solution is clear. The desired amount of azacitidine solution should be withdrawn and injected into 50 to 100 milliliters of either 0.9% Sodium Chloride or Lactated Ringer's Injection.

5.5.2.3 Storage and Stability

Store un-reconstituted vials at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) (See USP Controlled Room Temperature). Azacitidine reconstituted for subcutaneous administration may be stored for up to 1 hour at 25°C (77°F) or for up to 8 hours between 2-8°C (36 and 46°F). The constituted solutions hydrolyze at room temperature and should be used within 30 minutes for delivery of maximum potency. Administration of Azacitidine reconstituted for intravenous administration must be completed within 1 hour of reconstitution. The reconstituted azacitidine may be stored for up to 1 hour at 25°C (77°).

5.5.2.4 Route of Administration

Route of administration is via subcutaneous or intravenous injections. Injections are to be given by trained nursing staff at each institution. Reconstituted solutions of azacitidine are unstable. Upon reconstitution, the material should be injected within 30 minutes. To provide a homogeneous suspension, the contents of the syringe must be re-suspended by inverting the syringe 2-3 times and vigorously rolling the syringe between the palms for 30 seconds immediately prior to administration until a uniform, cloudy suspension is achieved. For intravenous administration, the total dose of diluted azacitidine solution should be administered over 10 to 40 minutes. Administration must be completed within 1 hour of the reconstitution of the azacitidine vial.

5.5.2.5 Adverse Events Associated with Azacitidine

Hematologic toxicities include leukopenia, neutropenia, thrombocytopenia, and anemia. GI toxicities include nausea and vomiting, diarrhea, stomatitis and mucositis. Increased liver function tests and renal tubular acidosis can be seen. Rarely, pulmonary edema, arrhythmia, pericarditis, hypotension, hepatic coma, CNS and neuromuscular toxicity have been seen. Rash, allergic reactions, fever, conjunctivitis and alopecia have also been noted. Local

injection reactions are common and include burning, pain, violaceous (purple) discoloration lasting up to ten days, and persistent brown discoloration. In clinical studies, the most commonly occurring adverse reactions were nausea (71%), anemia (70%), thrombocytopenia (66%), vomiting (54%), pyrexia (52%), leukopenia (48%), diarrhea (36%), fatigue (36%), injection site erythema (35%), constipation (34%), neutropenia (32%) and ecchymosis (31%). Other adverse reactions included dizziness (19%), chest pain (16%), febrile neutropenia (16%), myalgia (16%), injection site reaction (14%) and malaise (11%).

5.6 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 is be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.6.1 Acceptable Concomitant Medications

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

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

5.6.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy with the exception of hydroxyurea (to be stopped ≥12 hours prior to first chemotherapy dose on study). If the WBC increases > 30,000/µl during cycle 1, hydroxyurea may be resumed, to be discontinued prior to day 1 of cycle 2. If the WBC increases to > 30,000/µl prior to cycle 3, the patient will discontinue study treatment (unless increase in WBC is not leukemia-related, such as with steroid use, growth factors or infection).
- 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, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

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

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

5.7 Rescue Medications & Supportive Care

5.7.1 Antiemetics

Antiemetics will be used according to standard practices with the exception of systemic steroids (i.e. dexamethasone). Use of systemic steroids during this trial is not allowed. Any 5-HT3 receptor inhibitor, or alternative antiemetics such as prochlorperazine, haloperidol, lorazepam, or similar as needed, will be used during azacitidine treatment and pembrolizumab administration as needed.

5.7.2 Antimicrobial Prophylaxis

Patients will receive prophylaxis against gram-negative gastrointestinal infections, candidiasis or other fungal infections, and herpes simplex virus, as per standard of care.

5.7.3 Colony Stimulating Factors

The routine use of colony stimulating factors is not allowed. The use of colony stimulating factors in the presence of severe or life-threatening infection should be discussed with the principal investigator before implementation.

5.7.4 Prevention and Management of Tumor Lysis Syndrome

Tumor lysis may occur as part of initial cytoreductive therapy for AML. Tumor lysis labs consisting of comprehensive metabolic panel, uric acid, phosphate, and LDH will be monitored at least twice a week during the first week of treatment or more frequently if clinically indicated and according to standard practice.

The following prophylaxis regimen is suggested prior to and during chemotherapy administration:

- To prevent hyperuricemia, all patients without known allergy can receive Allopurinol 300 – 600 mg orally daily with chemotherapy as long as clinically indicated based on the laboratory parameters and the risk of tumor lysis. Rasburicase can be used per institutional policy for hyperuricemia. Screening for G6PD deficiency should be obtained in susceptible population before beginning rasburicase.

- To decrease the risk of hyperphosphatemia, an oral phosphate binder per institutional practice (e.g. sevelamer 400-800 mg) can be administered orally every 4 to 6 hours with the start of chemotherapy and continue as long as clinically indicate based on the laboratory parameters and the risk of tumor lysis.

5.7.5 Supportive Care Guidelines for Pembrolizumab

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

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

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

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

    Administer additional anti-inflammatory measures, as needed.

- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

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

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis**, treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

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

- **Hypophysitis**:

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

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

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

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

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

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

  Table 6 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 6. 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 permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
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.		

5.8 Diet/Activity/Other Considerations

5.8.1 Diet

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

5.8.2 Contraception

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

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

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

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

OR

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

OR

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

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

(1) practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

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

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

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

Subjects should be informed that taking the study medications may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.8.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab and azacitidine, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck

if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

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

5.8.4 Use in Nursing Women

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

5.9 Subject Withdrawal/Discontinuation Criteria

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

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Failure to achieve WBC $\leq 30 \times 10^9$/L off hydroxyurea after 2 cycles of therapy.

  Note: If increase in WBC is not leukemia-related such as seen with the use of steroids, infections, or growth factors and does not require hydroxyurea patient can remain on the study.
- Confirmed disease progression or relapse

  Note: Please see Section 7.1.2.6 for explanation.
- Unacceptable adverse experiences as described in Section 5.2.1.4 and 5.2.1.5
- 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 (interrupted or uninterrupted) with azacitidine and pembrolizumab. *Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop azacitidine and pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.5.5*
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.3.2). 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.

5.9.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment after achievement of CR/CRi is not recommended and the goal would be to administer up to 24 months of treatment; however, discontinuation of treatment may be considered at the discretion of the investigator for subjects who have attained a confirmed CR/CRi that have been treated for at least 6 cycles with azacitidine/pembrolizumab and had at least four treatments (cycles) with azacitidine/pembrolizumab beyond the date when the initial CR/CRi was declared. Subjects who then experience disease relapse may be eligible for up to one year of additional treatment with azacitidine/pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of azacitidine/pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.5.5.

5.10 Subject Replacement Strategy

Safety Run In Phase:

- If laboratory abnormalities and clinical situation exist on day 8 during cycle 1 precluding pembrolizumab administration, pembrolizumab administration can be delayed for 2 weeks, up to day 22, provided laboratory abnormalities correct themselves to the levels defined in Section 5.1 and clinical situation resolves/improves.

Patients who are ineligible for pembrolizumab administration by day 22 of cycle 1 will be removed from the study and replaced by another subject.

- If subject discontinue study treatment for any reason other than drug-related toxicity prior to completing 2 study cycles, this subject will be replaced.

- If subject discontinue study treatment before receiving a single dose of pembrolizumab, this subject will be replaced.

5.11 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

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

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screening Phase**	**Treatment Cycles**								**End of Treatment**			**Post-Treatment**
						To be repeated beyond Cycle 3				Discontinuation	Safety Follow-up	Follow Up Visits[j]	Survival Follow-Up
Treatment Cycle/Title:	Main Study Screening	C1 and C2 D1	C1 and C2 D2-7	C1 D8	C1 and C2 D15, D22	C3 D1	C3 D2-7	C3 D8, D15	C3 D22				
Scheduling Window (Days):	-14 to -1	±3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discontinuation	30 days post discon (± 4 days)	Every 8 weeks post discon[j]	Every 12 weeks
Administrative Procedures													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X				X							
Azacitidine Administration		X[a]	X[a]			X[a]	X[a]						
Pembrolizumab Administration		X[a]		X[a]	X[a]	X[a]		X[a]	X[a]				
Post-study anticancer therapy status	X												X
Survival Status											X	X	X
Clinical Procedures/Assessments													
Review Adverse Events		X	X	X	X	X		X[b]	X[b]	X	X		
Full Physical Examination	X	X				X				X	X		
Directed Physical Examination		X		X[b]	X[b]			X[b]	X[b]	X	X	X	
Vital Signs and Weight[g]	X	X	X	X[b]	X	X	X	X[b]	X[b]	X	X	X	

Trial Period:	**Screening Phase**	**Treatment Cycles**								**End of Treatment**			**Post-Treatment**
						To be repeated beyond Cycle 3				Discontinuation	Safety Follow-up	Follow Up Visits[j]	Survival Follow-Up
Treatment Cycle/Title:	Main Study Screening	C1 and C2 D1	C1 and C2 D2-7	C1 D8	C1 and C2 D15, D22	C3 D1	C3 D2-7	C3 D8, D15	C3 D22				
Scheduling Window (Days):	-14 to -1	±3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discontinuation	30 days post discon (± 4 days)	Every 8 weeks post discon[j]	Every 12 weeks
Height	X												
ECOG Performance Status	X	X				X						X	
Electrocardiogram	X												
Echocardiogram[k]	X												
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Pregnancy Test – Urine or Serum β-HCG[h]	X					X							
PT/INR, aPTT, fibrinogen	X	X		X	X	X							
CBC with Differential	X	X		X	X	X		X[C]	X[c]	X	X	X	
Comprehensive Serum Chemistry Panel	X	X		X	X	X		X[C]	X[c]	X	X	X	
Uric Acid, Phosphate, Magnesium, LDH	X	X		X	X	X		X[c]	X[c]	X	X	X	
T3, FT4 and TSH	X					X[i]							
Urinalysis	X												
Efficacy Measurements													
Bone Marrow Aspirate/Biopsy[d,f]	X[d,f]				X[d,f]				X[d,f]	X[d,f]		X[d,f]	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood													
Correlative Studies Peripheral Blood Collection	X[e]	X[e]		X[e]	X[e]	X[e]				X[e]		X[e]	

Trial Period:	**Screening Phase**	**Treatment Cycles**								**End of Treatment**			**Post-Treatment**
						To be repeated beyond Cycle 3				Discontinuation	Safety Follow-up	Follow Up Visits[j]	Survival Follow-Up
Treatment Cycle/Title:	Main Study Screening	C1 and C2 D1	C1 and C2 D2-7	C1 D8	C1 and C2 D15, D22	C3 D1	C3 D2-7	C3 D8, D15	C3 D22				
Scheduling Window (Days):	-14 to -1	±3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discontinuation	30 days post discon (± 4 days)	Every 8 weeks post discon[j]	Every 12 weeks
Correlative Studies Bone Marrow Collection	X[d,f]				X[d,f]				X[d,f]	X[d,f]		X[d,f]	

[a] Azacitidine should be administered without interruption in each cycle. Pembrolizumab to be administered starting on day 8 cycle 1 every 3 weeks. (See Section 5.2.1.4., 5.2.1.5, and 5.2.1.5.1 for specific exceptions)

[b]During cycle 1 and 2, patients will be seen weekly. In subsequent cycles, the frequency of study required visits will be reduced to d1 prior to azacitidine and prior to each pembrolizumab administration. However, patients may be seen more frequently as needed according to standard of care for management of patients with AML as determined and at the discretion of investigator/treating physician.

[c] During cycle 1 and 2, patients will have laboratory tests weekly. However, patients may have laboratory tests more frequently (twice or more per week) as needed according to standard of care for management of patients with AML as determined and at the discretion of the investigator/treating physician. In subsequent cycles, patient is required to have laboratory tests on day 1 and days of pembrolizumab administration in that cycle. However, patients may have laboratory tests more frequently (once or more per week) as needed according to standard of care for management of patients with AML as determined and at the discretion of the investigator/treating physician.

[d]Bone marrow aspirate and biopsy will be done as per standard of care: pre-treatment, after the completion of cycle 2, cycle 6, and every 3 months thereafter (up to 1 yr). If necessary (in the absence of count recovery as defined in Section 5.2.1.3 and 5.2.1.5.2) bone marrow as per standard of care will be done after cycle 1. In a second year, bone marrow aspirate and biopsy can be performed every 4-6 months and at the end of treatment. Bone marrow aspirate and biopsies will be performed every 4-6 months in a follow up phase. Bone marrow aspirate and biopsies can be performed at any time to evaluate for hematologic toxicity (Section 5.2.1.5.3) or if disease progression/relapse is suspected either during treatment or in follow up, or at treatment discontinuation. Each bone marrow should include morphologic assessment (aspirate/biopsy), flow cytometry, and karyotype

and/or molecular study if indicated. If karyotype was performed within 3 months prior to day 1 of treatment, it does not need to be repeated pre-treatment.

[e]For peripheral blood samples for research, 5x8.5mL light yellow top ACD tubes (or 4x10mL green top heparin tubes) of blood (~40mLs) will be collected pre-treatment on D1 of cycle 1 (for cycle 1 sample can be collected any time within 14 days prior to treatment) and cycle 2, on D8 of cycle 1 and D22 of cycle 2 (or on days of pembrolizumab administration in either cycle). Starting with cycle 3, peripheral blood samples will be collected only on day 1 of every other cycle (Cycle 3, 5, etc), at the time of the follow up visits. All samples should be collected prior to azacitidine/pembrolizumab administration if on a treatment day. Repeat blood sample at any time if there is a suspicion of leukemia recurrence either during maintenance phase or in follow-up, or at treatment discontinuation.

[f]The schedule of bone marrow procedures performed according to standard of care is delineated under [d]. Each time a bone marrow biopsy is done as per standard of care, additional ~40 mL of bone marrow will be collected in 5x8.5mL light yellow top ACD tubes (or 4x10mL green top heparin tubes).).

[g] Weight should be recorder only prior to start of each Cycle. Vital signs including temperature, pulse, respiratory rate, and blood pressure will be checked immediately before pembrolizumab infusion.

[h]Pregnancy test will be repeated every 3 cycles in women of childbearing potential.

[i]Thyroid function monitoring every 3 cycles.

[j] This applies only to patients who completed treatment and were not taken from study for progressive disease and did not start any other treatment.

[k] If an echocardiogram was conducted within the 3 months prior to the screening date and the patient has not received any chemotherapy in the time between and has not experienced any cardiac symptoms or events (heart attack, chest pain, arrhythmia, etc), then the screening echocardiogram does not need to be repeated, unless it is clinically indicated per investigator's discretion.

* In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participant's risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

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

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

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

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

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available

that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

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

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

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

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

7.1.1.4.2 Concomitant Medications

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

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

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.

7.1.1.6 Assignment of Screening Number

The research nurse or data manager at the Coordinating Center, Johns Hopkins, should be contacted to confirm a treatment slot is available before approaching a subject.

All subjects must be registered with the Clinical Research Office at Johns Hopkins SKCCC before enrollment to study.

To register a patient, the following documents should be completed by the research nurse or data manager and faxed *(410-614-2449)* or e-mailed *onc-coordcnt@jhmi.edu* to the Study Coordinator:

- Signed patient consent form
- Registration form
- Source documents

Subjects will be assigned a screening number (ID) at screening. The screening ID will be used on all correlative samples.

Treatment may not begin until eligibility has been confirmed by the Coordinating Center.

Confirmation will be scanned and e-mailed, or faxed to the registering site. The screening ID number will become the study ID.

7.1.1.7 Assignment of Randomization Number

This study has no randomization. However, there are two separate Cohorts of patients, Cohort 1 and Cohort 2. Each Cohort will have a unique study ID.

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

Compliance to the study procedures will be assessed at each visit and documented in the source document.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

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

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

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period and prior to each cycle. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.1.2.3 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.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, and blood pressure. Weight will be measured at screening and prior to start (day 1) of each Cycle. Height will be measured at screening only.

7.1.2.5 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 cycle and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Assessment of Disease

Bone marrow aspirate and biopsy and peripheral blood counts will be used to assess disease as per the Trial Flow Chart. Response will be assessed by standard international criteria proposed by the European LeukemiaNet Guidelines (update 2017) in AML[63] and by IWG Modified Response Criteria (2006) for MDS (for definition of HI)[64].

Complete remission (CR): Bone marrow blasts <5%; absence of circulating blasts or blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count ≥1,000/mcL; platelet count ≥100,000/mcL.

Complete remission with incomplete recovery (CRi): All CR criteria except for residual neutropenia (<1,000/mcL) or thrombocytopenia (<100,000/mcL).

Complete remission without minimal residual disease (MRD): If studied pre-treatment, CR with negativity for a genetic marker by realtime quantitative polymerase chain reaction (RT-qPCR), or CR with negativity by multi-color flow cytometry. Sensitivities vary by marker tested, and by method used. Since this study does not use centralized laboratory, MRD status will be recorded if available for each patient that achieves CR or CRi.

Morphologic leukemia-free state (MLFS): Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required (marrow should not be merely aplastic (marrow should not be merely "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%).

Partial response (PR): bone marrow blasts 5-25% and decrease of pretreatment bone marrow blast % by >50%; all hematologic criteria of CR.

Hematologic Improvement (HI)-major:
Hematologic improvement –unconfirmed will be documented following the first cycle after which it is achieved. If hematologic improvement persists after the following cycle, the response will be changed to hematologic improvement –confirmed. Responses of hematologic improvement confirmed may be recorded prior to the formal response assessment. It is understood that after the four-month or more likely six-month assessment, some patients with hematologic improvement will have responses upgraded to complete and partial responses.

For the purpose of this study, the criteria for HI-major developed for the grading of response in MDS (IWG criteria) will be applied to patients with AML and will be considered responses.

HI-major response (must have at least one of the three listed below) must persist for a minimum of two months to be considered a confirmed response.

HI-major Erythroid:
For patients with pretreatment hemoglobin <11 g/dL, greater than 2 g/dL increase in hemoglobin; for RBC transfusion-dependent patients, transfusion independence.

HI-major Platelet:
For patients with a pretreatment platelet count less than 100,000/μL, an absolute increase of 30,000/μL or more; for platelet transfusion dependent patients, stabilization of platelet counts and platelet transfusion independence.

HI-major Neutrophil:
For absolute neutrophil count (ANC) less than 1000/μL before therapy, at least a 100% increase, or an absolute increase of more than 500/μL, whichever is greater.

Stable disease (SD): Absence of CR, CRi, PR, MLFS; and criteria for PD not met. Period of stable disease should last at least 3 months.

Progressive disease: Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood:
• >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 months; without at least a 100% improvement in ANC to an absolute level >500/mcL, and/or platelet count to >50,000/μL non-transfused]; or
• >50% increase in peripheral blasts (WBC x % blasts) to >25,000/μl (in the absence of differentiation syndrome); or
• New extramedullary disease

Progression is not a useful concept for monitoring in AML. Further, pembrolizumab is expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months to be evident. Azacitidine is a low-intensity chemotherapy agent that usually requires at least 4-6 cycles of therapy to exert its full anti-leukemia effect. Thus, some patients may have objective increase in leukemia burden or other disease parameters within weeks following the start of pembrolizumab/azacitidine dosing. Such patients may not have had sufficient time to develop the required immune activation or for azacitidine/pembrolizumab to exert their anti-leukemia effect. In conventional studies, such increase in leukemia burden (bone marrow, peripheral blood, extramedullary disease) or other disease parameters during the first 2-4 months of the study would constitute progressive disease as per above criteria and lead to premature discontinuation of therapy, thus disregarding the potential for subsequent immune-mediated clinical response. Therefore, patients with leukemia progression by bone marrow or peripheral blood laboratory parameters prior to completion of 6 cycles of therapy but without rapid clinical deterioration and who do not require additional immediate therapy,

may continue to be treated with pembrolizumab /azacitidine and clinically observed to allow detection of a subsequent tumor response. Subjects that meet the PD criteria and continue on study therapy must discontinue azacitidine/pembrolizumab if there are no signs of disease stabilization by end of 6 cycles of therapy (approximately 6 months). The date of progression will be defined as of the first observation date of progressive disease in those patients who do not upgrade response with continuous therapy.

However, we are still using progression of AML as a reason for early discontinuation of study treatment (before completing 6 cycles) with the following criteria:
1. Progression of the leukemia such that clinical sequelae would result from a high WBC blast count such as hyperleukocytosis, or due to increasing size of spleen or extramedullary disease..
2. Failure to achieve WBC $\leq 30 \times 10^9$/L off hydroxyurea after 2 cycles of therapy.
Note: If increase in WBC is not leukemia-related such as seen with the use of steroids, infections, or growth factors and does not require hydroxyurea patient can remain on the study.
3. New CNS leukemia or new extramedullary disease.
4. Progressive disease is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms. Patient can be removed from the study before cycle 6 at the discretion of the investigator / treating physician if in their assessment further treatment on the study would not be in patient's best interest. This should be discussed with the Protocol Chair and the reason for study treatment discontinuation at any time should be documented and confirmed by BM assessment. (*Note:* the goal is to administer at minimum 2 cycles of therapy before initial assessment.)

Relapse:
Relapse following CR/CRi is defined as:
Peripheral Blood Counts-Reappearance of blasts in the blood that persist and are not considered to be due to count or marrow recovery.
Bone Marrow Aspirate and Biopsy-Presence of ≥ 5% blasts, not attributable to another cause (e.g., bone marrow regeneration).If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, and if unclear if increase in the blasts is due to regeneration, reactive process or leukemia, then a repeat bone marrow performed ≥ 1 week later documenting ≥ 5% blasts is necessary to meet the criteria for relapse.

Molecular Relapse (after CR_{MRD-}): If studied pre-treatment, reoccurrence of MRD as assessed by quantitative RTqPCR or by multi-color flow cytometry. Sensitivities vary by marker tested, and by method used. Since this study does not use centralized laboratory, MRD status will be recorded but clinical relapse parameters will be used for survival endpoint assessment.

Progression after Hematologic Improvement
One or more of the following: a 50% or greater decrement from maximum response levels in granulocytes or platelets, a reduction in hemoglobin concentration by at least 2 g/dL, or transfusion dependence not due to other toxicities and bone marrow blasts ≥ 5%.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Tumor tissue collection and blood collection will be performed as the Trial Flow Chart and processed as detailed in in the Appendix 11.5.

7.1.3 Laboratory Procedures/Assessments

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

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 7.

Table 7. 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, fibrinogen
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		PK
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

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

‡ If considered standard of care in your region.

Laboratory tests for screening or entry should be performed within 14 days prior to the first dose of treatment. However, repeat laboratory tests are required within 72 hours prior to the first dose of treatment in Cycle 1. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.3.1 Pharmacokinetic/Pharmacodynamic Evaluations

7.1.3.1.1 Blood Collection for Serum Pembrolizumab- N/A

7.1.3.1.2 Blood Collection for Anti-Pembrolizumab Antibodies- N/A

7.1.3.1.3 Correlative Studies Procedures (Exploratory Biomarkers)

Samples will be immediately processed following our Standard Operating Procedure (Appendix 11.4) in the laboratory of Dr. Leo Luznik. Participating institutions will have an option to process specimens locally and batch ship or to ship specimens within 24 hours of collection to the laboratory of Dr. Luznik.

The pharmacodynamics of azacitidine and pembrolizumab treatment will be assessed by examining (quantifying) biomarkers in peripheral blood and bone marrow (tumor tissue). Additional biomarkers may be analyzed in peripheral blood and tumor tissue collected prior to and after initiation of therapy in order to identify markers that may predict response to treatment. The proposed studies will be done in collaboration with Univ of North Carolina. Detailed schedules of pharmacodynamic evaluations are provided in Trial Flow Chart Section 6.0.

7.1.3.1.3.1 Flow Cytometry Studies

We will perform multi-color flow cytometry on PB and BM specimens before and after AZA and AZA/pembrolizumab treatment using three pre-determined panels of mAbs including but not limited to those specific for CD3, CD4, CD8, PD-1, CD45RA, CCR7, CD27, CD28, Ki-67, T-bet, CD127, CD25, FoxP3, HLA-DR, CTLA-4, TNFRII, ICOS, TIM3, LAG-3, CD160, 2B4, BTLA, KLRG-1, CD16, CD14, and CD56. This multi-color approach allows phenotypic separation of human $CD4^{+}FoxP3^{+}$ T cells into three distinct subpopulations as well as assessment of their proliferative status (% Ki-67 positive cells). The expression of HLA-DR, TNFRII and ICOS will be measured to characterize their activation status. The expression of co-inhibitory molecules (PD-1, TIM3, LAG-3, CD160, 2B4, BTLA, KLRG-1) will also be examined in relation to different $CD4^{+}$ and $CD8^{+}$ T cell subpopulations (expressing CD45RA, CCR7, CD27, and CD28). This panel will be combined with assessment of effector cytokines (IL-2, IFN-γ, IL-17) or canonical transcription factors (T-bet, Eomes, Blimp-1). Additionally, natural killer cells (NK), NK-T cells, and myeloid- derived suppressor cells (MDSCs) will be enumerated using the following gating strategies: CD14+HLA-$DR^{neg/low}$ (MDSCs); CD16 and CD56 (NK/NK-T cells). We will also use a highly multiplexed flow cytometry panels to analyze expression of co-inhibitory molecules ligands (PD-L1, PD-L2, galectin-9, CD80,

CD86) on MDSCs, leukemia blasts, and leukemia stem cells. We will analyze three distinct AML subpopulations defined as $CD33^+CD117^+CD14^-$ (AML progenitors) $CD33^+CD117^-CD14^-$ (AML myelo/monoblasts), $CD33^+CD117^-CD14^+$ (AML promonocytes)[59] and $CD34^+CD38^-ALDH^{intermediate}$ (leukemia stem cells),[65] when feasible. Our group has extensive experience in characterizing immune recovery in AML patients during and after chemotherapy and allo-SCT[66,67].

7.1.3.1.3.2 Immune Profiling of T cell Repertoire

The studies proposed will evaluate the T cell receptor (TCR) diversity in T cells isolated from PB and BM from AML patients before and after treatment with AZA/pembrolizumab on the parent clinical trial. We will determine TCR diversity and clonal composition using a molecular and computational approach based on high-throughput DNA sequencing of rearranged TCRβ CDR3 regions from T cell genomic DNA. This approach allows direct measurement of the TCRβ CDR3 region sequence diversity in any arbitrarily complex population of T cells, and also permits quantitative description of the clonal composition of the population. The Luznik laboratory in collaboration with the E. Warren laboratory from Fred Hutchinson Cancer Research Center (FHCRC) is routinely performing this comprehensive in-depth analysis of the TCR repertoire in patients with MDS/AML treated with ipilimumab and have performed extensive monitoring and tracking of the TCR repertoires in patients undergoing allo-SCT (manuscript in preparation). We are utilizing an established multiplex PCR strategy to amplify the CDR3 region of the TCR, spanning the variable region formed by the junction of the V, D and J segments and their associated non-template insertions followed by Illumina-based sequencing methodology, an well-characterized methodology developed by Adaptive Technology (http://www.adaptivebiotech.com/technology/)[68]. Sequencing is followed by comprehensive bioinformatics analyses focused on determining the diversity of the T cell and B cell repertoires as well as the entropy and clonality of each repertoire consistent with previous studies[69]. Through these ongoing studies we have developed substantial experience not only in using and analyzing DNA retrieved from unsorted PBMCs but also from sorted T cells subpopulations (naïve vs memory vs regulatory), paired PB and BM samples and as well from DNA retrieved from FFPE archived tissues. We hypothesize that a primary action of AZA/pembrolizumab will be to disrupt the skewed T cell pattern present in AML patients prior to treatment toward increased TCR diversity and the emergence of unique TCR clonotypes that may be relevant in the anti-leukemia response. The long-term goal of this work is to uncover antigen- specific responses that, together with the other proposed correlative studies, will provide deeper insight into the mechanisms involved in anti-leukemic immune responses after AZA/pembrolizumab treatment.

7.1.3.1.3.3 T cell Methylation Analysis

We will examine IL-2 and PD-1 promoter methylation status in T cell subsets to gain insight into the changes in DNA methylation and expression of IL-2 and PD-1 before and after treatment. We will perform bisulfite sequencing to assess the level of DNA methylation at the individual CpG sites of the IL-2 and PD-1 promoter in T cells. Both $CD4^+$ and $CD8^+$ T cells from PB and BM will be analyzed. $CD4^+$ and $CD8^+$ T cells will be isolated by either magnetic cell separation of by flow cytometric sorting. Sequencing of at least 10 individual clones from

each sample will be performed. All independent experiments will be duplicated to avoid any PCR amplification bias. These studies will be coupled with those measuring mRNA production of IL-2, PD-1 and other effector cytokines. The overarching goal of these studies is to determining the relationship between IL-2 and PD-1 promoter DNA methylation and T cell differentiation status and its relation to the response to AZA/pembrolizumab treatment.

7.1.3.1.3.4 Assessment of serum pro-inflammatory and inhibitory cytokines

We will investigate changes in the cytokine levels in patients before and after treatment with azacitidine/pembrolizumab. We predict that levels of pro-inflammatory cytokines (Il-12, IFN-γ, RANTES) will be higher while those of inhibitory ones (IL-10, TGF-β) will be lower in patients treated with azacitidine/pembrolizumab.

7.1.3.1.3.5 DNA Sequencing and Gene expression profiling

Bone marrow aspirates will be used to perform RNA sequencing, or to examine mRNA gene expression by gene array technology and/or quantitative real-time polymerase chain reaction (qPCR) to detect expression of selected immune related genes before and after treatment. In addition, whole or targeted exome sequencing on the leukemia cells may be performed (lymphocyte control) for detection of immunogenic neoepitopes.

7.1.3.1.3.6 Characterization of tumor-infiltrating lymphocytes (TILs) and tumor antigens

Immunohistochemistry (IHC) will be used to assess the number and composition of immune infiltrates in order to define the immune cell subsets present within bone marrow before and after exposure to azacitidine and pembrolizumab These IHC analyses will include, but not necessarily be limited to, the following markers: CD4, CD8, FOXp3, PD-L1, and PD-L2. Correlations between gene expression and IHC expression will be made between assays performed if deemed to be informative.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR as specified in Section 5.9.1 or b) complete 24 months of treatment with azacitidine and pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.4.2 Blinding/Unblinding

N/A

7.1.5 Visit Requirements

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

7.1.5.1 Screening

7.1.5.1.1 Screening Period (day -14 to day -1)

Unless otherwise noted, evaluations may be performed up to 2 weeks prior to Cycle 1 Day 1 of treatment. Informed consent should be obtained prior to any screening procedure.

Clinical evaluation: demographics and medical history, prior and concomitant medications, full physical examination to include height (baseline only) and weight, ECOG performance status.

Laboratory studies:

- **Pregnancy Test**: A serum or urine pregnancy test (β-HCG) is required for all women of childbearing potential at screening within 72 hours prior to the first dose of azacitdine. If urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- **CBC with differential and platelets**
- **Serum Chemistries (include Comprehensive Chemistry, uric acid, phosphate, magnesium):** These include the following parameters: sodium, potassium, chloride, bicarbonate (or CO2), LDH, BUN, serum creatinine, glucose, calcium, magnesium, phosphorus, uric acid, total protein, and albumin
- **LFTs:** These include total bilirubin (direct if total bilirubin is elevated), alkaline phosphatase, AST (SGOT), ALT (SGPT)
- **Thyroid Function Tests:** These include TSH and if clinically appropriate free T3 and T4
- **Coagulation Tests:** These include PT/INR, aPTT, and fibrinogen
- **Urinalysis**
- **Electrocardiogram**
- **Echocardiogram**
  - If an echocardiogram was conducted within the 3 months prior to the screening date and the patient has not received any chemotherapy in the time between and has not experienced any cardiac symptoms or events (heart attack, chest pain, arrhythmia etc), then the screening

echocardiogram does not need to be repeated, unless it is clinically indicated per investigator's discretion.

Tissue Acquisition/Disease Assessment: Primary tumor will be collected by peripheral blood and bone marrow biopsy. Bone marrow biopsy and blood sample should be obtained within 14 days prior to day 1 of treatment.

7.1.5.2 Treatment Period

Subjects will be followed with provider visits, physical exams, adverse event monitoring, laboratory tests, disease assessments as per the Trial Flow Chart 6.0. Collection of specimens for exploratory correlative studies will be done according to Trial Flow Chart 6.0

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. 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. Subjects who are eligible for retreatment with azacitidine/pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.5.4 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 8 weeks (± 14 days) as per Trial Flow Chart including by bone marrow exam every 4-6 months to monitor disease status as appropriate. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with azacitidine/pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with azacitidine/pembrolizumab according to the criteria in Section 7.1.5.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression/relapse. Details are provided in Section 5.9.1. The same procedures will need to be followed for Retreatment as per Trial Flow Chart for Initial Treatment.

7.1.5.4.1 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.

7.1.5.5 Second Course Phase (Retreatment Period)

Subjects who stop azacitidine/pembrolizumab with CR/CRi may be eligible for up to one year of additional azacitidine/pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
    - Stopped initial treatment with azacitidine/pembrolizumab after attaining an investigator-determined confirmed CR/CRi
        - Was treated for at least 6 cycles with azacitidine/pembrolizumab before discontinuing therapy
        - Received at least four treatments with azacitidine/pembrolizumab beyond the date when the initial CR/CRi was declared

OR

    - Had SD, MLFS, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined bone marrow confirmed disease progression after stopping their initial treatment with azacitidine/pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of azacitidine/pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate counts and organ function as detailed in Section 5.1.2/5.1.3
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.

- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.8.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.

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

- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might 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.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Trial Flow Chart and should follow the same procedure as for initial treatment.

7.2 Assessing and Recording Adverse Events

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

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

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

Adverse events may occur during the course of the use of Merck product in clinical trials or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period 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.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.

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

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose of pembrolizumab or azacitidine by 20% over the prescribed daily dose. No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

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

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

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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies 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 Coordinating Center (Sponsor) and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

7.2.3.1 Responsibility for Data Submission

Study participants are responsible for submitting data and/or data forms to the Coordinating Center within two weeks of the completion of the time point. Adverse event review will be performed at a minimum every two weeks on the submission by the coordinating center contact and the study chair.

7.2.3.2 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions. AEs reported through MedWatch must also be reported in routine study data submissions.

7.2.3.2.1 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting (i.e., MedWatch). The following AEs must be reported through the routine reporting mechanism.

Adverse Events	CTCAE v4.0
Anemia	Anemia
Leukopenia	White blood cell decreased
Neutropenic Fever	Febrile neutropenia with or without hospitalization
Infection	Infection with Grade 3 or 4 neutrophils with or without hospitalization
Thrombocytopenia	Platelet count decreased

Grades 3 and 4 adverse events (including hospitalization/prolonged hospitalization) of expected events list do not require expedited reporting:

- Progression of the malignancy should NOT be reported as an AE/SAE unless it is considered to be drug related by the investigator.
- Hospitalization due to signs and symptoms of malignancy progression does NOT require reporting as an SAE.

7.2.3.3 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of study drugs 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 an other important medical event

- Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same

timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Refer to Table 7 for additional details regarding each of the above criteria.

7.2.3.3.1 Reporting Adverse Events to the Institutional Review Board

The Principal Investigator is required to notify his/her Institutional Review Board (IRB) of a serious adverse event according to institutional policy. The requirements for IRB Protocol Problem Reporting at Johns Hopkins are can be found at this website: http://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/guidelines/

7.2.3.3.2 Reporting Adverse Events to the Coordinating Center (Sponsor) and to Merck Global Safety

Participating sites will report to the Coordinating Center (Sponsor) all events meeting the criteria of serious adverse events utilizing the MedWatch 3500A form within 24 hours of the site becoming aware of the event and to Merck Global Safety within 2 working days. The Coordinating Center, JHU, as the sponsor of the study, will make the final determination regarding FDA submission. Thus, the Coordinating Center will be responsible for reporting to the FDA.

For the time period beginning when the consent form is signed until treatment allocation/randomization, 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 (Section 7.2.3.5) that occurs to any subject must be reported within 24 hours to the Coordinating Center (Sponsor) and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, 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 (Section 7.2.3.5) whether or not related to the Merck product, must be reported within 24 hours to the Coordinating Center (Sponsor) and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Coordinating Center and to Merck Global Safety.

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

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

7.2.3.3.3 FDA Reporting Requirements

Unexpected Adverse Event: An AE is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7 Calendar-Day Telephone or Fax IND Safety Report
Serious adverse events (SAEs) that are **unexpected**, and at least **possibly associated** to the study drugs, as assessed by the Sponsor-Investigator, should be reported promptly to the Food and Drug Administration (FDA) by telephone or by fax. **Fatal or life threatening SAEs** that meet the criteria for reporting to the FDA **must be reported to the FDA within 7 calendar days after awareness of the event.**

15 Calendar-Day Written IND Safety Report
The Sponsor-Investigator is required to notify the FDA, and all participating investigators in a written IND Safety Report, of any serious, unexpected adverse event considered by the Sponsor-Investigator to be possibly related to the use of study drugs within 15 calendar-days of first learning of the event. If applicable, the Sponsor-Investigator must also notify the FDA, and all participating investigators, of any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity within 15 calendar-days of first learning of the event.

All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to

the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

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

7.2.3.4 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

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

7.2.3.5 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 7.2.3.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless

there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

7.2.4 Evaluating Adverse Events

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

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

Table 7. Evaluating Adverse Events

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

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a preexisting condition that has not worsened is not a serious adverse event A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); 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) (although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. 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 to the Coordinating Center and to Merck within 2 working days.	
	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 Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor'sproduct(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's 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 MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.	
No, there is not a reasonable possibility of Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause other than the Merck product. (Also entered for a subject with overdose without an associated AE.)	

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations and as documented in the Section 7.2.

7.2.6 Reporting Protocol Deviation

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

The term "protocol deviation" is not defined by either the HHS human subjects regulations (45 CFR 46) or the FDA human subjects regulations (21 CFR 50). For JHM purposes, a protocol deviation is a minor or administrative departure (see definitions below) from the protocol procedures approved by the IRB that was made by the PI without prior IRB approval. Please note: Eligibility exceptions (or eligibility waivers granted by a Sponsor) for enrollment of a specific individual who does not meet the inclusion/exclusion criteria in the IRB approved protocol are not deviations. Eligibility exceptions are considered changes in research that require IRB review and approval before a subject who does not meet the approved protocol inclusion/exclusion criteria may be enrolled.

Reporting Protocol Deviations to the JHM IRB and the Sponsor

There are several types of deviations from protocol procedures recognized by the JHM IRB, and each type has a different IRB reporting requirement:

A. Protocol deviations that constitute unanticipated problems involving risks require prompt reporting to the JHM IRB: A protocol deviation that constitutes an "unanticipated problem involving risks to subjects or to others" (see Policy No. 103.6(b) for the definition of an unanticipated problem) must be reported promptly to the IRB, as follows: *1. Emergency deviations:* When a deviation occurs in an ***emergency situation*****,** such as when a departure from the protocol is required to protect the life or physical well-being of a participant. The Sponsor (JHU Principal Investigator) and the reviewing IRB must be notified as soon as possible, but not later than 5 days after the ***emergency*** situation occurred (21 CFR 812.150(a)(4)). Once IRB's response is received, this should be forwarded to the Coordinating Center Regulatory Specialist.

2. Major, non-emergent deviations without prior approval: A planned deviation that is non-emergent and represents a major change in the protocol as approved by the IRB. The Sponsor (JHU Principal Investigator) and the IRB must approve the request before the proposed change is implemented. Once IRB's response is received, this should be forwarded to the Coordinating Center Regulatory Specialist. If a major, non-emergent deviation occurs without prior IRB approval the event is considered non-compliance. Non-compliance must be reported to the IRB promptly.

B. Protocol deviations that are only minor or administrative: At JHM, minor or administrative protocol deviations are defined as those which do not "affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects." If a protocol deviation occurs which meets this definition, the deviation should be reported to the JHM IRB at the time the continuing review application is submitted. Participating sites should record deviation on the

deviation form and provide it to the Sponsor and to the IRB according to the local IRB requirements. Examples of minor or administrative deviations could include: follow up visits that occurred outside the protocol required time frame because of the participant's schedule, or blood samples obtained at times close to but not precisely at the time points specified in the protocol.

Protocol Deviation Reporting Requirements for Commercially Sponsored Research

Sponsored research agreements may require the PI to notify the sponsor of all unplanned deviations or departures from IRB approved protocol procedures. Sponsor reporting requirements for deviations may differ from JHM IRB reporting requirements. It is the PI's responsibility to comply with the reporting requirements outlined in the signed contract.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

This multi-center, nonrandomized, open-label Phase II study will enroll two cohorts of patients: 1) Cohort 1: relapsed/refractory AML, and 2) Cohort 2: newly diagnosed AML in older (≥65 years) patients not candidates for induction chemotherapy.

8.2 Statistical Analysis Plan

8.2.1 Safety Run-In

Each cohort will be enrolled separately with distinct null hypotheses and statistical plans. Since azacitidine and pembrolizumab have not been tested previously in combination in AML patients, we plan first to start enrollment with Cohort 1 (a safety run-in phase). Recognizing that Cohort 1 may also include patients who relapsed after allogeneic hematopoietic stem cell transplant (alloHSCT) and thus may have differential sensitivity (tolerability, immune-related adverse events [IRAEs]) to this drug combination, we will start enrollment in Cohort 1 to include patients who did not have prior alloHSCT.

The primary objective of the safety run-in is to determine the safety and tolerability of AZA followed by pembrolizumab in relapsed and refractory AML using a 3+3 design. We plan to monitor for DLTs in cycles 1, 2 and 3 as some of immunological side effects associated with pembrolizumab may occur with the delay. The first three patients will be enrolled and receive planned azacitidine and pembrolizumab in cycle 1. If 0 or 1 patient of the first three patients has a DLT (dose limiting toxicity) in cycle 1, we will enroll an additional 3 patients to better assess for toxicities. If less than 2 among first 6 patients experiences DLT in the cycle 1-3, we will open enrollment in Cohort 1 for all relapsed/refractory patients including those with prior history of alloHSCT and will also start to enroll patients in Cohort 2. If ≥2 of the first 3 patients or ≥2 of 6 patients have DLTs during cycle 1, we will perform safety (detailed toxicity) review to assess for disease versus treatment-related toxicities. Given that pembrolizumab is given only once in cycle 1 (day 8), we will first dose de-escalate azacitidine as detailed in Section 2.2. However, if the safety data analysis suggests that the toxicities are more likely pembrolizumab related, an alternative approach we may take is to exclude pembrolizumab in cycle 1 and start pembrolizumab dosing in cycle 2 when AML is better controlled but maintain azacitidine dose/schedule as described in Section 2.2. If

≤1 of the 6 patients experience DLTs during cycle 1 but ≥ 2 of 6 patients experience DLTs during cycle 2 and cycle 3, or cycle 1, 2 and 3, we will first dose de-escalate pembrolizumab as described in Section 2.2 and maintain azacitidine dose/schedule. When each dose de-escalation occurs (maximum 2), additional 6 patients will be enrolled at that dose level. For the details of dose de-escalation, please see Section 2.1 and 2.2 Trial Diagram.

Only after a safe dose/schedule is defined will we start enrollment in the phase 2 cohorts. A full safety assessment of the combination of AZA and pembrolizumab will be performed in all patients after 1, 2 and 3 cycles and in subsequent cycles according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Patients who are treated at dose/schedule selected for Phase 2 will be included in the primary efficacy analysis and the analyses of secondary and exploratory objectives.

8.2.2 Sample Size

The primary objective of this study is to estimate the complete response (CR/CRi) rate in each cohort. We estimated sample sizes for the 2 cohorts using a one-sample, one-sided binomial test, with Type I and Type II errors of 0.05 and 0.2, respectively. For Cohort 1, the null and expected response rates were taken to be 0.1 and 0.25, respectively. For Cohort 2, the null and expected response rates were taken to be 0.17 and 0.34, respectively. The sample sizes were calculated to be 38 (which will be rounded to 40) and 40 for Cohorts 1 and 2, respectively.

We used an efficient and flexible design based on Bayesian predictive probability (Lee and Liu, 2008). This design controls the type I and type II error rates and also allows flexible monitoring for futility and efficacy of the new treatment. Furthermore, this design also has better sampling properties than Simon's 2-stage design, i.e. probabilities of early termination for futility under the null hypothesis and for efficacy under the alternate hypothesis are higher and the expected sample size is lower than Simon's 2-stage design.

The following input parameters were assumed in our design: minimum and maximum number of patients was 10 and 40, respectively. Type I and II errors were 0.1 and 0.2, respectively. For cohort 1, the treatment effect for current standard was 0.1 (i.e., probability of CR/CRi) and expected treatment effect for the new treatment was 0.25. For cohort 2, the treatment effect for current standard was 0.17 and expected treatment effect for the new treatment was 0.34. A beta (0.25, 0.75) distribution was assumed for the prior for the expected effect of the new treatment. This is a weak prior, which has negligible effect on the stopping boundaries.

The stopping boundaries for futility are given in the tables below for cohorts 1 and 2. For example, the first row of Table 1 indicates that we would stop the trial for futility if we see 0 responses (CR/CRi) out of the first 10 patients after 6 cycles of therapy (to be evaluable for futility pts must complete at least 2 cycles of therapy); if the study was not stopped after 10 patients, the next evaluation will be done after 14 patients (2^{nd} row). Study will be stopped for futility if we see 1 response or less for futility. If the study continues, the next evaluation happens after 21 patients (3^{rd} row). Study will be stopped if we see 2 or fewer responses in the first 21 patients.

Cohort 1: Interim monitoring boundaries for stopping for futility.

Patient. No.	Futility boundary (less than or equal to)
10	0
14	1
21	2
27	3
32	4
36	5
40	6

Cohort 2: Interim monitoring boundaries for stopping for futility.

Patient. No.	Futility boundary (less than or equal to)
10	0
11	1
16	2
20	3
24	4
27	5
31	6
34	7
36	8
39	9
40	10

For cohort 1, the expected sample size under the null and alternative hypotheses is 17.7 and 18.3, respectively. For cohort 2, these were 19.2 and 21.0.

8.2.2.1 Early stopping guideline for toxicity

Toxicity will be monitored after every 5 patients for each cohort. If it becomes evident that the proportion of patients being removed from the study for toxicity convincingly exceeds 20%, the study will be halted for a safety consultation. The toxicity stopping rule will hold enrollment if the posterior probability of failure being larger than 0.20 is 70% or higher. The prior for this monitoring rule is beta(0.5,2). This means that our prior guess at the proportion of failures is 20%, and there is 90% probability that this proportion is between 0.01% and 65.8%. The monitoring rule and operating characteristics are given below.

- Stop if 2 out of 5 patients removed from study: Pr(Risk>0.2 | Data) = 0.77
- Stop if 3 out of 10 patients removed from study: Pr(Risk>0.2 | Data) = 0.715

- Stop if 5 out of 15 patients removed from study: Pr(Risk>0.2 | Data) = 0.852
- Stop if 6 out of 20 patients removed from study: Pr(Risk>0.2 | Data) = 0.823
- Stop if 7 out of 25 patients removed from study: Pr(Risk>0.2 | Data) = 0.8
- Stop if 8 out of 30 patients removed from study: Pr(Risk>0.2 | Data) = 0.781
- Stop if 9 out of 35 patients removed from study: Pr(Risk>0.2 | Data) = 0.766
- Stop if 10 out of 40 patients removed from study: Pr(Risk>0.2 | Data) = 0.752

Operating Characteristics of stopping rule for toxicity, based on 5000 simulations:

True Toxicity Rate	Probability of declaring treatment too toxic	Average sample size
5%	2.9%	34.1
10%	11.5%	31.8
15%	28.9%	27.6
20%	51.1%	22.8
25%	73.1%	17.8
30%	86.8%	14.0
35%	95.9%	10.7
40%	98.8%	8.6

8.2.2.2 Early Stopping for Safety

The clinical failure of acute graft versus host disease will be monitored after every 5 patients who had a prior history of alloHSCT. If it becomes evident that the proportion of acute graft versus host disease grade 2-4 is greater than 33% the study would also be held for a safety consultation.

The acute GVHD grade 2-4 stopping rule will hold enrollment if the posterior probability of failure being larger than 0.33 is 70% or higher. The prior for this monitoring rule is beta(1,2). This means that our prior guess at the proportion of failures is 33%, and there is 90% probability that this proportion is between 2.5% and 77.6%. The monitoring rule and operating characteristics are given below.

- Stop if 3 out of 5 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.832
- Stop if 5 out of 10 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.829
- Stop if 7 out of 15 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.836
- Stop if 8 out of 20 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.719
- Stop if 10 out of 25 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.746
- Stop if 12 out of 30 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.77
- Stop if 14 out of 35 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.79
- Stop if 15 out of 40 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.709
- Stop if 17 out of 45 patients with gr2-4 aGVHD: Pr(Risk>0.33 | Data) = 0.735

True acute GVHD grade 2-4 rate	Probability of stopping for excess GVHD grade 2-4	Average Sample Size
10%	1.0%	39.7
15%	3.2%	39.0
20%	9.2%	37.3
25%	22.3%	33.9
30%	40.7%	29.4
35%	62.7%	24.2
40%	80.8%	19.1
45%	92.7%	14.5

8.2.3 Analysis of Primary Endpoint

The primary objective of this study is to estimate the complete remission (CR/CRi) rate of AZA followed by pembrolizumab in 1) relapsed/refractory AML patients, and 2) older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy. The CR/CRi rate will be reported with an exact binomial, two-sided, 95% confidence interval, separately by each cohort. We will test for heterogeneity of the treatment effect between cohorts using an interaction test, and if there is no evidence of heterogeneity, we will pool all patients treated at the phase II dose/schedule and estimate the CR/CRi rate with an exact binomial, two-sided, 95% confidence interval.

8.2.4 Analysis of Secondary Endpoints

The toxicity of AZA followed by pembrolizumab in 1) relapsed/refractory AML patients, and 2) older (≥65 years) newly diagnosed AML patients not candidates for intensive induction chemotherapy will described separately for each cohort using summary statistics.

The overall response rate, defined as CR/CRi/partial response (PR)/ morphologic leukemia-free state (MLFS)/hematologic improvement (HI) of AZA followed by pembrolizumab, will be estimated for each cohort separately and reported with exact 95% confidence intervals.

Disease-free survival (DFS) and overall survival (OS) will be estimated and plotted for patients who achieve a CR/CRi, separately by cohort, using the Kaplan Meier method. DFS will be defined as the time from CR/CRi to either progression, relapse, death (event) or last follow-up date (censored). OS will be defined as the time from study initiation to either death (event) or last follow-up date (censored).

Progression-free survival (PFS), time to progression, and OS will be estimated and plotted for all patients, separately by cohort, using the Kaplan Meier method. PFS will be defined as the time from study initiation to progression or death (event) or last follow-up date (censored). The cumulative incidence of progression will be estimated for each cohort (Prentice RL and Kalbfleisch JD. The

analysis of failure times in the presence of competing risks, Biometrics, 34: 541-554. 1978). Death will be considered a competing risk for progression.

8.2.5 Analysis of Correlative Endpoints

We will perform multi-color flow cytometry on PB and BM specimens before and after AZA and AZA/pembrolizumab treatment using three pre-determined panels of mAbs including but not limited to those specific for CD3, CD4, CD8, PD-1, CD45RA, CCR7, CD27, CD28, Ki-67, T-bet, CD127, CD25, FoxP3, HLA-DR, CTLA-4, TNFRII, ICOS, TIM3, LAG-3, CD160, 2B4, BTLA, KLRG-1, CD16, CD14, and CD56. The analyses of pre- and post-treatment PB and BM specimens for immune parameters will be descriptive and graphical in nature. Data will be summarized for each cohort separately. The change in flow values at each time point after AZA and AZA/pembrolizumab relative to the baseline value will be will checked for skewness and log-transformed as appropriate. The changes will be modeled using mixed-effects, linear regression models that included fixed effects for time and a random effect for the patient to account for within-patient correlation of measurements. To explore potential differences in the change in flow values across patient subgroups, interaction terms will be included in the regression models. Post-treatment PD changes in gene expression and methylation in immune gene signaling circuits in tumor biopsies and PB will be summarized with descriptive statistics and correlated with clinical outcomes.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

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

Table 7 Product Descriptions

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

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

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

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The subject has a right to protection against invasion of privacy. In compliance with United States federal regulations, the Investigator may permit representatives of Merck and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

10.2 Compliance with Financial Disclosure Requirements

All investigators on the study are expected to maintain up-to-date financial disclosures. Investigators at Johns Hopkins will report their financial disclosures through the Johns Hopkins electronic disclosure monitoring system.

10.3 Compliance with Law, Audit and Debarment

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

The SKCCC at Johns Hopkins will be coordinating center for this study. The following documentation must be provided to the Clinical Research Office (CRO) at the Johns Hopkins SKCCC prior to a participating site being authorized to enroll patients:

- Letter of Understanding
- IRB protocol approval letter: the letter must include the protocol name, version, and dates of protocol approval and expiration
- IRB approved consent form: the entire consent form must be submitted and must include the protocol name and date of approval
- IRB membership list, if available
- OHRP Federal Wide Assurance number
- Form FDA 1572 appropriately filled out and signed with appropriate documentation (NOTE: this is required if JHU holds the IND. Otherwise, the Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol is sufficient to ensure compliance)
- Curriculum vitae, signed and dated within the last two years, of all investigators and sub-investigators
- Medical license for all investigators and sub-investigators
- Financial disclosure forms, if appropriate
- Human Subjects Training certification for all study personnel
- Laboratory certification and normal ranges

This protocol will adhere to the policies and requirements of the Multicenter Guidelines. The specific responsibilities of the Principal Investigator (Protocol Chair) and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix 11.6.

The Protocol Chair (Principal Investigator at JHU SKCCC- Coordinating Center) will be responsible for preparing documents for submission to the JHU IRB and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study. Should amendments to the protocol be required, the amendments will be originated and documented by the Protocol Chair (Principal Investigator at JHU- SKCCC – Coordinating Center). It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be

required. The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

The Principal Investigator at the participating site must submit the JHU IRB approved protocol, informed consent, and amendment to their institution's IRB for approval. For multi-center studies, any participating site must submit their informed consent revisions to the JHU-SKCCC Regulatory Associate prior to submission to their IRB.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

The Principal Investigator is also responsible for notifying the IRB of any serious deviations from the protocol, or anything else that may involve added risk to subjects. Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB prior to use.

10.4 Compliance with Trial Registration and Results Posting Requirements

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

10.5 Quality Management System

This is a DSMP Level II study under the SKCCC Monitoring Plan (9/22/2011). A Level II study requires both internal and external data monitoring. The Principal Investigator is responsible for internal monitoring for both safety and data quality. External data monitoring will be performed by the SKCCC at Johns Hopkins Clinical Research Office Quality Assurance Program (CRO QA). A DSMP Level 2 study requires that 100% of all subjects will be reviewed for consent and eligibility, while at least 30% of all subjects will be monitored in their entirety. Scheduled monitoring visits will occur at least every 6 months, but may occur more (or less) frequently, depending on the rate of accrual. Data and safety monitoring oversight will be conducted by the SKCCC at Johns Hopkins Safety Monitoring Committee. Per the SKCCC at Johns Hopkins Safety Monitoring Plan, the CRO QA will forward summaries of all monitoring reports to the Safety Monitoring Committee for review. All reportable anticipated and unanticipated protocol events/problems and amendments that are submitted to the IRB will also be reviewed by the Safety Monitoring Committee Chair (or designee) and QA manager. Monitoring review plan: The PI will review data to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial. The PI will review safety reports and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study. The PI will be responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the annual report submitted to the IRB and the semi-annual report to the SMC. Content of the report at a

minimum should include year-to-date and full trial data on: accrual and eligibility, protocol compliance, treatment administration, toxicity and ADR reports, response, survival, regulatory compliance, compliance with prearranged statistical goals.

10.6 Data Management

All information will be collected on study-specific case report forms (CRFs) by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator. Case report forms will be provided to the data managers at each participating center. All data relating to a particular cycle of therapy must be completed within two weeks of the completion of the time point. The importance of protocol adherence and accurate data collection requires that each center have a specifically designated research nurse and data manager assigned to the study. The Principal Investigator and Research Nurse at SKCCC will be available by phone, e-mail, and pager for protocol and patient management questions. The data manager at SKCCC will be responsible for ensuring compliance with data reporting. Upon patient registration (accomplished by faxing a form to SKCCC), the SKCCC data manager will establish a projected calendar for patient treatment and submission of data to SKCCC. This calendar will be transmitted to the data manager at the treating center. If an expected data collection form is not received a week following the expected "due date", the SKCCC manager will contact the data manager at the treating center. If the data is 30 days overdue, a warning letter will be sent to the data manager and principal investigator at the treating center. If data is greater than 60 days delinquent, the center's ability to enroll new patients will be suspended until the situation is rectified, and a corrective plan is established.

10.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region; or if an application is not approved for drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA so notified." In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

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)

11.3 Response Evaluation Criteria in Acute Myeloid Leukemia

Response will be assessed using the International European LeukemiaNet Guidelines in AML2017 [63] and IWG Modified Response Criteria (2006) for MDS (for definition of HI)[64].

Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2016 :blood-2016-08-733196; Updated guidelines for 2017 available at http://www.bloodjournal.org/content/early/2016/11/28/blood-2016-08-733196).

Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108(2); 419-25.

11.4 Operating Procedures for Specimen Collection

Peripheral blood and bone marrow specimen collection will be performed as delineated in the Trial Flow Chart Section 6.0. and at the times when peripheral blood and bone marrow collections are performed for clinical care. Please see details in the Laboratory Manual.

Bone marrow: 5x8.5mL will be collected in light yellow top ACD tubes (or 4x10mL green top heparin tubes) (~40 mL) . Re-adjustment of the direction of bone marrow aspirate needle should take a place after each 10 cc is collected to prevent hemodilution. At the time of collection, green top tubes must be thoroughly mixed to prevent clotting.

Peripheral blood: 5x8.5mL light yellow topACD tubes (or 4x10mL green top heparin tubes) of blood (~40mLs) will be collected at each time point.

Specimens should be labeled with the patient's study number (given at the time of registration), study number, sample collection date and time, and sample source (PB or BM). Sample collection date and time, and sample source (PB or BM) will be recorded on correlative collection worksheet and copy included with delivery. All data should be kept in laboratory log. At each sampling time, BM and PB mononuclear cells (PBMC) will be processed via Ficoll density gradient centrifugation. The washed cells will be counted, triaged for DNA isolation, and viably cryopreserved using a controlled-rate freezer with transfer to the vapor phase of liquid nitrogen for long-term storage.

The Sidney Kimmel Comprehensive Cancer Center (SKCCC) specimens should be delivered to the Luznik laboratory immediately after collection.

Specimen collection / processing (Participating Sites). Participating sites should collect PB and BM specimens as outlined in the Trial Flow Chart 6.0. Participating sites have two options:

1) To process specimens following the SOP described below. Samples will be stored at each study site. Any samples collected under the clinical protocol will be batch

shipped on dry ice based on discussions with the participating site PI, Study Chair and Dr. Luznik.

2) If participating site does not have capability of processing the specimens as described below, then specimens can be shipped overnight to Dr. Luznik laboratory using cold shipping packages (boxes) provided by Fedex. Please notify PI (Study Chair), study coordinator and Dr. Luznik's laboratory of specimen shipment.

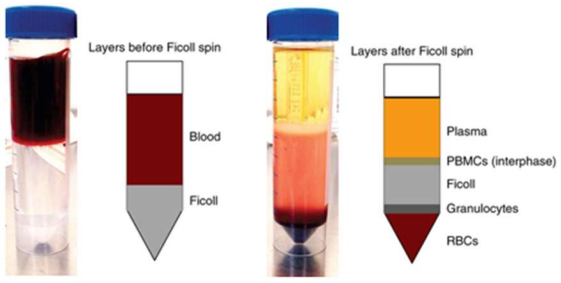
Shipping of Specimen(s)
The samples will be processed, stored and analyzed in Dr. Luznik's laboratory at the SKCCC at Johns Hopkins University. Upon request of the Study Chair they will be batch shipped to SKCCC. Samples will be shipped or delivered to Dr. Luznik laboratory: 1650 Orleans Street, CRB1 Rm 216, Baltimore MD 21287. Tel: (410) 955-8567

Freezing Protocol (SOP)

1. Prepare 50mL falcon tubes with Ficoll density grade media (Histopaque)
    - Ratio Ficoll : blood should be 1:1
    - Do not exceed 20mL Ficoll to 20mL blood (i.e. total volume of 40mL)

2. Slowly layer blood on top of Ficoll
    - Use whole blood or marrow aspirate, do not dilute with PBS or anything else
    - Be careful not to mix the two layers

3. Centrifuge: spin at the following settings:
    - 1240rpm (=364G)
    - 20 Celsius
    - Break off
    - 30min



4. Prepare 6 small Eppendorf tubes
    - Take off 6 x 500ul of plasma (top layer; blood only; not for BM aspirate) and put it into Eppendorf tubes
    - Be careful not to get into any layer but the plasma layer
    - Long term storage in -80 Celsius freezer

5. Soak up PBMC layer with transferring pipette and put it into a new 50mL Falcon tube
    - Fill up with PBS up to 45mL, vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

6. First wash
    - Dump supernatant
    - In case of visible RBC contamination add 1Ml of lysing buffer to the cell pellet, vortex, and leave for 1 minute. Then add 20mL of PBS
    - If there is no RBC contamination only add 20mL of PBS and vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

7. Second wash
    - Dump supernatant
    - just add 20mL of PBS and vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

8. Count cells
    - Dump supernatant
    - add 1mL of PBS and vortex thoroughly
    - take 10ul out and mix with 90ul of trypan blue
    - count in hematocytometer

9. Prepare freezing tubes and media
    - Put cells in the meantime on ice
    - Freezing media: FBS containing 10% DMSO
    - 1.8mL vials labeled with study #, timepoint, cell count, date, type of specimen,

10. Final spin
    - Add 20ml of PBS to the cells, vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4°C, break high, for 5 minutes

11. Freeze cells - General rules
    - PBMCs: 1.5mL freezing media per 10 million cells (=1 vial)
    - BMMCs: 1.5mL freezing media per 30 million cells (=1 vial)

12. Freezing
    - Put vials into Mr. Frosty® (container with Ethanol, that gradually cools down samples) and put into -80 Celsius fridge for about 24 hours
    - After 24 hrs (up to 48hrs) transfer vials into liquid nitrogen tanks (to minimize temperature fluctuations make sure you transport them there on dry ice)

Considerations regarding freezing and shipping

- Samples should optimally be processed the same day
- If there is a sample coming in late, keep it in the fridge (in 4◦C) overnight and process them early the next morning.
- Samples processed should be stored in -80 Celsius for 24-48 hours and get transferred into liquid nitrogen thereafter
- As soon as there is a good amount of samples accumulated, samples should be shipped (Fedex) in batches on dry ice to

Luznik Lab

Hopkins CRB1, Room 216

1650 Orleans Street

Baltimore, MD 21287

- If there are no liquid nitrogen tanks available, it's ok to keep samples in -80 Celsius for up to 2 weeks, but no longer. Shipments to Hopkins should then be done more frequently.

Important considerations:
1. All work should be done using standard BSL2 procedures (blood and body fluid precautions).
2. Maintain a clean workspace and use a containment laminar airflow hood when possible.
3. Minimize the chance of contamination.
4. Work quickly but methodically.
5. Keep tubes closed as much as possible and work quickly.
6. Change gloves frequently and maintain situational awareness.

11.5 Multicenter Guidelines

If an institution wishes to collaborate with other participating institutions in performing a sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison for the study. The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

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