SPONSOR: Guillermo Garcia-Manero MD, Department of Leukemia, MD Anderson Cancer Center.

TITLE: A phase II study of the combination of azacitidine and pembrolizumab for patients with MDS

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1.0 TRIAL SUMMARY

Abbreviated Title	**Combination of azacitidine and pembrolizumab in MDS**
Trial Phase	**II**
Clinical Indication	**MDS**
Trial Type	**interventional**
Type of control	**none**
Route of administration	**I.V.**
Trial Blinding	**none**
Treatment Groups	**2 groups: previously treated and relapsed/refractory**
Number of trial subjects	**N=40**
Estimated enrollment period	**24 months**
Estimated duration of trial	**36 months**
Duration of Participation	**36 months**

2.0 TRIAL DESIGN

This is a phase II design of the combination of azacitidine and pembrolizumab (also known as MK3475 in the text) for patients with myelodysplastic syndrome (MDS). Two different cohorts of patients will be studied: cohort #1 previously untreated and cohort #2 patients with hypomethylating agent (HMA) failure.

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

Objective:

1-To assess the safety of the combination of azacitidine and MK3475 in patients with higher risk MDS.

2- To explore the clinical activity (response, survival effect) of the combination of azacitidine with MK-3475 in patients with higher risk myelodysplastic syndrome

Hypothesis: The hypothesis is based on the concept that prior exposure to azacitidine may trigger expression of PD1/PDL1 and therefore “prime” leukemia cells to the effect of immune check point inhibitors. This mechanism may also be involved in resistance to hypomethylating agents.

3.2 Exploratory Objective

Objective: To study the biological effects of the combination of azacitidine and prembrolizumab in patients with MDS treated on this study.

4.0 BACKGROUND & RATIONALE

4.1 Myelodysplastic syndromes (MDS)

The myelodysplastic syndromes (MDS) are a group of heterogeneous malignancies that manifest clinically by peripheral cytopenias and an increase risk of transformation to acute mylogenous leukemia (AML). Patients with MDS are in general divided into those with lower risk disease and those with higher risk disease. The standard of care for most patients with high risk MDS is a hypomethylating agent such as azacitidine (1, 2). Data from the now classic aza-001trial (1) suggest that hypomethylating agents are associated with improved survival compared to supportive care. Longer follow up and experience with these compounds indicates that a large majority of the patients treated with these agents will eventually lose response to the hypomethylating agents. This phenomenon is called hypomethylating failure. The survival of such patients is very poor estimated to be 4 to 6 months (3, 4). Therefore the need for new agents or combinations in patients with MDS both as front line (to improve survival and delay failure) and in patients with HMA failure to improve survival. It should be noted that at this moment there are no drugs approved for HMA failure MDS (2).

4.1.1 Pembrolizumab Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-

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

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

4.1.1 Azacitidine

5-azacytidine, an analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and deoxyribonucleic acid (DNA) synthesis and metabolism. Since the early 1970s, 5-azacytidine has been investigated primarily in the US for the treatment of acute leukemia. Clinical studies have focused mainly on patients with disease refractory to conventional chemotherapy. Results of these investigations demonstrated activity of 5-azacytidine in the treatment of AML. Clinical studies subsequently evaluated the effects of 5-azacytidine in a variety of other malignant and hematologic disorders, including solid tumors, hemoglobinopathies (eg, thalassemia and sickle cell anemia), and MDS. In 1984, the Cancer and Leukemia Group B (CALGB) began a series of clinical studies with 5-azacytidine in patients with MDS. These studies, in addition

to other supportive data, led to the approval of Vidaza® (5-azacytidine) in May 2004 for the treatment of MDS.

5-azacytidine inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT). Increased methylation of DNA (hypermethylation) may result in the silencing of critical genes responsible for cell growth control and differentiation. Hypermethylation of CpG islands spanning the promoter regions of tumor suppressor genes is commonly associated with cancers. It is now widely recognized that hypermethylation of DNA is frequently associated with myelodysplastic syndromes and other cancers, such as renal, melanoma, breast, colorectal, non-small cell lungand hematologic malignancies. 5-azacytidine is believed to exert its antineoplastic effects through hypomethylation and cytotoxicity on abnormal hematopoietic cells in the bone marrow.(5-9) Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of 5-azacytidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.

The cytotoxicity of 5-azacytidine is proportional to dose and exposure time. Although the mechanisms of cytotoxicity are complex and multifaceted, there is general agreement that incorporation of 5-azacytidine into DNA and ribonucleic acid (RNA), and inhibition of protein synthesis, are critically important. Cytotoxicity is greatest in cells that are proliferating (S phase) and metabolically active.(5) Cytotoxic effects may also be mediated through induction of the DNA damage response pathways. Nonproliferating cells are relatively insensitive to 5-azacytidine.

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys. Most of the studies were performed during the 1970s and early 1980s according to existing guidelines and standards in place during that period. The preclinical studies identified the bone marrow, liver, kidneys, and lymphoid tissues (spleen, lymph nodes, and thymus) as the main target organs of toxicity for 5-azacytidine.(10) In single-dose studies, the lethal dose of 5-azacytidine after intravenous (IV) administration in mice, rats, and dogs was approximately 250 mg/m^2. Repeated daily dosing appears to increase the toxicity of 5-azacytidine. The genotoxicity of 5-azacytidine is consistent with that of other nucleoside analogs that interact with nucleic acids. Likewise, similar to other agents with cytostatic properties, 5-azacytidine was embryotoxic and reduced the reproductive performance in mice and rats.

Limited 5-azacytidine pharmacokinetic data are currently available. Based on human plasma concentrations of total radioactivity (which represents parent drug plus circulating metabolites), 5-azacytidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing.(10) 5-azacytidine and/or its by-products are then rapidly cleared by the kidneys. The half-lives and percent radioactivity recovered in urine are similar following IV and SC routes of administration. The effects of renal or hepatic impairment, gender, age, or race on the pharmacokinetics of 5-azacytidine have not been studied. A single dose (75 mg/m^2) SC versus IV crossover study in 6 MDS subjects revealed an approximate bioavailability of 89% for the SC dose (range 52%

to 128%) with mean half-lives of 0.69 hour and 0.36 hour after SC and IV administration, respectively. These results demonstrated that 5-azacytidine is rapidly and nearly completely absorbed after SC administration and that elimination is also rapid. The apparent SC clearance (167 L/h or 2791 mL/min) and systemic IV clearance (147 L/h) of 5-azacytidine exceeded the glomerular filtration rate (approximately 125 mL/min) and total renal blood flow (1200 mL/min) in healthy subjects. This indicates that non-renal elimination (eg, metabolism, hydrolysis, and/or degradation) plays a role in the elimination of parent 5-azacytidine. In addition, 5-azacytidine 75 mg/m^2 was generally well-tolerated after single SC injection or IV infusion over 10 minutes. There will be a washout period of one month if the patient is on another combination of hypomethlating agents.

A number of studies have looked at different parenteral doses and schedules of 5-azacytidine, finding maximum tolerated doses of up to 500 mg/m^2 when administered weekly.

During the two decades between the start of the CALGB studies and the approval of 5-azacytidine, a new understanding of MDS has developed, such as the World Health Organization (WHO) diagnostic criteria, the International Prognostic Scoring System (IPSS), and the International Working Group (IWG) response criteria. Silverman et al. reevaluated the combined data (N = 309) from 3 of the CALGB studies using the WHO classification system for MDS and AML and the IWG response criteria.(11) Using the IWG response criteria in MDS patients, response rates were between 40% and 70% in 5-azacytidine treated patients. Ten to 17% of patients achieved a complete remission; partial remission was rare; and 23% to 36% of patients had a hematologic improvement. In patients with AML (N = 103), 35% to 48% had hematologic improvement or better responses. The median survival time for 27 patients assigned to 5-azacytidine was 19.3 months compared with 12.9 months for the 25 patients assigned to observation.(11)

A randomized international Phase III trial (Study 5-azacytidine PH GL 2003 CL 001) for higher-risk MDS patients, classified by FAB as RAEB, RAEB-T, or CMML with 0-29% marrow blasts, with an IPSS of Intermediate -2 or High by central pathology/cytogenetic review was recently reported.(12) Patients were randomized to 5-azacytidine (75 mg/m^2/day x 7days in 28 day cycles) or conventional care regimens (CCR), where CCR was pre-selected by the Investigator as best supportive care (transfusions, antibiotics, and G-CSF for neutropenic infection), low-dose cytarabine (20 mg/m^2/day x 14 days in 28 day cycles); or standard chemotherapy (conventional induction/consolidation). Patients were stratified by FAB/IPSS and randomized 1:1 to 5-azacytidine or CCR. This trial did not allow erythropoietin. Three hundred fifty eight patients (70% male) were randomized at 79 centers to 5-azacytidine (N=179) or CCR (N=179): best supportive care only (N=105, 59%), low-dose cytarabine (N=49, 27%), or standard chemotherapy (N=25, 14%). Median age was 69 years (range 38-88 years). The 5-azacytidine and CCR groups were comparable for baseline patient characteristics. At baseline, 95% of patients were higher risk: RAEB (58%), RAEB-T/WHO AML (34%), CMML (3%), and other (5%). By IPSS, 87% were higher risk: Intermediate -2 (40%), High (47%), and 13% Indeterminate/other. 5-azacytidine was administered for a median of 9 cycles; low-dose cytarabine for 4 cycles. Median follow-up

for the survival analysis was 21.1 months. 5-azacytidine demonstrated statistically superior overall survival compared to CCR, with a median overall survival of 24.4 months vs. 15 months for CCR (stratified log-rank p=0.0001, hazard ration 0.58). Two-year survival approximately doubled in the 5-azacytidine arm compared to CCR: 51% vs. 26% (p<0.0001). 5-azacytidine was well tolerated with safety data consistent with previous reports.

4.1.2 Preclinical and Clinical Trial Data

There is little preclinical data using PD1 or PDL1 inhibitors in MDS. Concept is discussed below in the Rationale section below (#4.2). At this point, one study of MK3475 has included patients with MDS. In this trial, over 15 patients with MDS and HMA failure were treated. No significant toxicities have been documented thus far in this patient cohort.

4.2 Rationale

There is significant interest on the use of immune checkpoint inhibitors for human cancer. Over the last two or three years this new class of drugs have become the center of some of the most interesting clinical trials for patients with diverse solid tumor malignancies leading to the approval of multiple agents blocking either PD1 or PDL1 I different solid tumors malignancies.

There is less experience in patients with MDS or acute leukemias with this class of compounds. Our group studied the expression of the PD1, PDL1, CTLA and PDL2 in CD34 positive cells from patients with MDS and AML **(13)**. We were able to demonstrate that a significant fraction of patients with MDS express these molecules in their leukemia cells. We were also able to demonstrate the cellular location in human bone marrow specimens. More importantly, we were also able to show that exposure of cell lines of leukemic origin to a hypomethylating agents resulted in increased expression of the PD1 and PDL1. Furthermore, we were able to document that in patients with MDS treated with hypomethylating agents there was a sequential increase in the expression of PD1 and PDL1 particularly in patients that had failed hypomethylating agents.

Our initial hypothesis is that using inhibitors of the PD1/PDL1 pathway in patients with MDS may be associated with significant clinical benefit. Based on this, initially we were able to develop a multicenter exploratory trial with single agent MK3475 for patients with HMA failure of over 15 patients. This study has been completed but not reported yet. Initial results indicate that monotherapy with MK3475 was safe in patients with MDS. We did not observe any major significant toxicity, either hematological or non-hematological. No complete remissions have been documented beyond a hematological improvement. But a number of patients are still on therapy for over 12 months. This is important as the median survival of this group of patients is expected to be less than 6 months.

This data indicated that MK3475 is safe and has potential monotherapy clinical activity in patients with higher risk MDS after HMA failure.

Based on this clinical experience and the translational data described above, we hypothesized that the combination of MK3475 and azacitidine should be safe and active in MDS.

4.2.1 Rationale for the Trial and Selected Subject Population

Our hypothesis is that hematopoietic cells from patients with MDS express PDL1 after treatment with azacitidine and may therefore benefit from immune checkpoint therapy. We plan to study two different cohorts of patients. One will consist of a group of patients with previously untreated disease and the second cohort of patients with prior HMA exposure. These two cohorts will be treated in parallel (see statistical design). The reason to treat these two groups of patients in two different cohorts is due to the fact that patients with HMA failure are known to have a very distinct natural history than previously untreated patients(3). The survival of the groups of patients with HMA failure is documented to be 4 to 6 months (3). Also the expectation of response to any therapy has been significant lower in the group of patients with HMA failure (3).

4.2.2 Rationale for Dose Selection/Regimen/Modification for MK3475

An open-label Phase I trial (Protocol 001) is being 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, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the 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.

4.2.3 Rationale for the dose azacitidine

The standard dose of azacitidine is 75 mg/m^2 IV or SC daily for 7 days. There is no clear response advantage to SC or IV administration, although toxicity may differ: skin toxicity is not uncommon with SC administration. Also, in some centers it is not possible to administer azacitidine daily for 7 days with no interruption and in this case is not uncommon to interrupt therapy during the weekends and resume for two additional days.

4.2.4 Rationale for Endpoints

Our endpoints will include both response and survival assessments as well as toxicity.

4.2.4.1 Efficacy and Toxicity Endpoints

We will use standard response criteria universally accepted for MDS: IWG 2006 (14). For toxicity we will use standard adverse reporting criteria including leukemia specific language (Refer to Appendix C in PDOL).

4.2.4.2 Biomarker Research

Correlative studies will be considered exploratory and will be performed whenever possible. This will be voluntary. Patients will have opportunity to refuse participation on these studies. If a patient's baseline correlative bone marrow or blood sample is not collected for any reason, no additional correlative bone marrow or blood samples will be collected on the patient. Additionally, any samples that are missed are not considered a protocol deviation.

Gene sequencing.

Patients will be consented for genomic analysis according to institutional guidelines. At MD Anderson, patients not consented through the Apollo program (PI Futreal) will be offer consenting under protocol LAB01-473 at baseline and their genomes will be sequenced using the T300 platform. 5 cc of blood in a green tube will be required for this analysis.

These samples will be obtained at baseline and on Cycle 1 day 28 (+/- 3 days) (including analysis of the bone marrow) every 3 cycles thereafter, and at the time of best morphological response.

Immune assessment.

Tumor tissue, blood samples and bone marrow aspirate will be collected on a separate IRB approved laboratory protocol (PA13-0291) for immune monitoring under the supervision of the Immunotherapy Platform at MD Anderson Cancer Center. In tumor tissues, immunohistochemical studies will be performed to evaluate tumor and immunological cell markers such as CD4 and CD8 T cells. In peripheral blood, we will also evaluate tumor and immune cell populations including but not limited to CD4 and CD8 T cells in pre and post therapy samples.

Peripheral blood

Up to 150 mL (within 24 hours) of peripheral blood will be collected under an IRB-approved laboratory protocol (PA13-0291) for testing of biomarkers described in this clinical protocol at the following time points:

- At screening
- Prior to each dose of MK3475 (pembrolizumab) during the first 3 cycles
- At time of best morphological response
- At progression

The treating physician or designee will have the option to cancel the laboratory protocol collection for patient safety without protocol deviation.

Analysis of hematopoetic stem cell populations

We will also analyze the effect of therapy at the level of different stem cell compartments (15). This will be done in the laboratory of Simona Colla at MD Anderson Cancer Center. This will be done at baseline and Cycle 1 Day 28 and then every 3 months thereafter.

Because we will use a number of different assays, the variability of which is not necessarily established, the intention of the correlative studies is not to draw statistical conclusions in terms of the optimal biological dose of the agents studied here. Rather, they should be considered pilot studies that will help understand the in vivo mechanism of action of the agents studied, and may help in the development of future studies of this or other combinations.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Patients with MDS with at least Int-1 disease per IPSS criteria (16) will be enrolled in this trial.

5.1.2 Subject Inclusion Criteria

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

1-Voluntary signed informed consent before performance of any study related procedure not part of normal medical care indicating that the patient is aware of the investigation and nature of this study in keeping with Institutional policies and with the understanding that the consent may be withdrawn with the subject at any time without prejudice to future medical care.

2-INT-1 or higher risk MDS defined by IPSS criteria (16)**.**

3-More or equal than 18 years of age at the time of signing consent.

4-Patients can or cannot have receive prior therapy with hypomethylating agent but will be allocated to specific patient cohorts based on their prior exposure. Patients that had received prior hypomethylating agent therapy should have at least received 6 cycles of therapy and not achieved any response or had progressed after any given number of cycles.

5-ECOG performance of 0 to 1.

6-Male patients, even if surgically sterilized, should agree to practice effective barrier contraception for the entire study treatment period and through 120 days after the last dose of the study treatment or agree to completely abstain from heterosexually intercourse. Female patients who are postmenopausal for at least one year before the screening visit or are surgical sterile or if they are of child bearing potential must have a negative pregnancy test within 72 hours of treatment of the start date and agree to practice two effective methods of contraception forms at the same time from the time of signing the informed consent through 120 days of the last dose of the study treatment or agree to completely abstain from heterosexual intercourse.

7-Willingness and ability to comply with scheduled visits treatment plans, laboratory tests and other study procedures

8-Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤2.0 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 2.0 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
[a]Creatinine clearance should be calculated per institutional standard.	

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

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

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

12-Patients must receive a minimum dose of azacitidine of 75mg/(m)(2) dose.

5.1.3 Subject Exclusion Criteria

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

1-Significant medical psychiatric cognitive or other conditions that may compromise the patient ability to understand the patient information to give informed consent to comply with the study protocol or to complete the study.

2-Any severe or concurrent disease or condition including uncontrolled systemic infection, congestive heart failure, angina pectoris or cardiac arrhythmia and autoimmune processes that in the opinion of the investigator would make the patient inappropriate for study participation.

3-Patients with known hypersensitivity to 5-azacitdine or MK3475 or any of their excipients.

4-Prior history of stem cell transplantation.

5-For patients in the relapse or refractory cohort, any other therapy not being a hypomethylating agent after HMA failure or more than 4 months since completion of last cycle of hypomethylating agent. Please note that hypomethylating agent may include second generation compounds such as SGI-110, oral decitabine or oral azacitidine and will also include combinations with investigational agents.

6-Treatment with other investigational agents including chemotherapy, immunotherapy, or radiation therapy within a month prior to the start of this clinical trial

7-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.

8-Has a diagnosis of immunodeficiency or is receiving systemic corticosteroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Physiologic doses of corticosteroids may be allowed for patients with adrenal insufficiency eligible.

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

10-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.

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

- Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and 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.

12-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.

13-Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

14-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 (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

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

16-Has an active infection requiring systemic therapy.

17-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.

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

19- 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.

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

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

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

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

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

5.2 Trial Treatments

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

Patients will be treated with the combination of azacitidine and MK3475. The dose of azacitidine will be 75 milligrams per meter squared IV or SC daily for 7 consecutive days following a standard administration guidelines for this compound. MK3475 will be administered at a dose of 200 milligrams every three weeks starting on Cycle 1 Day 1 and will continue every three weeks thereafter independent of azacitidine dosing. A cycle will be considered as 4 weeks and will be based upon the azacitidine dosing schedule. Cycles will be repeated for as long as possible unless evidence of significant toxicity or progression to AML (defined by more than 30% blasts in the marrow), unless there is frank proliferation in peripheral blood (in the opinion of the treating physician), or if it is not safe for the patient. Patients will receive the first course of therapy without interruption regardless of the degree of myelosuppression. Patients can continue to receive therapy indefinitely until one of the criteria outlined below occurs. After the first course of therapy the interval between cycles of therapy can be spaced out at the discretion of the treating physician, but will otherwise be scheduled as close to possible to the initial 4 weeks. If there is evidence of prolonged myelosuppression defined by absolute neutrophil count of less than 1x10/dl and a platelet count of less than 30 x 10/dL for more than 42 days with evidence of hypocellular marrow (defined as marrow cellularity of less than 5%), subsequent courses of the azacitidine will be given at the next lower dose after the counts have recovered to an ANC of more than1x 10/dL and platelet more than 30 x dL. Cycles will be repeated as long as possible until toxicity or lack of response for at least 6 cycles of combination therapy.

In the absence of treatment delays due to early trial termination as described in Section 5.10, treatment may continue indefinitely until one of the following criteria applies:

1-Clinically significant progressive disease

2-Possibility of undergoing allogeneic stem cell transplant

3-Clinical significant intercurrent illness that prevents further administration of treatment

4-Patient request or general or specific changes in the patient condition that render the patient unacceptable for further treatment in the judgment of the investigator or treating physician

Table 2. Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Starting on day 1 of initial treatment and every 3 weeks thereafter	Experimental
Azacitidine	75 mg/m^2	Q4W	IV or SC infusion	Day 1 to 7 of each 4 week cycle	Standard

Please note that in this schedule, pembrolizumab and azacitidine run as to parallel treatment schedules.

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.

We will use the standard dosing of Azacitidine.

5.2.1.2 Dose Modification of MK3475

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

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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/ Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue **NOTE: G**rade 4 hypophysitis does not require stopping therapy with pembrolizumab as long as the pituitary hormones are replaced.	Permanently discontinue NOTE: **G**rade 4 hypophysitis does not require stopping therapy with pembrolizumab as long as the pituitary hormones are replaced.
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

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

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

[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

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

5.2.1.3 Dose Modification of azacitidine

Hematological toxicities will be attributed primarily to Azacitidine and preferentially will be dose reduced according to Table 4 below:

Table 4: Dose Adjustments for Grade 3 – 4 Hematological Toxicity

	Azacitidine IV/SC (x 7 days)

Level – 1	50 mg/m^2/day
Level – 2	25 mg/m^2/day

Further adjustments of azacitidine can be implemented as clinically indicated (i.e reducing to 5 days).

5.2.2 Timing of Dose Administration

Therapy can be administered in an outpatient or inpatient bases depending on clinical needs. Azacitidine will be administered on day 1 to 7. Therapy will be repeated every 4 weeks. MK3475 (Pembrolizumab) will be administered starting on Cycle 1 Day 1 and every 3 weeks thereafter. Azacitidine 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.

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

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

Azacitidine will be administered using standard guidelines.

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

This is not a randomized trial. Patients will be allocated based on their disease status.

5.4 Stratification

There is no stratification on this trial. Patients will be allocated based on their disease status.

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

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

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

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on PDMS/CORe 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.

- The protocol specific data will be entered into PDMS/CORe, the electronic case report form.
- The Investigator or physician designee is responsible for providing source documentation and assigning attribution for all AEs.

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 as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

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

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

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. An endocrine consult will be obtained for patients with type 1 diabetes, hypophysitis and hyperthyroidism to evaluate the cause and appropriate treatment. 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.
    - 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.

- **Type 1 diabetes mellitus (T1DM) (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA). An Endocrine consult will be requested.**

  - 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**, an Endocrine consult will be requested:

  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started. 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. Replacement of appropriate hormones may be required as the steroid dose is tapered. Note: **G**rade 4 hypophysitis does not require stopping therapy with pembrolizumab as long as the pituitary hormones are replaced.

- **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. An Endocrine consult will be requested to evaluate the cause of hyperthyroidism and the recommended treatment.

- **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. 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.

- **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.

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

Table 5 Infusion Reaction Treatment Guidelines

NCI CTCAE v4.0 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	**Stop Infusion and monitor symptoms.**	Subject may be premedicated 1.5h (±

NCI CTCAE v4.0 Grade	Treatment	Premedication at subsequent dosing
Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	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.**	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).
<u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids	No subsequent dosing

NCI CTCAE v4.0 Grade	Treatment	Premedication at subsequent dosing
(e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	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.** Note: **G**rade 4 hypophysitis does not require stopping therapy with pembrolizumab as long as the pituitary hormones are replaced.	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

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

5.7.2 Contraception

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

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

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

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women

not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

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

OR

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

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

(1) practice abstinence[†] from heterosexual activity;

OR

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

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

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

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

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

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, 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 medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

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

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

5.7.4 Use in Nursing Women

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

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be

withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved
- Unacceptable adverse experiences
- 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 uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment with Medical Monitor approval
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1). 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, every 12 weeks, for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.8.1 Discontinuation of Study Therapy after CR

Therapy should not be discontinued unless medically indicated or per patient decision.

5.9 Subject Replacement Strategy

Only patients that consent to therapy but are actually not treated with one dose of MK3475 will be consider for replacement on this trial.

5.10 Clinical Criteria for Early Trial Termination

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

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

2. Poor adherence to protocol and regulatory requirements

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

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

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

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart – Azacitidine Schedule (Cycle = Q4W)

Trial Period:	**Screening Phase**		**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	To be repeated beyond 8 cycles: 5	6	7	8	Discon	Safety Follow-up	Follow-Up Visits	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Administrative Procedures														
Days			1, 8, 15, 22	1	1	1	1	1	1	1				
Pre-screening Consent	X													
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X													
Azacitidine Administration Days 1 to 7[e]			X	X	X	X	X	X	X	X				
Post-study anticancer therapy status											X			
Survival Status											X	X	X	X
Clinical Procedures/Assessments														

Trial Period:	**Screening Phase**		**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	To be repeated beyond 8 cycles 5	6	7	8	Discon	Safety Follow-up	Follow-Up Visits	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
Days			**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**				
Review Adverse Events			X	X	X	X	X	X	X	X	X			
Full Physical Examination	X		X	X	X	X	X	X	X	X	X			
Directed Physical Examination														
Vital Signs and Weight	X		X	X	X	X	X	X	X	X	X			
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory														
Days			**1, 8, 15, 22**	**1**	**1**	**1**	**1**	**1**	**1**	**1**				
Pregnancy Test – Urine or Serum □-HCG	X		X*											
CBC with Differential[a,e]			X	X	X	X	X	X	X	X				
Comprehensive Serum Chemistry Panel[b,e]			X	X	X	X	X	X	X	X				
Efficacy Measurements														
Bone marrow examination[c] (aspirate and biopsy (later when indicated) and includes		X	X		X			X						

Trial Period:	**Screening Phase**		**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	To be repeated beyond 8 cycles 5	6	7	8	Discon	Safety Follow-up	Follow-Up Visits	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 12 weeks post discon	Every 12 weeks
cytogenetics, flow cytometry and standard genomics as indicated)														
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood														
Correlative Studies Blood Collection[c]		X	X	X	X									
Stem cell population analysis (from bone marrow aspirate)[c]		X	X		X			X						
Genomics (from bone marrow aspiarate)[d]		X	X		X			X						

* Pregnancy test will be repeated if screening tests performed more than 72 hours prior to first dose of study drug.
a. CBC with differential: performed on Screening, days 1, 8, 15, and 22 of Cycle 1. On each subsequent cycle, CBC with differential should be done as clinically indicated.
b. Comprehensive Serum Chemistry Panel: performed on Screening, days 1, 8, 15, and 22 of Cycle 1. On each subsequent cycle, CBC with differential should be done as clinically indicated. Coagulation tests (PT/aPTT) should only be done on Cycle 1 Day 1.
c. Bone marrow biopsy and/or aspiration collected at screening, between days 22 to 28 of Cycles 1 and 3, then every 3 cycles thereafter, additional bone marrow evaluations could be performed as clinically indicated. Bone Marrow Correlatives will be collected at the same time.
d. Correlative studies blood collection will be collected: at screen, prior to each of MK3475 dose during the first 3 cycles, at time of best morphological response and at progression
e After Cycle 1, azacitidine may be administered and routine laboratory tests may be performed/ordered by patient's local physician, if the patient is not scheduled to return to MD Anderson on those days.

6.2 Study Flow Chart - Pembrolizumab (MK3475) Schedule (Q3W)

If Azacitidine and MK3475 dosing fall on the same day: CBC with differential, Comprehensive serum chemistry panel, and thyroid function testing (TSH, FT4, T3) should be done on day 1 (+/-3days) before any doses of study drugs are administered.

Trial Period	**Weeks of Treatment**								End of Treatment
Weeks	**1**	**4**	**7**	**10**	**13**	**16**	**19**	**22+**	Discontinuation
Scheduling Window (days)	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	At time of Discontinuation
Day	1	1	1	1	1	1	1	1	
Review Adverse Events	X	X	X	X	X	X	X	X	X
Full Physical Examination									X
Directed Physical Examination	X	X	X	X	X	X	X	X	
Vital Signs and Weight	X	X	X	X	X	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X	X	X
CBC with Differential	X	X	X	X	X	X	X	X	
Comprehensive Serum Chemistry Panel	X	X	X	X	X	X	X	X	
T3, FT4 and TSH	X	X	X	X	X	X	X	X	
MK-3475 Dosing	X	X	X	X	X	X	X	X	

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

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

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

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

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

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

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

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

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

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

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs 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.

Per institutional guidelines

7.1.1.7 Assignment of Randomization Number N/A

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

Per institutional guidelines.

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 (seeAppendix E in PDOL). 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. Clinically significant abnormal findings should be recorded as medical history.

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, weight and blood pressure. 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 Appendix D in PDOL) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

There is no indication for imaging studies for patients with MDS. Bone marrow specimens to evaluate response and when agreed for exploratory correlatives studies will be performed at baseline and on after cycle 1, cycle 3 and then every 3 months. Bone marrow studies will include aspirate, biopsy (when indicated). Samples will be obtained also for cytogenetic and molecular analysis following institutional guidelines.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Patients will be consented to institutional consents for sample acquisition and analysis as described in the text and pertinent tables.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below in Table 6.

Table 6 Laboratory Tests

Hematology	**Chemistry**	**Urinalysis**	**Other**
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	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 Creatinine		

† 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.

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 or b) complete 24 months of treatment with pembrolizumab may discontinue treatment. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (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

Per table 6.1

7.1.5.1.1 Screening Period

Per table 6.1

7.1.5.2 Treatment Period

Per table 6.1

7.1.5.3 Post-Treatment Visits

Per table 6.1

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.

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 about every 6-8 weeks (± 7 days) as clinically indicated to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

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.

7.2 Assessing and Recording Adverse Events

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

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

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

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

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

All adverse events will be recorded in PDMS/CORe from the time the consent form is signed through 30 days following cessation of treatment and at each examination.

- The protocol specific data will be entered into PDMS/CORe, the electronic case report.
- The Investigator or physician designee is responsible for providing source documentation and assigning attribution for all AEs.

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

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

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

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

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

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

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

7.2.3 Immediate Reporting of Adverse Events

7.2.3.1 Serious Adverse Events

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

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

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

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices".

Reporting to FDA:

- **Serious adverse events will be forwarded to FDA according to 21 CFR 312.32.**

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

- **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 6 for additional details regarding each of the above criteria.

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, 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 serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the 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 Sponsor 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

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.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be 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.

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 in PDMS/CORe.

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 Sponsor 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 test drug	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's product(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 THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported in PDMS/CORe by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**	

Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

This is a Phase II open-label study designed to evaluate the safety and efficacy of the combination of MK3475 and 5-azacitidine in patients with MDS. Two different patient groups (hypomethylating failure group and previously untreated MDS group) will be studied. Each cohort will be composed of N=20 patients. Accrual will be parallel for each patient group.

The primary efficacy outcome for each cohort is the overall response rate (ORR) defined as CR + PR + hematological improvement (HI). Overall response will be assessed after 6 cycles of treatment; cycle length is 28 days. A maximum of 20 patients will be enrolled in each cohort (total for study N=40).

Hypomethylating failure MDS cohort

The target ORR with the experimental treatment is 20%. There is no standard treatment for this patient population, we use a null ORR of 5%. The regimen of the experimental treatment will be considered worthy of further investigation if it elicits an increase in ORR to 20% with acceptable toxicity. A >30% therapy related non-hematological grade 3/4 toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions below, were constructed that meet the following two conditions,

1) Stop if Prob{p(ORR, E) < p(ORR, H) + 0.15 | data} > 0.99, or
2) Stop if Prob{p(TOX, E) > 0.30 | data} > 0.95,

where p(ORR, E) and p(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 1%) that ORR rate of the experimental treatment is greater than the ORR rate of standard treatment by 15%. The second condition will stop the study early if excessive therapy-related non-hematological grade 3/4 toxicity (>30%) is highly probable (i.e., probability >95%) for the experimental treatment. Monitoring for toxicity and futility will start with the first patient, and cohort size of 5.

The monitoring rule for the toxicity rate, based on these assumptions and monitoring conditions above is found in Table 8. For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.

Table 8. Stop accrual if the number of drug-related non-hematological grade 3/4 toxicities is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	5	10	15	20
# patients with toxicities	4-5	6-10	8-15	Always stop

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 9. For example, accrual will cease if no patients experience an overall response within 6 cycles in the first 10 patients treated.

Table 9. Stop accrual if the number with response is less than or equal to indicated (i.e., # patients with overall response) among the number of patients evaluated

# patients evaluated	5	10-15	20
# patients with response	Never	0	Always stop

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 10). In order to utilize the software for the design, a response constant rate of 5% and beta (0.1, 1.9) prior were assumed for the standard treatment response rate and experimental treatment response prior distribution, respectively. A delta 15% was assumed. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the combination treatment was 20% and the true toxicity rate was 30% was 18%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 82% when the true ORR was 5% and 61% when true ORR rate was 20%.

Table 10. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.05	0.5990
	0.10	0.3491
	0.15	0.1973
	0.20	0.1079
	0.25	0.0569

Table 10. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment		
True Toxicity Rate	True ORR	Prob(stop the trial early)
0.20	0.05	0.6040
	0.10	0.3573
	0.15	0.2075
	0.20	0.1192
	0.25	0.0688
0.30	0.05	0.6326
	0.10	0.4037
	0.15	0.2647
	0.20	**0.1828**
	0.25	0.1360
0.40	0.05	0.7085
	0.10	0.5268
	0.15	0.4166
	0.20	0.3516
	0.25	0.3145
0.50	0.05	0.8230
	0.10	0.7126
	0.15	0.6456
	0.20	0.6062
	0.25	0.5836

Previously untreated MDS cohort

The target ORR with the experimental treatment is 70%, assuming the standard treatment ORR is 50%. The regimen of the experimental treatment will be considered worthy of further investigation if it elicits an increase in ORR to 70% with acceptable toxicity. A >30% therapy related non-hematological grade 3/4 toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions below, were constructed that meet the following two conditions,

3) Stop if Prob{p(ORR, E) < p(ORR,H) + 0.20 | data} > 0.95, or
4) Stop if Prob{p(TOX, E) > 0.30 | data} > 0.95,

where p(ORR, E) and p(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 5%) that ORR rate of the experimental treatment is greater than the ORR rate of standard treatment by 20%. The second condition will stop the study early if excessive therapy-related

non-hematological grade 3/4 toxicity (>30%) is highly probable (i.e., probability >95%) for the experimental treatment. Monitoring for toxicity and futility will start with the first patient, and cohort size of 5.

The monitoring rule for the toxicity rate, based on these assumptions and monitoring conditions above is found in Table 11. For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.

Table 11. Stop accrual if the number of drug-related non-hematological grade 3/4 toxicities is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	5	10	15	20
# patients with toxicities	4-5	6-10	8-15	Always stop

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 12. For example, accrual will cease if 1 patients experience an overall response within 6 cycles in the first 5 patients treated.

Table 12. Stop accrual if the number with response is less than or equal to indicated (i.e., # patients with overall response) among the number of patients evaluated

# patients evaluated	5	10	15	20
# patients with response	0-1	0-4	0-7	Always stop

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 13) In order to utilize the software for the design, a response constant rate of 50% and beta (1.0, 1.0) priors were assumed for the standard treatment response rate and experimental treatment response prior distribution, respectively. A delta 20% was assumed. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the experimental treatment was 70% and the true toxicity rate was 30% was 16%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 81% when the true ORR was 50%, and 60% when true ORR rate was 70%.

Table 13. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.50	0.5590
	0.60	0.2740
	0.70	0.0850

Table 13. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment		
True Toxicity Rate	True ORR	Prob(stop the trial early)
	0.80	0.0138
	0.90	0.0012
0.20	0.50	0.5646
	0.60	0.2831
	0.70	0.0965
	0.80	0.0262
	0.90	0.0138
0.30	0.50	0.5960
	0.60	0.3349
	0.70	**0.1618**
	0.80	0.0965
	0.90	0.0850
0.40	0.50	0.6795
	0.60	0.4723
	0.70	0.3349
	0.80	0.2831
	0.90	0.2740
0.50	0.50	0.8053
	0.60	0.6795
	0.70	0.5960
	0.80	0.5646
	0.90	0.5590

8.2 Statistical Analysis Plan

Statistical Analysis Plan

All patients who received any dose of the study agent will be included in the analysis for efficacy and safety. Drop out of patients will count as failure for the efficacy analysis. Demographic/clinical characteristics (i.e. including duration of response) and safety data of the patients will be summarized using descriptive statistics such as mean, standard deviation, median and range. . For the primary efficacy analysis, we will estimate the ORR (cohort 1) and ORR (cohort 2) for the combination treatment, along with the 95% credible interval. For the efficacy of cohort 1, a sample size of 20 patients ensures a posterior 95% credible interval for ORR of (0.06, 0.37), if the trial is not terminated early, under the assumption of a 20% ORR. For the efficacy of cohort 2, a sample size of 20 patients ensures a posterior 95% credible interval for ORR of (0.48, 0.85), if the trial is not terminated early, under the assumption of a 70% ORR. The association between ORR and patient's clinical characteristics will be

examined by Wilcoxon's rank sum test or Fisher's exact test, as appropriate. Toxicity type, severity and attribution will be summarized for each patient using frequency tables. The distribution of time-to-event endpoints (EFS and OS) will be estimated using the method of Kaplan and Meier. Comparisons of time-to-event endpoints by important subgroups will be made using the log-rank tests. Paired t-tests will be used to determine the immunological and molecular changes in the peripheral blood and bone marrow from baseline to the time of response, and to the time of disease progression.

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 14.

Table 14. 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.

9.6 Additional information on azacitidine

Chemical Name: 4-amino-1-(2-deoxy-ß-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one

Other Names: 4-Amino-1-ß-D-ribofuranosyl-1,3,5-triazin-2(1H)-one, 5 AZC, 5-AC, 5-AzaC, 5-azacytidine, 5-AZCR, Antibiotic U 18496, Azacytidine, ladakamycin, Mylosar, NSC-102816, U-18496, WR-183027, Azacitidine

Classification: Antimetabolite, DNA hypomethylating agent

CAS Registry Number: 320-67-2

Molecular Formula: C8H12N4O5 **M.W.:** 244.21

Approximate Solubility: soluble (soluble in caustic soda)

Mode of Action:

Azacitidine is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer

cells that are no longer responsive to normal growth control mechanisms. Non-proliferating cells are relatively insensitive to azacitidine.

Pharmacokinetics:

The pharmacokinetics of azacitidine were studied in six MDS patients following a single 75 mg/m2 subcutaneous (SC) dose and a single 75 mg/m2 intravenous (IV) dose. Azacitidine is rapidly absorbed after SC administration; the peak plasma azacitidine concentration of 750 ± 403 ng/ml occurred in 0.5 hour. The bioavailability of SC azacitidine relative to IV azacitidine is approximately 89%, based on area under the curve. Mean volume of distribution following IV dosing is 76 ± 26 L. Mean apparent SC clearance is 167 ± 49 L/hour and mean half-life after SC administration is 41 ± 8 minutes.

Published studies indicate that urinary excretion is the primary route of elimination of azacitidine and its metabolites. Following IV administration of radioactive azacitidine to 5 cancer patients, the cumulative urinary excretion was 85% of the radioactive dose. Fecal excretion accounted for <1% of administered radioactivity over three days. Mean excretion of radioactivity in urine following SC administration of 14C-azacitidine was 50%. The mean elimination half-lives of total radioactivity (azacitidine and its metabolites) were similar after IV and SC administrations, about 4 hours.

Drug-Drug Interactions:

Drug interaction studies with 5-azacitidine have not been conducted. An in vitro study of 5-azacitidine incubation in human liver fractions indicated that azacitidine may be metabolized by the liver. Whether azacitidine metabolism may be affected by known microsomal enzyme inhibitors or inducers has not been studied. The potential of azacitidine to inhibit cytochrome P450 (CYP) enzymes is not known. In vitro studies with human cultured hepatocytes indicate that azacitidine at concentrations of 1.0 µM to 100 µM does not induce CYP 1A2, 2C19, or 3A4/5. Hydroxurea is known to inhibit ribonuclease reducatase, a key enzyme required for the incorporation of 5-azacytidine residues into DNA. Therefore, hydroxyurea may potentially limit the clinical activity of 5-azacytidine and its use during this clinical trial should be avoided.

Route of Administration: Intravenous

Preparation: Azacitidine is a cytotoxic drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing suspensions. If reconstituted azacitidine comes into contact with the skin, immediately and thoroughly wash with soap and water. If it comes into contact with mucous membranes, flush thoroughly with water.

How Supplied: Azacitidine is supplied as 100 mg vials of lyophilized powder.

Storage: Store un-reconstituted vials at 25º C (77º F); excursions permitted to 15º-30º C (59º-86º F) (See USP Controlled Room Temperature). There is no need to protect azacitidine from exposure to light.

Handling and Disposal: Procedures for proper handling and disposal of anticancer drugs should be applied.

Reported Adverse Events and Potential Risks:

In clinical studies, the most commonly occurring adverse reactions were nausea (70.5%), anemia (69.5%), thrombocytopenia (65.5%), vomiting (54.1%), pyrexia (51.8%), leucopenia (48.2%), diarrhea (36.4%), fatigue (35.9%), injection site erythema (35.0%), constipation (33.6%), neutropenia (32.3%), and ecchymosis (30.5%). Other adverse reactions included dizziness (18.6%), chest pain (16.4%), febrile neutropenia (16.4%), myalgia (15.9%), injection site reaction (13.6%), aggravated fatigue (12.7%) and malaise (10.9%).

Because treatment with Azacitidine is associated with neutropenia and thrombocytopenia, complete blood counts should be performed as needed to monitor response and toxicity, but at a minimum, prior to each dosing cycle.

Because azacitidine is potentially hepatotoxic in patients with pre-existing hepatic impairment, caution is needed in patients with liver disease. In addition, azacitidine and its metabolites are substantially excreted by the kidneys and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, it may be useful to monitor renal function.

Azacitidine may cause fetal harm. While receiving treatment with Azacitidine, women of childbearing potential should avoid becoming pregnant, and men should avoid fathering a child. In addition, women treated with Azacitidine should not nurse.

Reconstitution Recommendations for Azacitidine for IV Administration

General Cautions:

Azacitidine's active agent, azacitidine, is hydrolytically unstable. Administration of reconstituted drug product must be completed within one hour of reconstitution. Azacitidine is incompatible with 5% Dextrose solutions, Hespan, or solutions containing Bicarbonate. At no time should diluents with a pH below 6.0 be used.

Procedure:

1. Reconstitute each Azacitidine drug product vial with 10mL of Sterile Water for Injection, USP (SWFI).

2. Shake/roll the vial until all solids are dissolved as determined by visual inspection. Vigorous shaking is allowed if required to ensure complete dissolution. The reconstituted solution should be clear.

3. The concentration of the reconstituted drug product is 10 mg/mL Azacitidine.

4. Withdraw and dilute the required amount of reconstituted drug product in an appropriate container with either 0.9% Sodium Chloride Injection, USP, or Lactated Ringer's Injection, USP. The final volume of the diluted drug product should be no greater than 100mL.

5. If a patient requires more than one vial of Azacitidine, reconstitute each vial as described above and dilute all required, reconstituted Azacitidine into a single container with a total volume not to exceed 100mL. (Final concentration should be <10 mg/mL).

6. The reconstituted and diluted Azacitidine should be administered intravenously over a 10 - 40 minute period. **The administration must be completed within one hour of the reconstitution time.**

7. Follow the administration with a 10 mL normal saline flush.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

Per institutional rules

10.2 Compliance with Financial Disclosure Requirements

Per institutional rules

10.3 Compliance with Law, Audit and Debarment

Per institutional rules

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

Per institutional rules

10.6 Data Management

Per institutional rules

11.0 REFERENCES

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