CLINICAL RESEARCH PROJECT

Protocol # 17-H-0026
Drug Names: Pembrolizumab, Decitabine
IND: 131826
Closed to accrual: November 30, 2017

Title: Pembrolizumab and Decitabine for Refractory or Relapsed Acute Myeloid Leukemia

Short Title: PD-AML

Keywords: Pembrolizumab, Decitabine, AML.

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Subjects of Study:

Number	Sex	Age-range
10	M/F	18-99

(Maximal accrual including replacement subjects: 15)

Ionizing Radiation for Research:	No
Off-Site Project:	No
Multi-center trial:	No
DSMB Involvement:	Yes
Tech Transfer:	Yes
IND:	Yes (IND number 131826)
Version:	11.0

PRECIS

This is a pilot study to determine the feasibility of a novel combination of Pembrolizumab and Decitabine in relapsed/refractory adult AML patients. While both Pembrolizumab and Decitabine are FDA approved agents, this study will explore giving these drugs in combination for this population of patients.

Abbreviated Title	PD-AML
Trial Phase	Pilot
Clinical Indication	Relapsed or Refractory Acute Myeloid Leukemia
Trial Type	Single arm pilot study
Type of control	Historical
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	Single-arm
Number of trial subjects	Ten
Estimated enrollment period	December 2016-December 2017
Estimated duration of trial	December 2016 – December 2019
Duration of Participation	Up to two years
Estimated average length of treatment per patient	Six months

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1.0 OBJECTIVES

1.1. Primary Objective

To determine the feasibility of a novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

1.2. Secondary Objectives

To explore the efficacy of a novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

To determine time to first response for the novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

To determine time to best response for the novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

To determine duration of response for the novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

To determine duration of best response for the novel combination of Pembrolizumab and Decitabine in relapsed/refractory AML patients.

1.3. Exploratory Laboratory Objectives

Investigate changes in AML clonal composition in relapsed or refractory patients treated with Pembrolizumab and Decitabine.

Investigate measurable changes in immune parameters associated with clinical efficacy and/or toxicity (eg: autoimmunity) using repeated disease site sampling.

Investigate changes in AML disease burden during treatment of relapsed/refractory AML patients with Pembrolizumab and Decitabine.

Investigate association of clinical response with marrow infiltrating lymphocytes (MILs), mutational burden in AML and PDL-1 expression.

2.0 BACKGROUND & RATIONALE

2.1. Pharmaceutical and Therapeutic Background

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

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

PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. The FDA recently also granted accelerated approval for pembrolizumab to treat patients with advanced (metastatic) non-small cell lung cancer (NSCLC) whose disease has progressed after other treatments and with tumors that express PD-L1.

2.2. Rationale for the Trial and Selected Subject Population

Approximately 20,000 patients will be diagnosed with acute myeloid leukemia (AML), with greater than 10,000 AML patient deaths in the United States during 2015. The majority of those AML patients will ultimately be diagnosed with relapsed or refractory disease (RR-AML), which is generally fatal. Response rates for patients who receive intensive salvage therapy after relapse, which offers the best chance for response, range from 30-50%. Response rates for those with refractory disease are even lower, typically under 10%. While a variety of different treatment regimens have been studied in an effort to improve outcomes of patients with RR-AML there appears to be no single superior approach. **There is no current standard of care for adult relapsed or refractory AML other than offering referral to an appropriate clinical trial.**

The powerful "graft versus leukemia" effect thought responsible for the therapeutic effect of allo-HSCT in reducing relapse rates of high-risk AML patients, and provides the rationale for the use of immune therapy with Pembrolizumab. While pembrolizumab has been tested in many cancer types and already has FDA approvals for use in melanoma, non-small cell lung cancer and head and neck cancer, this pilot trial will be the first use of pembrolizumab immunotherapy in AML.

There is strong pre-clinical rationale for the combination of pembrolizumab (anti-PD1 immune checkpoint therapy) and decitabine (hypomethylating epigenetic modulating therapy) in patients with AML. 1) There are now reports from two groups suggesting that therapy with hypomethylating agents in MDS/AML patients results in up-regulation of PD-1/PD-L1 checkpoints potentially resulting in therapeutic resistance by inhibiting immune based control of disease[1,2]. 2) Pre-clinical data from Bert Vogelstein and colleagues at Johns Hopkins suggest that co-treatment with epigenetic-modulating drugs and checkpoint inhibitors markedly improved treatment outcomes in BALB/c mouse models with 4T1 and large CT26 tumors compared with the use of checkpoint inhibitors alone[3]. 3) Low dose decitabine appears to modulate natural killer cell anti-AML function

by increased expression of killer immunoglobulin-like receptors (KIR) and the activating receptor NKp44 on NK cells[4], while also up-regulating NKG2D-specific UL16-binding protein (ULBP) ligands on AML cells potentially sensitizing to increased NK mediated killing[5]. 4) Epigenetic therapy with DNA methyltransferase inhibitor has been shown to upregulate cancer-testis antigen (CTA) expression in AML[6,7], and to be associated with induction of CTA-specific cytotoxic T lymphocytes (CTLs) with the majority (8/11) having an induced CTL immune response also having a major clinical response[7]. 5) PD-L1 expression is seen by IHC on 35-50% of human AML[8] and levels are higher in relapsed than primary AML[9]. 6) PD-1 (hi) and TIM-3(+) T cells are associated with (and found prior to) AML relapse post allo-HSCT, with a functional phenotype characterized by the inability to produce interleukin 2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in both mice[10] and humans[11]. 6) PD-1 knockout mice have improved survival and augmented immune response compared with wild-type mice in an AML challenge model[12] and PD-1 pathway blocking antibody treatment restored CD8+ T cell function and resulted in improved survival in the same AML model[10]. 7) Recent evidence suggests that treatment with DNA methyltransferase inhibitors (like decitabine) may cause an up-regulation of immune signaling via a type I interferon response due to expression and recognition of double stranded RNA including from endogenous retrovirus genes[13,14]. In patients treated with immune checkpoint therapy this same viral defense expression signature has been associated with durable clinical response. 8) Treatment with DNA methyltransferase inhibitor sensitized to immune checkpoint therapy in a mouse cancer model[14]. Taken together these findings provide strong pre-clinical rationale for the combination of hypomethylating agent and immune checkpoint therapy used in our trial[15,16].

Finally, AML has historically been approached as a homogeneous diagnostic entity with a resulting "one size fits all" treatment strategy, often resulting in disappointing outcomes[17]. In reality, the acute myeloid leukemias are a heterogeneous group of diseases with distinct molecular and phenotypic characteristics[18,19]. Even within a single patient AML may be polyclonal at any examined time-point, and this clonal composition can change over time with the clone predominant at presentation not necessarily the one responsible for relapse and death[20]. We therefore hypothesize that effective pembrolizumab therapy for refractory or relapsed AML in those who have not received allo-HSCT may be associated with changes in the clonal composition due to differences in immunogenicity between clones. This oligoclonal nature of AML biology, together with a blood and bone marrow distribution highly amenable to repeated sampling of the sites of disease burden, provides a near unique opportunity to investigate fundamental mechanisms underpinning treatment efficacy of this new class of immunotherapeutic drugs.

2.3. Pembrolizumab

2.3.1. Mechanism of Action

The programmed cell death 1 (PD-1) pathway represents a major immune control switch, which may be

engaged by tumor cells to overcome active T cell immune surveillance. 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 a type I transmembrane glycoproteins. Upon engagement of its ligands, PD-L1 and PD-L2, the cytoplasmic tail of PD-1 recruits tyrosine phosphatases SHP-1 and SHP-2 to the immunoreceptor tyrosine-based switch motif (ITSM) motif, and dephosphorylates effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The ligands for PD-1 are constitutively expressed or can be induced in a variety of cell types. 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/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab (KEYTRUDA®, MK-3475; previously known as SCH 900475 and ORG 307488-0 [herein referred to as pembrolizumab unless otherwise noted]) is a potent and highly selective humanized monoclonal antibody of the immunoglobulin G4 (IgG4)/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection.

Pembrolizumab potentiates existing immune responses only in the presence of antigen-receptor stimulation and does not nonspecifically activate all T cells. Pembrolizumab was tested for its capacity to enhance T cell activity in vitro using blood cells from healthy volunteers stimulated with staphylococcus enterotoxin B (SEB). Pembrolizumab enhanced IL-2 production over control on average 2- to 4-fold at the highest antibody concentration tested (25 μg/mL). In addition to IL-2, levels of tumor necrosis factor alpha (TNFα), IL-17, IL-6, and interferon gamma (IFNγ) increased by 1.2 to 1.7 folds after the addition of pembrolizumab to SEB-stimulated whole blood cells.

2.3.2. Pharmacokinetics

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. 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 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.

2.3.3. Metabolism and Clearance

No traditional metabolism studies were conducted with pembrolizumab per current ICH S (R1) guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals. However, in vivo studies were conducted in C.B17 SCID mice to demonstrate the lack of Fab-arm or half molecule exchange for pembrolizumab. IgG4 wild type molecules can undergo in vitro and in vivo molecular rearrangement called Fab -arm (or half molecule) exchange by swapping their half molecule with other IgG4 half molecules, thereby generating bispecific or hybrid antibodies. A point mutation (S228P) in the core hinge region in IgG4 has been shown to be sufficient to prevent the Fab-arm exchange. The results supported that pembrolizumab, which has a hinge mutation from S to P at position 228, did not form detectable hybrid antibodies with co-administered wild type IgG4 molecules in vivo in SCID mice. This observation is consistent with the results of extensive *in vitro* characterization and indicates that pembrolizumab is not likely to engage in Fab-arm exchange in humans.

Renal Impairment

The effect of renal impairment on the clearance (CL) of pembrolizumab was evaluated by population PK analyses in patients with mild (eGFR 60 to 89 mL/min/1.73 m^2; n=210), moderate (eGFR 30 to 59mL/min/1.73m^2; n=43), or severe (eGFR 15 to 29mL/min/1.73 m^2; n=2) renal impairment compared to patients with normal (eGFR greater than or equal to 90 mL/min/1.73m^2; n=221) renal function. No clinically important differences in the CL of pembrolizumab were found between patients with renal impairment and patients with normal renal function.

Hepatic Impairment

The effect of hepatic impairment on the CL of pembrolizumab was evaluated by population PK analyses in patients with mild hepatic impairment (total bilirubin (TB) less than or equal to upper limit of normal (ULN) and AST greater than ULN or TB between 1 and 1.5 times ULN and any AST; n=59) compared to patients with normal hepatic function (TB and AST less than or equal to ULN; n=410). No clinically important differences in the CL of pembrolizumab were found between patients with mild hepatic impairment and normal hepatic function. Pembrolizumab has not been studied in patients with moderate (TB greater than 1.5 to 3 times ULN and any AST) or severe (TB greater than 3 times ULN and any AST) hepatic impairment.

2.3.4. Clinical experiences

The efficacy of pembrolizumab was investigated in other solid tumors, including melanoma and lung cancer. In a multicenter randomized phase II trial in metastatic melanoma, patients were randomized to pembrolizumab 2mg/kg Q3W and 10mg/kg Q3W versus standard-of-care in a 1:1:1 ratio. 361 patients received pembrolizumab and 179 received chemotherapy. 6-month PFS rates were 34.3% (95% CI: 27.4 to 41.3%) and 37.7% (95% CI: 30.6% to 44.8%) for pembrolizumab 2 mg/kg and 10 mg/kg, respectively, versus 15.6% (95% CI: 10.5% to 21.5%) for the control arm. ORR per RECIST 1.1 by IRO were 21% in the pembrolizumab 2 mg/kg arm, 25% in the 10 mg/kg arm, and 4% in the chemotherapy arm ($p<0.0001$ for each pembrolizumab dose versus chemotherapy).

Pembrolizumab is approved by the U.S. FDA for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAFV600 mutation positive, a BRAF inhibitor. The FDA also recently also granted accelerated approval for pembrolizumab to treat patients with advanced (metastatic) non-small cell lung cancer (NSCLC) whose disease has progressed after other treatments and with tumors that express PD-L1 and also patients with recurrent or metastatic recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy. Clinical trials using pembrolizumab in other solid and hematologic malignancies are now ongoing.

2.4. Decitabine

Decitabine is a nucleoside metabolic inhibitor that is U.S. FDA approved for treatment of patients with myelodysplastic syndromes (MDS).

2.4.1. Mechanism of Action

Decitabine is believed to exert its antineoplastic effects after phosphorylation and direct incorporation into DNA and inhibition of DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. Decitabine inhibits DNA methylation *in vitro,* which is achieved at concentrations that do not cause major suppression of DNA synthesis. Decitabine-induced hypomethylation in neoplastic cells may restore normal function to genes that are critical for the control of cellular differentiation and proliferation. In rapidly dividing cells, the cytotoxicity of decitabine may also be attributed to the formation of covalent adducts between DNA methyltransferase and decitabine incorporated into DNA. Non-proliferating cells are relatively insensitive to decitabine.

2.4.2. Pharmacokinetics

Pharmacokinetic parameters were evaluated in patients. Eleven patients received 20 mg/m2 infused over 1 hour intravenously (treatment Option 2). Fourteen patients received 15 mg/m2 infused over 3 hours (treatment Option 1). PK parameters are shown in Table 1 below. Plasma concentration-time profiles after discontinuation of infusion showed a biexponential decline. The CL of decitabine was higher following treatment Option 2. Upon repeat doses there was no systemic accumulation of decitabine or any changes in PK parameters. Population PK analysis (N=35) showed that the cumulative AUC per cycle for treatment Option 2 was 2.3-fold lower than the cumulative AUC per cycle following treatment Option 1.

Table 1: Mean (CV% or 95% CI) Pharmacokinetic Parameters of Decitabine (from FDA Label 10/2014)

Dose	C_{max} (ng/mL)	$AUC_{0\infty}$ (ng·h/mL)	$T_{½}$ (h)	CL (L/h/m²)	$AUC_{Cumulative}$*** (ng·h/mL)
15 mg/m² 3-hr infusion every 8 hours for 3 days (Option 1)*	73.8 (66)	163 (62)	0.62 (49)	125 (53)	1332 (1010-1730)
20 mg/m² 1-hr infusion daily for 5 days (Option 2)**	147 (49)	115 (43)	0.54 (43)	210 (47)	570 (470-700)

*N=14, **N=11, ***N=35 Cumulative AUC per cycle

The exact route of elimination and metabolic fate of decitabine is not known in humans. One of the pathways of elimination of decitabine appears to be deamination by cytidine deaminase found principally in the liver but also in granulocytes, intestinal epithelium and whole blood.

2.4.3. Metabolism and Clearance

Geriatric Use

Of the total number of patients exposed to decitabine in the controlled clinical trial, 61 of 83 patients were age 65 and over, while 21 of 83 patients were age 75 and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

Renal Impairment

There are no data on the use of decitabine in patients with renal dysfunction; therefore, decitabine should be used with caution in these patients.

Hepatic Impairment

There are no data on the use of decitabine in patients with hepatic dysfunction; therefore, decitabine should be used with caution in these patients.

2.5. Summary of Clinical Safety

2.5.1. Pembrolizumab

Pooled safety data (N=2461) treatment with pembrolizumab is available on patients with melanoma or non-small cell lung cancer in 8 clinical trials: P001, P002, P011, P012, P013, P021, P023, and P028. In general, the safety profile observed in P011 (monotherapy arm), P012, P013, and P028 was similar to that observed in P001/P002.

In the pembrolizumab monotherapy trials, the most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, dyspnea, and anemia. In P021, the most commonly reported AEs in this population across the dose regimens were fatigue (49.2%), constipation and nausea (26.2% each), decreased appetite (23.1%), diarrhea (18.5%), and anemia and alopecia (15.4% each). In P023, the most commonly reported AEs experienced in this population across the dose regimens were neutropenia and thrombocytopenia (50.0% each), followed by anemia, respiratory tract infection, and back pain (30.0% each).

In the pembrolizumab monotherapy trials, the incidence of Grade 3-5 treatment-related AEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 subjects) in P001/P002. In the combination therapy trials, Grade 3-5 treatment-related AEs were reported in 23.1% (15 of 65 subjects in this population) in P021 and 50.0% (5 of 10 subjects in this population) in P023. The most commonly reported Grade 3-5 treatment-related AEs were anemia, ALT or AST increases, and colitis. In P021, the most common Grade 3-5 treatment-related AEs across dose regimens were AST increase (6.2%) and anemia and ALT increase (4.6% each). In P023, the only Grade 3-5 treatment-related AEs that occurred in more than 1 subject in this population across dose regimens were neutropenia (40.0%) and anemia (20.0%).

In the pembrolizumab monotherapy trials, most subjects who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 4.2% (18 of430 subjects in P028) to 12.3% (192 of 1562 subjects in P001/P002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a treatment-related AE were infrequent and ranged from 0% (no subjects in P011) to 4.5% (4 of 88 subjects in P013). The most commonly reported treatment-related AE leading to discontinuation was pneumonitis. In P021, most subjects continued treatment despite AEs, and only 4.6% discontinued due to an AE. Only 3.1% of subjects discontinued study treatment due to an AE that was considered related to study treatment by Investigators. Interstitial lung disease, allergic dermatitis and drug

eruption were the only treatment-related AEs resulting in discontinuation and were reported in 1 subject each (1.5%). In P023, no subjects discontinued due to an AE.

For more detailed information refer to the current version of the Investigator Brochure.

2.5.2. Decitabine

Decitabine was studied in 3 single-arm studies (N=66, N=98, N=99) and 1 controlled supportive care study (N=83 decitabine, N=81 supportive care). The data described below reflect exposure to decitabine in 83 patients in the MDS trial. In the trial, patients received 15 mg/m2 intravenously every 8 hours for 3 days every 6 weeks. The median number of decitabine cycles was 3 (range 0 to 9).

Most Commonly Occurring Adverse Reactions: neutropenia, thrombocytopenia, anemia, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea, and hyperglycemia.

Adverse Reactions Most Frequently (≥1%) Resulting in Clinical Intervention in the Phase 3 Trials in the decitabine Arm:

Discontinuation: thrombocytopenia, neutropenia, pneumonia, Mycobacterium avium complex infection, cardio-respiratory arrest, increased blood bilirubin, intracranial hemorrhage, abnormal liver function tests.

Dose Delayed: neutropenia, pulmonary edema, atrial fibrillation, central line infection, febrile neutropenia.

Dose Reduced: neutropenia, thrombocytopenia, anemia, lethargy, edema, tachycardia, depression, pharyngitis.

In the controlled trial using decitabine dosed at 15 mg/m2, administered by continuous intravenous infusion over 3 hours repeated every 8 hours for 3 days, the highest incidence of Grade 3 or Grade 4 adverse events in the decitabine arm were neutropenia (87%), thrombocytopenia (85%), febrile neutropenia (23%) and leukopenia (22%). Bone marrow suppression was the most frequent cause of dose reduction, delay and discontinuation. Six patients had fatal events associated with their underlying disease and myelosuppression (anemia, neutropenia, and thrombocytopenia) that were considered at least possibly related to drug treatment. Of the 83 decitabine-treated patients, 8 permanently discontinued therapy for adverse events; compared to 1 of 81 patients in the supportive care arm.

In a single-arm phase 1 study of patients with hematological malignancies (N=48) decitabine was dosed at 5, 10, 15 or 20mg/m^2 daily intravenously over one hour for 10 days (5 days on, 2 days off, 5 days on), approximately every six weeks[21]. 73% of these patients had AML and 14% had MDS. The treatment was well tolerated overall. Nonhematopoietic side effects are detailed in Table 2 below. Asymptomatic but severe

elevations in liver function tests, possibly related to therapy, were observed in 6 patients. In 5 cases, values returned to baseline within 2 weeks; the sixth patient died on day 21 with a bilirubin level of 290.7 μM and evidence of sepsis. Febrile episodes were noted in 26 patients (52%). These included fever of unknown origin in 8 patients (16%) and documented infections in 18 patients (36%): bacterial in 6, fungal in 1, others in 3, minor infections in 1, pneumonia in 12.

Myelosuppression was evaluated by studying time to CR and myelosuppression in patients receiving additional courses in remission. In patients achieving CR, the median time to platelet recovery more than 100 x 10^9/L was 39 days (range, 31-70 days), and the median time to granulocyte recovery more than 10^9/L was 45 days (range, 33-70 days). A total of 10 courses of therapy administered to 6 patients in remission were analyzable for myelosuppression. In these, the median platelet nadir was 45 x 10^9/L (range, 20-200 x 10^9/L), and, in the 6 courses with thrombocytopenia less than 100 x 10^9/L, median time to platelet recovery more than 100 x 10^9/L was 32 days (range, 24-58 days). The median nadir granulocyte count was 0.32 x 10^9/L (range, 0-3.0 x 10^9/L), and, in the 7 courses with granulocytopenia less than 10^9/L, median time to granulocyte recovery more than 10^9/L was 36 days (range, 32-45 days). Of these 10 courses, 8 were administered at the dose of 15 mg/m^2 for 10 days, precluding meaningful analysis of myelosuppression at lower versus higher doses.

Table 2: Non-hematological side effects with decitabine (50 cases, including two patients treated twice at different dose levels) from Phase I study[21].

Side effect	Grade II	Grades III/IV
Nausea, vomiting	2	1/–
Diarrhea	1	–/–
Skin rashes	1	–/–
Liver dysfunction		
Elevated enzymes	3	4/–
Increased bilirubin level	7	3/1
Creatinine elevation	5	0

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

This single arm Phase 1 trial at MD Anderson showed impressive efficacy with 16/52 responding (32%) including 9 with CR (18%). In the cohort of 17 patients receiving 15mg/m^2 on a 5 days per week for two consecutive weeks schedule (ie: same schedule we propose for this trial) the response rate was 65% with 35% CR[21]. A subsequent phase I/II trial used a decitabine dose of 15mg/m^2 for 10 consecutive days every 4 weeks in addition to the histone deacetylase inhibitor valproic acid[22]. In total 12 of 53 patients achieved a CR or CR

with incomplete platelet recovery (23% CR+CRp). A phase 1 study at Ohio State determined the optimal biological decitabine dose for AML (based on a gene re-expression assay) to be 20mg/m^2 when given daily for 10 consecutive days over 4 weeks with 11 of 21 patients achieving a response (52%) with a combined CR (19%) and CRi (19%) rate of 38%[23]. A follow-up phase 2 trial in older patients with previously untreated AML at this same dose and schedule demonstrated a CR of 25/53 (47%) and a CRi rate of 17% for an overall response rate of 65%. In contrast the response rates for the FDA approved dosing of 15 mg/m^2 three times daily for 3 days every 6 weeks (Phase 2, n=227 AML patients[24]) and 20 mg/m^2 for 5 days every 4 weeks (Phase 3, n=242 AML patients[25]) are more modest with CR rates of 13% and 18% respectively.

2.6. Rationale for Dose Selection/Regimen/Combination/Modifications

The intent of this pilot study is to determine the feasibility of adding pembrolizumab to an agent with known efficacy in myeloid malignancies. Decitabine is an FDA approved agent that is listed in the current National Comprehensive Cancer Network (NCCN) consensus ("standard of care") guidelines as therapy for relapsed/refractory AML (see Appendix F).

The proposed regimen is pembrolizumab 200 mg every 3 weeks for up to eight cycles with low dose decitabine 20mg/m^2 given on days 8 through 12 and 15 through 19 of alternative cycles (ie: cycles 1, 3, 5, 7).

Decitabine will be given according to the schedule and optimal biological dosing for AML of 20mg/m^2 as identified in the phase 1 study at Ohio State[23] (as described in section 2.5.2 above).

The rationale for the investigational fixed dose of pembrolizumab 200 mg every 3 weeks is described in section 2.3.2 above.

This combination of pembrolizumab and decitabine was selected based on several factors: 1) Largely non-overlapping adverse reaction profiles for these agents. 2) Both agents already FDA approved potentially allowing for rapid translation/adoption. 3) Both agents have previously evaluated dosing schedules that are compatible with one another (pembrolizumab every three weeks and decitabine every six weeks). 4) Theoretical possibility of synergy given respective mechanisms of action (see Section 2.2).

2.7. Rationale for Endpoints

This is a pilot study to assess the impact of the addition of pembrolizumab to decitabine for adult patients with relapsed/refractory AML.

Feasibility: Pembrolizumab and decitabine are both FDA approved agents. Pembrolizumab is approved for certain solid tumors, and decitabine for myelodysplastic syndrome (although commonly used off-label for AML). While the side effects of these agents appear to be non-overlapping, the combination of pembrolizumab and decitabine has not been tested. This pilot study will generate descriptive data on the adverse events

observed in relapsed and refractory AML patients using this combination (number, severity and duration of adverse events) according to the NCI-Common Terminology Criteria for Adverse Events (CTCAE) v4.0.

Efficacy: There is proof of principle in animal models that pembrolizumab may have activity against AML but no human studies. While this pilot study is not powered for formal assessment of efficacy, standard response criteria (see Appendix A) will be used at set time-points to provide descriptive data on efficacy of the pembrolizumab and decitabine combination.

Time to first response: Previous studies for single agent pembrolizumab in solid tumors reported an average time to first response occurred at first imaging for restaging at 12 weeks (Hamid *et. al.*, 2013). There is no information regarding time to first response for this pembrolizumab and decitabine combination in relapsed/relapsed AML patients.

Time to best response: There is no current information on the time to best response for this pembrolizumab and decitabine combination in relapsed/relapsed AML patients.

Duration of response: It is unknown, if any, how long the response duration of a response to this pembrolizumab and decitabine combination would be in relapsed/relapsed AML patients. It is possible this combination including pembrolizumab, due to novel mechanisms of action, may result in clinical responses in patients who are or would be refractory to traditional cytotoxic chemotherapy.

Duration of best response: as above.

Overall survival: Overall survival is unquestionably a clinical meaningful endpoint. A small pilot study such as this is not powered to provide statistical information on this endpoint, but descriptive data on outcomes will be reported and may provide anecdotal support for further trials.

2.8. Biomarker Research

One of the main intentions of this initial pilot study is to provide deep laboratory assessment of any observed clinical responses and toxicity in patients to help guide, plan and justify subsequent clinical trials for this indication.

The Intramural Research Program (IRP) of the National Institutes of Health (NIH) and the NIH Clinical Center (NIH-CC) offers unique resources; facilities and opportunities conduct such early translational clinical research. In addition, malignancies of the hematopoietic system such as Acute Myeloid Leukemia allow the repeated direct sampling to evaluate both changes in disease burden and character and to characterize any associated immune response and microenvironment. An integral component to this trial is the commitment to

collect, store and analyze biological specimens including paired blood and bone marrow samples on regular and frequent bases.

We will attempt to answer the following correlative research questions:

How does the clonal diversity of acute myeloid leukemia change during treatment with the combination of pembrolizumab and decitabine? This will be determined by next generation sequencing of AML associated somatic mutations prior to treatment, and tracking changes in variant allele frequency over time.

Are measurable changes in immune parameters associated with treatment, clinical efficacy and/or toxicity (eg: autoimmunity) using repeated disease site sampling of blood and bone marrow?

How much does AML disease burden change during treatment of relapsed/refractory AML patients with pembrolizumab and decitabine? A variety of high sensitivity tests for quantification of residual disease including flow cytometry and real-time quantitative RT-PCR will be used.

Are observed clinical responses associated with potentially predictive biomarkers such as marrow infiltrating lymphocytes (MILs), mutational burden in AML and PDL-1 expression?

3.0 STUDY DESIGN

This is an investigator sponsored pilot study to evaluate the feasibility of a novel combination of pembrolizumab and decitabine in adult patients with relapsed/refractory AML.

Secondary objectives determining, efficacy, time to first response, time to best response, duration of best response and overall survival. Treatment plan is discussed in detail in Section 5.0.

4.0 ELIGIBILITY ASSESSMENT AND ENROLLMENT

4.1. Inclusion Criteria

- Unequivocal diagnosis of relapsed or refractory acute myeloid leukemia (AML) confirmed by an NIH attending pathologist within 30 days of study enrollment (includes residual AML as confirmed by institutional standards by NIH pathologists, see Appendix A).
- Received at least one prior AML therapy-before study enrollment.
- Ability to comprehend the investigational nature of the study and provide informed consent.
- Be at least 18 years of age on day of signing informed consent.
- Availability of a physician willing to assume clinical care after completion of the study.
- Be willing to provide blood and bone marrow for research as described in the study.

- Have a performance status of less than or equal to 1 on the ECOG Performance Scale (Appendix **B)**.

- Demonstrate adequate organ function as defined in Table 3, all screening labs should be performed within 14 days of treatment initiation.

- 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.

- Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication. Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

- Male subjects of childbearing potential (Section 5.7.1) must agree to use an adequate method of contraception as outlined in Section 5.7.1- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy. Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

Table 3: Adequate Organ Function Laboratory Values

System	Laboratory Value
Renal	
Serum creatinine	≤1.5 X upper limit of normal (ULN)
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 3 X ULN

4.2. Exclusion Criteria

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

- Has a diagnosis of acute promyelocytic leukemia (APL)

- Has previously received an allogeneic hematopoietic stem cell transplant.

- Has received AML treatment with an investigational therapy or device within 4 weeks of the first dose of treatment.

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

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

- Has hypersensitivity to pembrolizumab or any of its excipients.

- Has hypersensitivity to decitabine or any of its excipients.

- Has received more than two prior cycles of decitabine.

- Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1.

- Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1.

  Note: Subjects who have received cytoreductive therapy with hydroxyurea at any time prior to study Day 1 are an exception to this criterion.

- Has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to previously administered AML therapy agents.

  - *Note: Subjects with ≤ Grade 2 neuropathy are an exception 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.*

- Has a known additional malignancy that is progressing or requires active treatment. Patients with basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer would not be excluded.

- Has known malignant central nervous system (CNS) involvement.

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

- Has history of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non-infectious pneumonitis.

- Has an active infection requiring intravenous systemic therapy.

- 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.

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

- 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.

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

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

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

- 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.*

5.0 TREATMENT PLAN

5.1. Treatment Schedule

Up to eight cycles of pembrolizumab will be given during the initial induction phase. Each cycle is 21 days. Decitabine will ordinarily be given on days 8 through 12 and 15 through 19 on alternative cycles (ie: cycles 1, 3, 5 and 7).

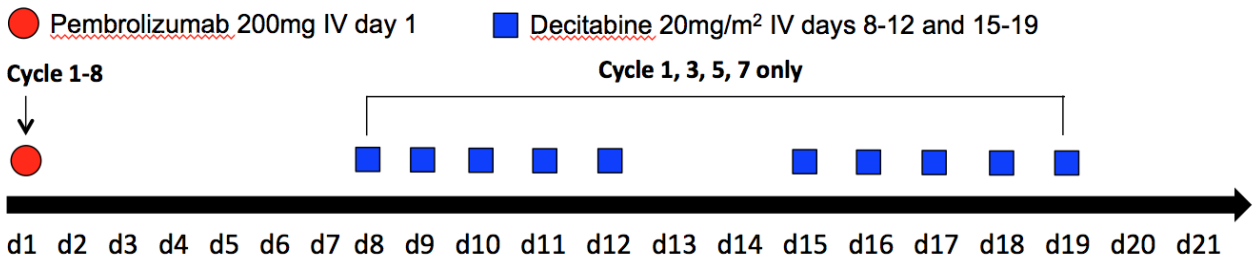

Figure 1: Induction Treatment Schema

This is a single institution pilot study of pembrolizumab and decitabine in adult patients with relapsed/refractory AML. The primary aim is to assess the feasibility of this novel pembrolizumab and decitabine combination in this patient population. This trial is open labeled, single arm study.

An optional continuation phase (see section 5.9) will be offered for those who have maintained a best response of at least stable disease (see Appendix A) after the initial eight cycles of the induction phase.

Table 4: Experimental Treatment Doses and Schedule.

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Decitabine	20 mg/m^2	Q6W	IV infusion	Days 8 through 12 and 15 through 19 of alternative cycles starting with cycle 1	Experimental

5.2. Dose Modification Guidelines For Drug-Related Adverse Events

5.2.1. Hematologic toxicities

Given the pathobiology of Acute Myeloid Leukemia, severe blood count abnormalities (up to grade 4 levels in leukocytes, neutrophils, lymphocytes, red blood cells and platelets) are expected and may be present at baseline.

Decitabine will be initiated on Cycle 1 and 3 regardless of peripheral blood count values. After this point, if hematologic recovery (absolute neutrophil count ≥1,000/μL and platelets ≥50,000/μL) does not occur six weeks after initiation of prior cycle of decitabine in complete remission patients, then subsequent decitabine doses will be modified to consist of five days of 20mg/m^2 given, at the discretion of the investigator, anytime during days 8-19 of a cycle.

In accordance with the FDA label and past clinical trial experience no dose modification will be made for pembrolizumab in the event of hematological toxicity.

5.2.2. Non-hematologic toxicities

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 5 below. See Section 5.6 for supportive care guidelines, including use of corticosteroids. **Decitabine** should be withheld for serum creatinine ≥2 mg/dL, an ALT ≥5 times upper limit

of normal (ULN) or total bilirubin ≥2 times ULN, or an uncontrolled infection. Decitabine may be resumed at resolution of these abnormalities.

5.2.3. Other acceptable dose delays

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.

Table 5: Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

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

	Grade 4	Permanently discontinue		fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-	• Monitor for signs and symptoms of thyroid

	Grade 3 or 4	Withhold or permanently discontinue[1]	blockers (eg, propranolol) or thionamides as appropriate	disorders.
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		
1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. **NOTE:** For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).				

5.3. Timing of Dose Administration

Pembrolizumab should be administered on Day 1 of each cycle after all procedures/assessments have been completed.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps, 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.

Decitabine will be administered at a dose of 20 mg/m2 by intravenous infusion over approximately1 hour repeated daily ordinarily on days 8 through 12 and 15 through 19 of alternative cycles (ie: cycles 1, 3, 5, 7). Decitabine may be given +/- 3 days, if needed for administrative or logistical issues. Decitabine should be repeated every 6 weeks. Patients may be premedicated with standard anti-emetic therapy. Every effort should be made to target infusion timing to be as close to 60 minutes as possible. However, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 60 minutes: -5 min/+10 min).

These timings may change in accordance with section 5.2 above.

5.4. Randomization or Treatment Allocation

All patients will receive the same treatment.

This is an open-label trial; therefore, the sponsor, investigator, care team and subject will all know the treatment administered.

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.

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 in the electronic medical record 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 will also be included in the electronic medical record.

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.

Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab or decitabine
- 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 investigator.

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.

There are no prohibited therapies during the Post-Treatment Active Surveillance follow-up stage.

5.6. Rescue Medications & Supportive Care

Supportive Care Guidelines

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

infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2 for dose modification.

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

Pneumonitis:

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

For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.

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

Diarrhea/Colitis:

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

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

For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.

For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

For **T1DM** or **Grade 3-4** Hyperglycemia

Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

Hypophysitis:

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

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

Hyperthyroidism or Hypothyroidism:

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

Grade 2 hyperthyroidism events (and **Grade 2-4** hypothyroidism):

In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.

In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

Grade 3-4 hyperthyroidism

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

Hepatic:

For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).

Treat with IV or oral corticosteroids

For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Renal Failure or Nephritis:

For **Grade 2** events, treat with corticosteroids.

For **Grade 3-4** events, treat with systemic corticosteroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

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

Table 6: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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:

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

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

OR

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 contraception regimens if sexually active:

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

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 this study and followed on an alternative hematology branch protocol (eg: 94-H-0010). 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 IRB 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 IRB and to Merck and followed as described section 9.1.3.

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.

5.8.1. Off-Treatment Criteria

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

- Unacceptable adverse experiences as described in Section 5.2
- Any grade 4 treatment-emergent adverse event
- Intercurrent illness that prevents further administration of treatment
- The subject has completed 35 cycles of pembrolizumab (ie: induction and continuation phase)
- The subject or legal representative (such as a parent or legal guardian) withdraws consent for treatment.
- Investigator's decision to withdraw the subject from treatment

5.8.2. Off-Study Criteria

A subject must be removed *from the study* for any of the following reasons:

- Confirmed disease progression despite at least two cycles of hypomethylating agent therapy and/or four doses of pembrolizumab. For these purposes disease progression is defined as greater than 50% relative increase in blasts in the peripheral blood or bone marrow or development of extramedullary disease (see Appendix A) at a therapy response assessment time-point (see Appendix G) compared to before start of treatment or relapse in those previously in complete remission (mCR/mCRi, CR/CRi).
- Patient meets off-treatment criteria but is not eligible, or declines, active surveillance phase
- The subject has a confirmed positive serum pregnancy test
- Subject becomes significantly noncompliant with study drug administration, study procedures, or study requirements, which might increase risk or substantially compromise the interpretation of study results.
- The subject is lost to follow-up
- The subject completes two years on study
- Administrative reasons
- The subject or legal representative (such as a parent or legal guardian) withdraws consent
- Investigator's decision to withdraw the subject from the study

The end of treatment and follow-up visit procedures are listed in Section 6.

The End of Treatment and Follow-up visit procedures are listed in Section 6. After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating another cancer treatment, withdrawing consent or becoming lost to follow-up. After study withdrawal each subject will be offered the opportunity to consent to an alternative hematology branch protocol (eg: 94-H-0010) to allow follow-up by telephone at approximately three monthly intervals for overall survival until death, withdrawal of consent, or the closing of the study protocol, whichever occurs first.

5.9. Optional Continuation Phase after Eight Cycles of Pembrolizumab

As described in section 5.1 this pilot study is intended to offer a total of eight doses of pembrolizumab on every three weekly cycles with decitabine given on alternative cycles as initial induction therapy.

In the event that a patient achieves a response of mCR, mCRi, CR, CRi, PR or SD (see Appendix A) at the end of cycle 8 assessment then additional cycles of pembrolizumab and decitabine therapy will be offered. These additional cycles will be given according to the same schedule (see section 5.1) with the below exceptions. Pembrolizumab in cycle 9 (ie: first cycle of continuation phase) may be delayed up to 7 days to allow post-induction phase staging to be completed. No more than 35 cycles (ie: two years) of therapy will be offered. Decitabine 20 mg/m2 will be given for not more than ten consecutive days every six weeks and may be prescribed and supplied by non-study physician.

5.10. Subject Replacement Strategy

If a patient discontinues treatment prior to receiving fourth dose of Pembrolizumab, due to patient or investigator action/decision, that patient may be replaced with another subject. Maximum accrual will be fifteen subjects.

5.11. Criteria for Early Trial Termination

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

- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects. Please see section 8.0 Biostatistical Considerations for stopping rule calculations.

6.0 CLINICAL MONITORING

6.1. Study Evaluations: Screening/Baseline

Screening: Screening period will consist of the 28 days prior to C1 starting. During this time, patients will complete the necessary screening studies and testing. These will ordinarily be performed, prior to a patient signing consent for this protocol, on another screening Hematology Branch protocol (eg: 97-H-0041).

- Complete medical history
- ECOG performance evaluation

- Physical exam
- Concomitant medication review
- Complete blood count (CBC) with differential
- Acute care & mineral panels (includes Na, K, Cl, CO2, creatinine, glucose, BUN, phosphorus, magnesium, albumin, and calcium)
- Total protein, CK, Uric Acid, and LDH
- Hepatic panel (includes alkaline phosphatase, ALT, AST, total bilirubin, and direct bilirubin)
- Reticulocyte count
- PT, PTT, fibrinogen
- Thyroid function tests
- Type and screen
- Urinalysis
- HLA typing
- EKG
- G6PD testing (as clinically indicated)
- CMV IgG, IgM and PCR
- Viral studies (serologies for HIV 1/2, hepatitis B and C). For individuals with a positive hepatitis B core antibody, HBV DNA PCR will be performed.
- Peripheral blood flow cytometry (AML and T/B/NK).

- For females of childbearing potential, one negative pregnancy tests sensitive to 50 mIU within 72 hours prior to starting study drug.

- Baseline non-contrast computerized tomography scans of chest and sinus will be performed prior to, or in first five days after treatment initiation. Further baseline imaging will not be routinely performed except for those with a history of extra-medullary myeloid disease (also known as granulocytic sarcoma, myeloid sarcoma, or chloroma), another malignancy or disease where imaging represents the clinical standard of care, or suspicion for extra-medullary myeloid disease or ongoing infection.

- Baseline bone marrow aspiration and biopsy
    - Morphology and immunohistochemistry
    - Cytogenetics (karyotype) and/or FISH.
    - AML molecular testing panel (e.g.: c-Kit, FLT3-ITD, NPM1 or CEBPA mutation)
    - Flow cytometry

6.2. On therapy evaluations during induction phase

Cycles 1-8: before starting each cycle (+/- 3 day window):

The following assessments will be performed prior to each cycle of pembrolizumab.

- Interval History
- ECOG performance status evaluation
- Structured adverse event assessment
- Concomitant medication review
- Physical exam
- Complete blood count with differential
- Acute care & mineral panels (includes Na, K, Cl, CO_2, creatinine, glucose, BUN, phosphorus, magnesium, albumin, and calcium)
- Total protein, CK, Uric Acid, and LDH
- Hepatic panel (includes alkaline phosphatase, ALT, AST, total bilirubin, and direct bilirubin)
- Reticulocyte count

- PT, PTT, fibrinogen
- Pregnancy test for females of childbearing potential

6.3. Clinical Assessments

Clinical endpoints on this trial are defined as below:

Table 7: Clinical Endpoints

Outcome	Included Patients	Point of Measurement	Definition
Feasibility	**All**	**Interim Analysis/Safety Follow-Up Visit** (see Section 6.5.1)	*Descriptive summary of all adverse events (number, severity and duration of adverse events) according to NCI-Common Terminology Criteria for Adverse Events (CTCAE) v4.0*
Efficacy	**All**	**Following cycles 2, 4, 6, 8 and at progression/off-study time-point**	*Descriptive summary of conventional response criteria as described in Appendix A. In addition any clinical information regarding MRD status (eg: flow cytometry, cytogenetics, PCR) will be captured at these time-points.*
Time to first response	**Responders** (mCR, mCRi,, CR, CRi, PR)	**As above**	*Defined as time from start of study to initial achievement of first response.*
Time to best response	**As above**	**As above**	*Defined as time from start of study to initial achievement of best response.*
Duration of response	**As above**	**As above**	*Defined as time from initial achievement of response to loss of response.*
Duration of best response	**As above**	**As above**	*Defined as time from initial achievement of best response to loss of best response.*
Overall survival	**All**	**Entry onto trial**	*Death from any cause*

Bone marrow examinations for clinical response assessment will be performed pre-treatment and after cycles 2, 4, 6 and 8 (or the progression/off-study time-point). Bone marrows after cycles 2, 4, 6 and 8 may be performed anytime in the week prior to the next cycle of Pembrolizumab (*ie:* the "after cycle 2" marrow may be performed prior to the pembrolizumab dose on day 1 of cycle 3 or anytime in the preceding week). Assessments will include clinical pathological assessment by morphology, flow cytometry and cytogenetics. An additional bone marrow for research purposes may be performed on or around day 8 of cycle 1. Additional bone marrows may be done after cycle 8, until progression as clinically indicated.

6.4. On therapy evaluations during optional continuation phase

Patients treated on the continuation phase will ordinarily be evaluated, including Structured adverse event assessment, as described in section 6.2, every 4 cycles (ie: every 12-13 weeks).

6.5. Post-Induction Treatment Follow-Up

6.5.1. Safety Follow-Up Visit (SFV)

The mandatory Safety Follow-Up Visit should be conducted after the eighth cycle of treatment (ie: end of induction phase), or (for patients discontinuing therapy prior to this point) 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 this Safety Follow-Up Visit should be recorded. Subjects with a persistent treatment related AE will be followed until the resolution of the AE or until the beginning of a new anti-neoplastic therapy, whichever occurs first. No new adverse event information will be collected after this safety follow-up visit.

6.5.1. Post-Treatment Active Surveillance Phase

Subjects who discontinue trial treatment in a complete remission or partial remission (mCR/mCRi/ CR/CRi/PR, see Appendix A) at the time of the safety follow-up visit will remain as a subject of this protocol but move into the Active Surveillance Phase and will be assessed every 1-3 months for up to two years (from the start of treatment) according to the NCCN consensus guidelines. This phase will continue until the start of new anti-neoplastic therapy, disease progression, death or end of the study at which point a subject would go off study and enter the post-study follow up stage.

6.5.2. Off Study Visit (all patients)

Subjects who meet off-study criterion listed above (Section 5.8) will be assessed in a final office study visit and referred back to his or her referring physician, consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) for consideration for standard therapy or evaluated for eligibility for another branch protocol, depending on what is considered to be in the best interest of the subject. Such subjects will, where possible, enter the post-study follow-up stage (below). For those who come off study in the first eight cycles this may also serve as the safety follow-up visit (see 6.5.1 above).

6.5.3. Post-study follow-up stage (all patients)

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. After a subject completes the study they will be offered the opportunity to consent to an alternative hematology branch protocol (eg: 94-H-0010) to allow follow-up contact, ordinarily by telephone, approximately every 13 weeks to assess for remission and survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.0 LABORATORY RESEARCH SAMPLES

7.1. Collection of Research Samples

Peripheral Blood samples: Volume collected for clinical and research purposes will not exceed 550 ml during any 8-week period.

Leukapheresis: A 4-5 liter leukapheresis procedure for research may be obtained prior to initiating therapy, and/or at the completion of the study, in consenting subjects.

Bone marrow biopsies and aspirate: Bone marrow biopsy and up to 25 mls of BM aspirate may be obtained for research at the time of the BM biopsy as described in Section 6.3.

Tissue for germline sequencing: A non-invasive buccal cheek swab and/or saliva samples may be collected. A skin punch biopsy may also be collected at the end of treatment.

Please see Appendix E for time-points where research samples are anticipated to be collected. Research blood collection at each of these time points is not obligatory and may be cancelled or reduced at the discretion of the

investigator on clinical and/or research grounds. Additional research samples may be collected at time of clinical event subject to the limits described above.

7.2. Research Testing

All or a selection of the following laboratory studies will be performed:

- Detection, quantification and characterization of protein, carbohydrate and/or lipid biomarkers.
- Evaluation of gene expression in unsorted and sorted biologic samples (e.g.: blood and marrow).
- Evaluation of protein expression in unsorted and sorted biologic samples.
- Evaluation of gene variants and mutations in unsorted and sorted biologic samples.
- Determination of epigenetic, histone and chromatin modifications.
- Evaluation of cellular phenotype in unsorted and sorted biologic samples.
- Evaluation of cellular function in unsorted and sorted biologic samples.
- Use of biologic samples to develop, test, and validate novel therapeutic agents.
- Use of biologic samples to develop, test, and validate surrogate predictive and prognostic markers of disease behavior.

A variety of molecular biology techniques including real-time quantitative PCR and sequencing of both DNA and RNA will be performed on research samples. Sequencing may involve single cell sanger sequencing, targeted panels, exome or genome level sequencing of sorted and unsorted biological samples (including from those enriched for tumor, and those predominantly tumor-free).

All the above laboratory studies are investigational. Studies described in Appendix D: NHLBI Hematology Branch Laboratory Research Studies may also be performed.

Sequencing results performed in a CLIA certified laboratory and ordered for a clinical indication as described in Section 6 will be returned to patients. If in the course of research analysis of whole genome/exome sequencing a gene variant of medical importance is found, the investigator will return to the IRB to discuss the next steps.

8.0 BIOSTATISTICAL CONSIDERATIONS

8.1. Primary statistical hypothesis

The primary statistical hypothesis is that pembrolizumab and decitabine can be co-administered safely in non-HSCT relapsed or refractory patients.

8.2. Sample size

The sample size is 10 patients. This is a pilot study.

8.3. Statistical safety monitoring

Given that the primary hypothesis is for feasibility, we will monitor the progress of the patients in the study. Following Geller *et. al.*[26], we use a Bayesian stopping rule for safety. For a given patient the safety endpoint is defined as grade 4 or 5 treatment related adverse event (excluding hematological toxicity, see section 9.1.2). This endpoint encompasses causes of death and severe toxicity from pembrolizumab and/or decitabine therapy during the induction phase (eight cycles) and, as such, is a suitable safety endpoint. Before the data are

collected, we assume that the prior distribution of the proportion of patients who will experience the safety endpoint follows a beta distribution. We combine the beta prior and the proportion data to form a beta posterior.

We anticipate the rate of treatment-related toxicities and deaths within the study period to be 50% or less. Since the proportion is a realization of a beta distribution, the proportion parameter is not a fixed quantity but a random variable. We assume the prior mean to be 0.3, and our prior knowledge is "worth" two patients. The conditions are satisfied where we set a=0.6 and b=1.4 for the beta distribution. Note that a+b is interpreted as the measure of contribution of the prior distribution. The subjective contribution of two patients, added to the sample of ten patients, is quite modest and does not exert undue influence on the data characteristics.

We set the stopping boundary for the event that the posterior probability, given accruing data, exceeds 0.8 when the posterior proportion of the specified events is greater than 0.5. That is, Prob(p>0.5|data)>0.8. The resulting boundaries are shown in the Table 8 below.

No. Patients	Stop if toxicities reaches or exceeds	Posterior Probability
3	3	0.86
5	4	0.81
7	5	0.78
8	6	0.86
10	7	0.83

We evaluated the performance of this stopping rule with computer simulations. In each simulation we generated a study with 10 independent Bernoulli trials, each with prior proportion p for having toxicity and compared the outcome with the stopping boundary. We repeated the simulation 100,000 times and counted the number of times the study was stopped. We did this for a number of different prior proportions to investigate whether our benchmark of 0.5 is appropriate (Table 9 below).

Proportion of SAE=p	0.2	0.35	0.5	0.65	0.8
Proportion of Stopped Studies	1.36%	9.74%	31.77%	64.73%	92.33%
Average number of subjects	9.92	9.49	8.44	6.76	4.83
Average number toxicities	1.99	3.33	4.22	4.39	3.86

These results suggest our stopping rule has a low probability of stopping a study when the proportion of toxicities is below the benchmark value of 50% toxicities is high when above this cut off.

8.4. Exploratory analyses

Given the size of the data, other analyses will be descriptive in nature and used to aid future research.

9.0 DATA AND SAFETY MONITORING

Principal Investigator: Accrual, efficacy and safety data will be monitored by the principal investigator Dr. Christopher S. Hourigan.

NHLBI's IRB: Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46. Quality assurance and control monitoring will be consistent with the NHLBI Division of Intramural Research Clinical Research Quality Assurance and Quality Control Policy.

NHLBI DSMB: The NHLBI Data And Safety Monitoring Board (DSMB) will review the protocol, progress report, accrual, efficacy and safety data at six- or twelve-month intervals as scheduled. All AEs and SAEs observed during the clinical trial and for which there is a relationship with the use of pembrolizumab and/or decitabine, or the conduct of the study will be reported to the DSMB at the regularly scheduled DSMB meeting. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

Merck: Merck will approve all amendments to the protocol or informed consent prior to submission to the IRB and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues. Accrual and safety data will also be monitored and reviewed annually by the IRB. An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to Merck Global Safety facsimile number: +1-215-993-1220.

FDA: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded by the Sponsor to FDA to:

Center for Drug Evaluation and Research
Food and Drug Administration
Division of Hematology Products
Office of Hematology Oncology Products
5901 B Ammendale Road
Beltsville, MD 20705-1266

9.1. Assessment of safety

Definitions

*For definitions please refer to policy 801 "*Reporting Research Events".

~~*Adverse event (AE):* Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:~~

~~Results in discontinuation from the study~~

~~Is associated with clinical signs or symptoms~~

~~Requires treatment or any other therapeutic intervention~~

Is associated with death or another serious adverse event, including hospitalization

Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious adverse event (SAE): An event is serious if it meets the definition of a SAE as follows, or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects;

Results in death

Is life-threatening (places the subject at immediate risk of death from the event as it occurred)

Results in in-patient hospitalization or prolongation of existing hospitalization

Results in a persistent or significant incapacity

Results in a congenital anomaly/birth defect

Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Suspected adverse reaction: Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

Unexpected adverse reaction: An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Unanticipated problem (UP): Any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected in terms of nature, severity, or frequency in relation to

a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and

b. the characteristics of the subject population being studied; and

2. Related or possibly related to participation in the research; and

3. Places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

UP that is not an AE: An unanticipated problem that does not fit the definition of an AE, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or

~~significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.~~

~~*Protocol deviation (PD):* Any change, divergence, or departure from the IRB approved research protocol.~~

~~*Non-compliance:* The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:~~

~~1. Serious non-compliance**:** Non-compliance that:~~

~~a. Increases risks, or causes harm, to participants~~

~~b. Decreases potential benefits to participants~~

~~c. Compromises the integrity of the NIH HRPP~~

~~d. Invalidates the study data~~

~~2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.~~

~~3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing.~~

~~Safety assessments will consist of monitoring and recording AEs and SAEs; measurements of protocol-specified hematology, clinical chemistry, and other laboratory variables; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug.~~

9.1.1. Timing

The AE reporting period for this study begins when the patient takes the first dose of study drug and ends with the safety follow-up visit. If an SAE is present at the safety follow-up visit or within 30 days of the last dose of study drug (whichever is later), it should be followed to resolution or until the Investigator assesses the subject as stable, a new anticancer therapy is initiated, or the subject is lost to follow-up or withdraws consent. Resolution/stable means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.

All adverse events will be recorded at each examination on the Adverse Event case report forms/worksheets from the time the consent form is signed through the safety follow-up visit.

9.1.2. Severity

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for grading the ***severity*** (intensity) of **non-hematologic** AEs (http://ctep.cancer.gov/reporting/ctc.html).

Given the pathobiology of Acute Myeloid Leukemia, severe blood count abnormalities (up to grade 4 levels in leukocytes, neutrophils, lymphocytes, red blood cells and platelets) are expected and may be present at baseline. Thus we will record these values in the protocol database, but will not report these abnormalities as adverse or serious adverse events unless the grade of toxicity (based on NCI-Common Terminology Criteria for Adverse Events, CTCAE v4.0) is grade 4 or above and was not present at baseline. If the myelosuppressive event is fatal (Grade 5), or if the Investigator finds the event to be medically important, then it will be collected as an SAE.

9.1.3. Pregnancy

A patient will be advised to immediately inform the investigator if the patient or patient's partner becomes pregnant from the time of consent to 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. If a subject inadvertently becomes pregnant while on treatment with the study regimen, 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.

Although pregnancy and lactation are not considered AEs, 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. Abortion, whether therapeutic, elective or spontaneous, will be reported as an SAE, which includes pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth. If the pregnancy continues to term, the outcome (health of infant) must also be reported.

The outcome of the pregnancy will be reported 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). Such events must be reported to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

9.1.4. Causality assessments

The following general guideline will be followed:

Table 10: Causality Assessment Guideline

Unrelated	Another cause of the AE is more plausible; a temporal sequence cannot be established with the onset of the AE and administration of the investigational product; or, a causal relationship is considered biologically implausible.
Unlikely to be Related:	A temporal sequence with the onset of the adverse event and administration of the investigational product is improbable, but not impossible. Concurrent or underlying disease, or the use of other drugs or procedures provide plausible explanations for the adverse event.
Possibly Related	There is a clinically plausible time sequence between onset of the AE and administration of the investigational product, but the adverse event could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the investigational product is one of several biologically plausible adverse event causes.
Probably Related:	There is a clinically plausible time sequence between onset of the adverse event and administration of the investigational product, and the adverse event is unlikely to be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Clinical response to withdrawal of the investigational product may indicate the adverse event is probably related. Rechallenge with the investigational drug is not required.
Definitely Related	The AE is clearly related to use of the investigational product.

9.2. Documentation

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study. There are ten scheduled patient evaluation time-points; at baseline, after cycles 1-8 and at the safety follow-up visit (see also Appendix G). In addition, all AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded in the patient's medical record.

9.3. NIH-IRB and Clinical Director Reporting

Expedited Reporting
Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review:
The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:
The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

9.4. Company Reporting

Reports to Merck:

A SAE is any AE occurring at any dose or during any use of Merck's product that:

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

Any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the sponsor and within 2 working days to Merck Global Safety.

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

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

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

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

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

9.5. FDA Reporting Criteria

9.5.1. IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a or other permitted method.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

9.5.2. FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

9.6. Events of Clinical Interest

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

Overdose: An overdose of pembrolizumab is 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 AE is associated with the overdose of pembrolizumab, the AE is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose pembrolizumab meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious ECI, using the terminology "accidental or intentional overdose without adverse effect."

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

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

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

Additional adverse events: A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document can be found in Appendix C and provides guidance regarding identification, evaluation and management of ECIs and immune-related AEs.

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

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

9.7. Protocol Amendments

Per the IST Agreement, any amendments to the protocol or informed consent form must be sent to Merck for review and approval prior to submission to the IRB. Written verification of IRB approval will be obtained before any amendment is implemented. Amendments that might change reporting criteria will be submitted to the FDA.

9.8. Publication Policy

Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). In addition to publication of primary pre-specified analysis once all subjects have completed or been removed from induction therapy stage (ie: 6mos), and also later at the 1 and 2 year since the start of treatment landmarks, given the pilot nature of this exploratory study interim analysis may also be performed and published for individual patients at other timespoints, for example in cases of exceptional response or notable toxicity.

Per the IST Agreement, the Investigator is required to submit to Merck a copy of a planned publication (abstract, poster, oral presentation or manuscript) prior to the submission thereof for publication or disclosure. Merck may provide scientific comments and suggestions understanding that the Investigator has sole editorial responsibility, and retains the authority to make the final determination on whether or not to incorporate Merck comments or requests for additional information.

9.9. Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.5 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subject's

charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

10.0 BIOSPECIMEN AND DATA MANAGEMENT PLAN

10.1. Storage

All samples will be stored in the laboratory of Dr. Hourigan. Collected samples will be coded prior to storage in the laboratory of the principal investigator following current NHLBI DIR BSI Policy.

Efforts to ensure protection of patient information include;

- Each sample is assigned a unique code.
- An electronic database is used to store patient information related to the coded samples
- The laboratory is located in a controlled access building and laboratory doors are kept locked. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.

10.2. Tracking

Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA or CTA.

10.3. End of study procedures

Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. Upon completion of the data analysis, the investigator will send to Merck a copy of the de-identified data set and final Clinical Study Report as requested.

10.4. Loss or destruction of samples or data

Should we become aware that a major breech in our plan for tracking and storage of samples or data has occurred, the IRB will be notified.

10.5. Biospecimen Management

Specimens and their derivatives (e.g., genomic material, cell lines) will be coded and stored in conformity with DIR Policy (e.g., BSI). Coded biospecimens may be sent to collaborators outside of the NIH with IRB approval

in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement. Biospecimens with subject personal identifiers may be sent to associate investigators and collaborators outside of the NIH only after approvals of both NHLBI and local IRBs, an executed reliance agreement with NHLBI's IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

10.6. Data Management

The principal investigator will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from the subjects' home physician. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards. Neither individual personal identifiers nor the key linking coded data to individuals will be released to Merck without prior IRB approval and an executed CDA or MTA. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA.

10.7. Data sharing and future use of data

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB approval. Future research use of data not defined in the research protocol may occur only after IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

10.8. Future use of biospecimens

Following analyses of biospecimens for primary research purposes, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB or OHSRP approval, as applicable. Biospecimens may be destroyed only when permitted by the clinical director and the IRB.

Any future research use of biospecimens not defined in the protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use requires IRB review and approval (for research collaborations) or submission of a determination to OHSRP (for non-collaborative research), and an executed transfer agreement. Unlinked biospecimens (no key to identify research subjects exists) to be shared outside of NIH for future research use requires submission of a determination to OHSRP and an executed transfer agreement. There are a few types of biospecimens that do not require IRB or OHSRP approval for future research use outside of NIH, such as specimens from deceased individuals (refer to OHSRP SOP5, Appendix 1 for complete list); an executed transfer agreement is required in these special cases. Refusal of a

research subject participant to allow for future use of identifiable biospecimens--other than required in the protocol or for appropriate regulatory purposes, e.g., by the FDA--will be honored.

11.0 HUMAN SUBJECT PROTECTION

The investigator(s) accept their responsibilities for protecting the rights and welfare of human research subjects and will permit, with reasonable advance notice and at reasonable times, the designated research monitors to monitor the conduct of the research, as well as to audit source documents to the extent necessary to verify compliance with FDA Good Clinical Practice and the approved protocol.

11.1. Rationale for subject selection

11.1.1. Predicted distribution by gender, age and race

AML is a rare neoplasm and is generally a disease of older adults with an average age at diagnosis of 67 years old. Epidemiologic studies suggest that the male: female ratio is approximately 5:3. The disease can occur in all races, with the highest incidence in non-Hispanic whites while Hispanic whites, blacks and Asian Pacific Islanders have a lower incidence. This study will be open to all patients who fit the inclusion criteria and provide informed consent to protocol participation. We would predict that distribution should be comparable to that seen on the NHLBI Hematology Branch screening protocol as follows:

- by gender: 33% females; 66% males
- by age: ages 23-79, median 60
- by race: 2% Asian, 11% Black, 8% Hispanic, 79% White

11.1.2. Special Populations:

Justification for inclusion of patients with relapsed/refractory AML: The survival rates for those with relapsed or refractory AML are dismal and the majority of such patients, despite best available treatment, will die of their disease. Pembrolizumab is a promising immunotherapy agent now recently FDA-approved for the treatment for melanoma and NSCLC. There is some pre-clinical evidence that pembrolizumab may have activity in leukemia patients. We believe the combination described in this trial has potential for benefit in this population poorly served by current treatments.

Justification for exclusion of children: Patients under the age of 18 years are excluded from this study, as inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this study.

Justification for exclusion of pregnant women: Pregnant women or breastfeeding women are not eligible for inclusion in this study. Women who are pregnant and have AML are optimally managed in leukemia centers with on-site high-risk obstetrics unit support. Should a patient become pregnant while on study an attending investigator on this study will counsel the patient, discussing any risks of continuing the pregnancy, any possible effects on the fetus and risk to the patient of stopping therapy. Pregnancy in a subject receiving chemotherapy will be reported to the IRB within 24 hours. 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. There are no clinical studies in pregnant women, and it is unknown whether decitabine, pembrolizumab or their metabolites are excreted in human milk.

Justification for Exclusion of patients with impaired hepatic or renal function: No specific clinical studies have been conducted to date in patients with impaired hepatic or renal function. To minimize risks, patients enrolled must have adequate hepatic and renal function as defined in eligibility and exclusion criteria.

Those unable to consent: Subjects must be able to provide informed consent, and understand and comply with the treatment plan and follow-up in accordance with NIH SOP 14E - Research Involving Adults Who Are or May Be Unable to Consent".

Recruitment: The study will be listed on the clinicaltrials.gov, Clinical Center research studies and the National Heart, Lung and Blood Institute patient recruitment websites. If recruitment goals are not met recruitment plans will be developed by the Clinical Center Office of Patient Recruitment.

Payment for participation: Subjects will not be compensated for their participation in this study.
There is no payment for the biological samples obtained for research.

Reimbursement for protocol participation travel, food, and lodging will be consistent with NIH and NHLBI guidelines.

Competition with other Branch or NIH protocols: This pilot study will be one of only two active treatment protocols for patients with relapsed/refractory AML at the NIH clinical center. Both clinical protocols will be conducted by the Myeloid Malignancies Section and have the same Principal Investigator. This small pilot trial will have priority in the event that a patient is eligible for both protocols.

11.2. Risks and discomforts

11.2.1. Risks related to pembrolizumab

Guidelines for supportive management of adverse event of clinical interest appear in Section 5.6.

Cardiac

Pericarditis was identified in only 1 subject. The event was identified as serious, Grade 3, and was considered by the Investigator to be drug-related.

Colitis

Out of a total of 1562 subjects, colitis was identified in 21 subjects (1.3%), of which 16 (1.0%) were considered drug related. Serious colitis were identified in 0.9% of subjects. 0.6% were considered to have serious colitis related to the drug by investigators. Concomitant corticosteroid can be used to manage colitis. A high initial dose of corticosteroids was used in 12 subjects, while a low initial dose of corticosteroids was used in 2 subjects.

Dermatologic

Skin changes include pruritus, rash, rash generalised, rash maculo-papular, dermatitis exfoliative, toxic epidermal necrolysis, erythema multiforme, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, dermatitis bullous, dermatitis exfoliative generalised, epidermal necrosis, exfoliative rash, skin necrosis, toxic skin eruption, acquired epidermolysis bullosa,epidermolysis, and mucocutaneous ulceration. Out of a total of 1562 subjects, skin changes were identified in 20 subjects (1.3%), of which 15 (1.0%) were considered drug related. Serious events were identified in 0.4% of subjects, and serious events were considered by the Investigators to be drug related were identified in 0.2% of subjects overall.

Hematologic

A grade 3 autoimmune hemolytic anemia was identified in only 1 subject to date, and was considered by the Investigator to be drug-related.

Hepatic and pancreatic

Out of a total of 1562 subjects, hepatic adverse events were identified in 8 subjects (0.5%). All the hepatic adverse events were considered drug-related. Serious hepatic adverse events were identified in 2 subjects (0.1%). Out of a total of 1562 subjects, pancreatitis were identified in 2 subjects (0.1%). Both cases were considered serious and drug related. The 2 instances of pancreatitis were Grade 2 and Grade 3.

Endocrinologic

Type 1 diabetes mellitus AEOSI was identified in only 1 subject. The event was identif ied as serious, Grade 2, and was considered by the Investigator to be drug related. No corticosteroids were used to manage the subject's AEOSI.

Out of a total of 1562 subjects, hyperthyroidism was identified in 34 subjects (2.2%), of which 30 (1.9%) were considered drug related. Serious hyperthyroidism was identified in 0.3% of subjects, which were all considered by the Investigators to be drug related. Two of 34 subjects were treated with concomitant corticosteroid.

Out of a total of 1562 subjects, hypothyroidism was identified in 113 subjects (7.2%), of which 104 (6.7%) were considered drug related. Serious events were identified in 0.1% of subjects, which were all considered by the Investigators to be drug related. A high initial dose of corticosteroids was used in 1 subject, while a low initial dose of corticosteroids was used in 2 subjects.

Out of a total of 1562 subjects, thyroiditis was identified in 11 subjects (0.7%), all of which were considered drug related. None of the thyroiditis was considered by the Investigators to be serious. Thyroiditis was identified as the following preferred terms: "thyroiditis" in 7 subjects, and "autoimmune thyroiditis" in 4 subjects. Four subjects (0.3%) experienced Grade 1 thyroiditis and 3 subjects (0.2%) experienced Grade 2 thyroiditis. One subject (0.1%) experienced Grade 1 autoimmune thyroiditis and 3 subjects (0.2%) experienced Grade 2 autoimmune thyroiditis.

Out of a total of 1562 subjects, hypophysitis was identified in 11 subjects (0.7%), of which 9 (0.6%) were considered drug related. Serious events were identified in 0.4% of subjects, which were all considered by the Investigators to be drug related.

Out of a total of 1562 subjects, adrenal insufficiency was reported for 2 subjects (0.1%). Both events were identified as Grade 3 acute event, and were considered by the Investigators to be serious and not drug related.

Infusion reaction

Out of a total of 1562 subjects, infusion reaction was identified in 39 subjects (2.5%), of which 27 (1.7%) were considered drug related. Eight subjects (0.5%) experienced Grade 1 hypersensitivity, and 4 subjects (0.3%) experienced Grade 2 hypersensitivity. Four subjects (0.3%) experienced Grade1 drug hypersensitivity, and 3 subjects (0.2%) experienced Grade 2 drug hypersensitivity. Serious events were identified in 2 subjects (0.1%), and 1 of the two was considered by the Investigators to be drug related (<0.1%).

Neuromuscular

Myasthenic syndrome was quantitatively assessed using the following preferred terms: myasthenic syndrome, myasthenia gravis, myasthenia gravis crisis, and ocular myasthenia. Out of a total of 1562 subjects, myasthenic syndrome was identified in 2 subjects (0.1%). Both cases were considered drug related. The myasthenic syndrome was identified as serious for one subject (0.1%). The two instances of myasthenic syndrome were Grade 2 and Grade 3.

Uveitis

Out of a total of 1562 subjects, uveitis was identified in 8 subjects (0.5%), all of which were considered drug related. A serious uveitis was identified in 1 subject, and was considered by the Investigators to be drug related.

Pneumonitis

Out of a total of 1562 subjects, pneumonitis was identified in 45 subjects (2.9%), of which 42 (2.7%) were considered drug related. Serious events were identified in 1.2% of subjects, and all serious events were considered by the Investigators to be drug related. One subject died due to pneumonitis (interstitial lung disease). Fourteen subjects (0.9%) experienced Grade 1 pneumonitis, 15 subjects (1.0%) experienced Grade 2 pneumonitis, 11 subjects (0.7%) experienced Grade 3 pneumonitis, and 2 subjects (0.1%) experienced Grade 4 pneumonitis. For "interstitial lung disease," 1 subject (0.1%) experienced Grade 1 interstitial lung disease, 1 subject (0.1%) experienced Grade 3 interstitial lung disease, and 1 subject (0.1%) experienced Grade 5 interstitial lung disease. A high initial dose of corticosteroids was used in 21 subjects, while a low initial dose of corticosteroids was used in 5 subjects.

Vasculitis

Out of a total of 1562 subjects, vasculitis was identified in 2 subjects (0.1%); both cases were considered drug related. The events were identified as serious for one subject (0.1%). The two instances of vasculitis were Grade 1 and Grade 3.

Common adverse events

The most commonly reported treatment-emergent AEs in pembrolizumab monotherapy trials are fatigue (35.4%), nausea (13.8%), decreased appetite (13.8%), diarrhea (12.3%), constipation (10.8%), and AST increase (10.8%). In combination therapy trials, the most commonly reported treatment-emergent AEs across the dose regimens were neutropenia and thrombocytopenia (50.0% each), anemia (30.0%), dysphonia, hiccups, and pruritis (20.0% each). Most of these events also have been mild to moderate in severity. The incidence of grade 3-5 treatment-emergent AEs ranges from 6.8-12.0% in monotherapy trials, and 23.1-50.0% in combination therapy trials. The above is preliminary safety information. The information has been reviewed, but has not been completely monitored; therefore, changes may occur to the safety information may occur going forward. Safety information will continue to be collected and evaluated as the study continues.

Potential for Drug-Drug Interactions

No formal pharmacokinetic drug interaction studies have been conducted with pembrolizumab.

Contraindications

None.

11.2.2. Risks related to decitabine

In a single-arm MDS study (N=99) decitabine was dosed at 20 mg/m^2 intravenous, infused over one hour daily for 5 consecutive days of a four week cycle. Listed below are all adverse events reported, regardless of causality, occurring in at least 5% of patients.

Blood and lymphatic system disorders

- Anemia
  Febrile neutropenia
- Leukopenia
- Neutropenia
- Pancytopenia
- Thrombocythemia
- Thrombocytopenia

Cardiac disorders

- Cardiac failure congestive
- Tachycardia

Ear and labyrinth disorders

- Ear pain

Gastrointestinal disorders

- Abdominal pain
- Abdominal pain upper
- Constipation
- Diarrhea
- Dyspepsia
- Dysphagia
- Gastro-esophageal reflux disease
- Nausea
- Oral pain
- Stomatitis
- Toothache
- Vomiting

General disorders and administration site conditions

- Asthenia
- Chest pain
- Chills
- Fatigue
- Mucosal inflammation
- Edema
- Edema peripheral
- Pain
  Pyrexia

Infections and infestations

- Cellulitis
- Oral candidiasis
- Pneumonia
- Sinusitis
- Staphylococcal bacteremia
- Tooth abscess
- Upper respiratory tract infection
- Urinary tract infection

Injury, poisoning and procedural complications

- Contusion

Investigations

- Blood bilirubin increased
- Breath sounds abnormal
- Weight decreased

Metabolism and nutrition disorders

- Anorexia
- Decreased appetite
- Dehydration
- Hyperglycemia
- Hypokalemia
- Hypomagnesemia

Musculoskeletal and connective tissue disorders

- Arthralgia
- Back pain
- Bone pain
- Muscle spasms
- Muscular weakness
- Musculoskeletal pain
- Myalgia
- Pain in extremity

Nervous system disorders

- Dizziness
- Headache
- Psychiatric disorders
- Anxiety
- Confusional state
- Depression
- Insomnia

Respiratory, thoracic and mediastinal disorders

- Cough
- Dyspnea
- Epistaxis
- Pharyngolaryngeal pain
- Pleural effusion
- Sinus congestion

Skin and subcutaneous tissue disorders

- Dry skin
- Ecchymosis
- Erythema

- Night sweats
- Petechiae
- Pruritus
- Rash
- Skin lesion

Vascular disorders
- Hypertension
- Hypotension

Potential for Drug-Drug Interactions

Drug interaction studies with decitabine have not been conducted. In vitro studies in human liver microsomes suggest that decitabine is unlikely to inhibit or induce cytochrome P450 enzymes. In vitro metabolism studies have suggested that decitabine is not a substrate for human liver cytochrome P450 enzymes. As plasma protein binding of decitabine is negligible (<1%), interactions due to displacement of more highly protein bound drugs from plasma proteins are not expected.

Contraindications
None.

11.2.3. Risks related to blood draws

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

11.2.4. Risks related to CT scans

CT (computed tomography) scanning is performed as part of the clinical standard of care and does not have a research use in this protocol. CT uses special x-ray equipment to obtain image data from different angles around the body and then uses computer processing of the information to show a cross-section of body tissues and organs. Oral and/or intravenous contrast agents will not routinely be used on this study. Contrast agents may be used medically indicated to due unique clinical circumstances. These agents are usually well tolerated. However, some subjects will experience allergic reactions to intravenous contrast. To lower the risk of allergic reactions, low allergenic contrast agents are administered at NIH clinical center. In addition, subjects will be advised that approximately 2-7% of patients who receive contrast agents will experience a temporary reduction in kidney function lasting up to 2 weeks following infusion and that in rare instances, permanent renal damage can result from the use of the IV contrasting agent. Therefore, in subjects with impaired kidney function, we will not use intravenous contrast.

11.2.5. Risks related to pregnancy and nursing mothers

There are no clinical studies in pregnant women, and it is unknown whether pembrolizumab is excreted in human milk. Men and women of child-bearing potential must use highly effective contraception as described in section 5.7.2. Pregnant or breastfeeding women are not eligible for this protocol. If a female subject or the partner of a male subject becomes pregnant, the sponsor must be notified. Male subjects should refrain from sperm donation.

11.2.6. Risks related to central line placement

When indicated, a catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the placement procedure. Only trained experienced staff will place the line in order to minimize these procedure related risks.

The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Some patient may experience a vasovagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure). Very rarely (less than 1% of the time), the line placement may nick the lung causing it to

collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, patients will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will ideally remain in place until drug administration is complete.

11.2.7. Risks related to bone marrow and skin punch biopsy

The anesthetic can cause some temporary stinging and burning. A pulling sensation and discomfort may be felt as the sample is withdrawn. Although rare, there is a potential for bleeding at the site and local infection. Bleeding can be stopped by applying local pressure, and infection can be treated with antibiotics. In the long term minimal scarring may occur but in most cases the biopsy site is indistinguishable within a few months.

11.2.8. Risks related to transfusions

Some risks with the transfusion with blood and /or blood products include fever or allergic reactions. These risks are uncommon and are usually mild, but on rare occasions may be severe or life threatening. Extremely rare risks include infections with viruses, such as hepatitis or HIV or serious incompatibility reactions.

11.2.9. Risks related to leukapheresis procedure

The leukapheresis procedures will be performed in accordance with standard leukapheresis donation policies and procedures operative in the NIH department of transfusion medicine and will be in compliance with the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to leukapheresis procedures are rare, but include:

- Pain and hematoma at the needle placement site.
- Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 5% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.
- Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium solution.
- Subjects may receive hydroxyethyl starch (HES) to aid in the collection of cells. HES can rarely cause anaphylactoid reactions (hypersensitivity, mild influenza-like symptoms, bradycardia, tachycardia, bronchospasm, non-cardiogenic pulmonary edema), renal dysfunction, coagulopathy, and hypertension.

Prior to each leukapheresis, the potential risks associated with the procedure will be explained to the patient and a separate informed consent obtained.

11.2.10. Risks related to genetic testing

Accidental disclosure of genetic information derived from research testing will be unlikely as all samples and research data will be coded and kept separately from the patient demographic information. Individually identifiable genetic data directly related to this study will not be released to participants. If an unexpected but clinically relevant, inheritable DNA change (also known as an “incidental” or “secondary” finding) is discovered by research exome and/or genome sequencing we will consult with the IRB on the best way to handle this information and potentially share the need for confirmatory testing by a CLIA accredited facility with the patient. Patients may opt-out of receiving such notification of such secondary findings from research sequencing. With increasing technology, it is possible that a person may be identifiable from their sequence data submitted as part of a publication of this material. We will attempt to limit this, by again using only coded specimens.

11.3. Risks in Relation to Benefit

Relapsed and refractory Acute Myeloid Leukemia is fatal without treatment. Even with the best available treatment it is fatal in the majority of cases. The benefits to the adult patient on this study could be a control, reduction or elimination of detectable AML potentially resulting in an improved quality of life, decreased transfusion requirements, a decreased susceptibility to infections, become eligible to receive additional therapy such as allogeneic transplant and foremost a significant improvement in survival time. Potentially, treatment with other therapies could also be avoided or postponed.

As of November 30, 2017 this study was closed to new subject accrual and in subject follow up only. As of March 15, 2019, the study will continue in data analysis only and the level of risk is minimal.

11.4. Informed consent processes and procedures

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study.

At any time during participation in the protocol, should new information become available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

11.5. Conflict of interest

Merck is providing pembrolizumab and research funding for this study to NIH. No NIH investigator involved in this study receives any payment or other benefits from Merck. The principal investigator assures that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. No members of the research team reported a potential conflict of interest. There are no conflicts of interest with any financial organization regarding the material mentioned in this protocol.

11.6. Technical Transfer Agreements

The protocol will have the following tech transfer agreements: Cooperative Research and Development Agreement (CRADA) between NHLBI and Merck.

Material transfer agreement (MTA) with collaborators at Collaborative Health Initiative Research Program, Uniformed Services University School of Medicine who will conduct genomic sequencing on de-identified (coded) samples to determine molecular etiology of diseases encountered on this protocol.

Material transfer agreement (MTA) with collaborator at New York Genome Center who will conduct analysis of gene and protein expression on de-identified (coded) samples on this protocol.

12.0 PHARMACEUTICALS

12.1. Pembrolizumab

Background Information: Note for detailed and comprehensive background information please refer to the Investigator's Brochure.

Investigational Product Name and Description: Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is an IgG4 kappa immunoglobulin with an approximate molecular weight of 149 kDa.

Preparation: Pembrolizumab is provided as a white to off white lyophilized powder (50 mg/vial) or as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only. Pembrolizumab Powder for Solution for Infusion, 50 mg/vial, is reconstituted with sterile water for injection prior to use.

The lyophilized drug product after reconstitution with sterile water for injection, and the liquid drug product are a clear to opalescent solutions, essentially free of visible particles. The reconstituted lyophilized product and the liquid product are intended for IV administration. The reconstituted drug product solution or the liquid drug product can be further diluted with normal saline or 5% dextrose in the concentration range of 1 to 10 mg/mL in intravenous (IV) containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be immediately used to prepare the infusion solution in the IV bag and the infusion solution should be immediately administered. Diluted pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. In addition, IV bags can be stored at 2 to 8°C for up to a cumulative time of 20 hours. This recommendation is based on up to 24 hours of room temperature and up to 24 hours of refrigerated stability data of diluted pembrolizumab solutions in the IV bags.

Packaging, and Storage: The drug product is stored as a stable lyophilized powder or liquid solution under refrigerated conditions (2 to 8°C).

Administration: The prescribed dose of pembrolizumab is given through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter. It is not allowed to co-administer other drugs through the same infusion line.

Supply: The drug product pembrolizumab is manufactured and supplied by Merck.

Shipping:

National Institutes of Health
PHARM DEV SVC, Room 1C230
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196
Shipping Designee Name: Hope Decederfelt, RPh
Shipping Designee Phone No: (301) 496-1031
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: hcederfelt@nih.gov

12.2. Decitabine

Background Information: Note for detailed and comprehensive background information please refer to the FDA product label.

Product Name and Description: Decitabine (5-aza-2'-deoxycitidine) is an analogue of the natural nucleoside 2'-deoxycytidine. Decitabine is a fine, white to almost white powder with the molecular formula of C8H12N4O4 and a molecular weight of 228.21. Its chemical name is 4-amino-1-(2-deoxy-β- D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one.

Preparation: Decitabine is a cytotoxic drug and caution should be exercised when handling and preparing. Procedures for proper handling and disposal of antineoplastic drugs should be applied. should be aseptically reconstituted with 10 mL of Sterile Water for Injection (USP); upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7-7.3. Immediately after reconstitution, the solution should be further diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a final drug concentration of

0.1-1.0 mg/mL. Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2℃ - 8℃) infusion fluids and stored at 2℃ - 8℃ (36℉ - 46℉) for up to a maximum of 4 hours until administration.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if there is evidence of particulate matter or discoloration.

Packaging, and Storage: Decitabine is supplied as a sterile, lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Each vial contains 50 mg of decitabine.

Administration: Decitabine is administered at a dose of 20 mg/m^2 by intravenous infusion over approximately 1 hour repeated daily for 10 days. This cycle should be repeated every 6 weeks. Patients may be premedicated with standard anti-emetic therapy. Store vials at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Supply: Decitabine will be supplied by the NIH clinical center pharmacy. During the optional continuation phase decitabine may be supplied by another pharmacy.

12.3. Accountability procedures

Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials which are destroyed, and copies of these documents will be provided to the sponsor. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the sponsor at the end of the study after drug accountability is performed by the study monitor.

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APPENDIX A: AML Diagnostic and Treatment Response Criteria

Acute Myeloid Leukemia (AML) is a clonal expansion of myeloid blasts in bone marrow, blood or other tissue, ICD-O code 9861/3.

Diagnostic and Staging Criteria

Bone marrow cellularity: The volume of hematopoietic nucleated cells, expressed as a percentage of marrow volume less volume of fibrosis.

Blasts: For AML, the following cell types are considered equivalent to blasts and are included in the calculation of blast percentages. Note that erythroblasts are not counted as blasts in calculating blast percentages.

- Myeloblasts include both agranular and granular variants.
- Monoblasts and promonocytes for Acute Monoblastic and Monocytic Leukemia. Megakaryoblasts for Acute Megakaryoblastic Leukemia.

Bone Marrow Blast Percentage: calculated as the percent of blasts among all nucleated marrow cells.

Residual Disease: The NIH institutional standard for AML residual disease diagnosis is currently assessment with flow cytometry. Other clinically accepted methods of making this diagnosis exist (eg: Cytogenetics, FISH, RQ-PCR) and may be used at the discretion of the NIH attending pathologist.

Response Definitions for AML:

1. **Measurable residual disease (MRD) negative complete remission.** No evidence of residual disease-after clinical assessment by flow cytometry (plus any other technique used in initial diagnosis of residual disease, if applicable). < 5% bone marrow blasts, no Auer rods, no evidence of extramedullary disease. This may be with **(mCR)** or without count recovery to ANC ≥ 1,000/mcl and platelet count ≥ 100,000/mcl **(mCRi)**.
2. **Morphologic complete remission (CR):** ANC ≥ 1,000/mcl, platelet count ≥ 100,000/mcl, < 5% bone marrow blasts, no Auer rods, no evidence of extramedullary disease. (No requirements for marrow cellularity, hemoglobin concentration).
3. **Morphologic complete remission with incomplete blood count recovery (CRi):** Same as CR but ANC may be < 1,000/mcl and/or platelet count < 100,000/mcl.
   *Patients with both an ANC < 1000/mcl and platelet count < 100,000/ mcl with at least a 10% marrow cellularity will be reported as a Morphological Leukemia Free State (MLFS).
4. **Partial remission (PR):** ANC ≥ 1,000/mcl, platelet count > 100,000/mcl, and at least a 50% decrease in the percentage of marrow aspirate blasts to 5-25%, or marrow blasts < 5% with persistent Auer rods.
5. **Stable disease (SD):** The absence of a complete or partial response, and no progressive disease.
6. **Progressive disease (PD)**: Defined as ONE of the following:
   - Greater than 50% relative increase in blasts in the peripheral blood or bone marrow from best assessment with minimum threshold of 20% blasts in the marrow or $1.0x10^9$/L blasts in peripheral blood.
   - Development of biopsy proven extramedullary leukemia (if the subject has extramedullary disease at baseline, then PD will be defined by blood and marrow criteria or if new sites of extramedullary disease appear).
   - If subject who present with an initial marrow blast percentage sufficiently high to preclude the ability to base disease progression on a >50% increase in marrow blast percentage, disease progression should be based on peripheral blood criteria or development of extramedullary leukemia as above.
7. **Not Evaluable:** Data incomplete or inadequate for time-point or overall assessment.

Note: Patients entering this study already in morphologic complete remission (ie: CR or CRi) but with evidence of residual disease the only responses possible are mCR, mCRi, SD, PD or NE (see above).

APPENDIX B: ECOG Performance Status Scale

ECOG Performance Status Scale	
Grade	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.,* light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX C: Events of Clinical Interest

Pneumonitis (reported as ECI if ≥ Grade 2)		
Acute interstitial pneumonitis	Interstitial lung disease	Pneumonitis
Colitis (reported as ECI if ≥ Grade 2 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Intestinal Obstruction	Colitis	Colitis microscopic
Enterocolitis	Enterocolitis hemorrhagic	Gastrointestinal perforation
Necrotizing colitis	Diarrhea	
Endocrine (reported as ECI if ≥ Grade 3 or ≥ Grade 2 and resulting in dose modification or use of systemic steroids to treat the AE)		
Adrenal Insufficiency	Hyperthyroidism	Hypophysitis
Hypopituitarism	Hypothyroidism	Thyroid disorder
Thyroiditis	Hyperglycemia, if ≥Grade 3 and associated with ketosis or metabolic acidosis (DKA)	
Endocrine (reported as ECI)		
Type 1 diabetes mellitus (if new onset)		
Hematologic (reported as ECI if ≥ Grade 3 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Autoimmune hemolytic anemia	Aplastic anemia	Thrombotic Thrombocytopenic Purpura (TTP)
Idiopathic (or immune) Thrombocytopenia Purpura (ITP)	Disseminated Intravascular Coagulation (DIC)	Haemolytic Uraemic Syndrome (HUS)
Any Grade 4 anemia regardless of underlying mechanism		
Hepatic (reported as ECI if ≥ Grade 2, or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Hepatitis	Autoimmune hepatitis	Transaminase elevations (ALT and/or AST)
Infusion Reactions (reported as ECI for any grade)		
Allergic reaction	Anaphylaxis	Cytokine release syndrome
Serum sickness	Infusion reactions	Infusion-like reactions
Neurologic (reported as ECI for any grade)		
Autoimmune neuropathy	Guillain-Barre syndrome	Demyelinating polyneuropathy
Myasthenic syndrome		
Ocular (report as ECI if ≥ Grade 2 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Uveitis	Iritis	
Renal (reported as ECI if ≥ Grade 2)		
Nephritis	Nephritis autoimmune	Renal Failure
Renal failure acute	Creatinine elevations (report as ECI if ≥Grade 3 or any grade resulting in dose modification or use of systemic steroids to treat the AE)	
Skin (reported as ECI for any grade)		
Dermatitis exfoliative	Erythema multiforme	Stevens-Johnson syndrome
Toxic epidermal necrolysis		
Skin (reported as ECI if ≥ Grade 3)		
Pruritus	Rash	Rash generalized
Rash maculo-papular		
Any rash considered clinically significant in the physician's judgment		
Other (reported as ECI for any grade)		
Myocarditis	Pancreatitis	Pericarditis
Any other Grade 3 event which is considered immune-related by the physician		

APPENDIX D: NHLBI Hematology Branch Laboratory Research Studies

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett)**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No

A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No
B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No

C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2.*	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No

C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inoculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No

E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post-transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.	No	No
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No

F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

APPENDIX E: Anticipated Research Sample Collection

Bone Marrow

Bone marrow biopsy and aspirate for research (up to 25ml) will be collected at each of the following time-points:

Time-point	Clinical	Research
Prior to cycle 1	X	X
Cycle 1 Day 8		X
After cycle 2 *(ie: C3D1 or 7 days prior)*	X	X
After cycle 4 *(ie: C5D1 or 7 days prior)*	X	X
After cycle 6 *(ie: C7D1 or 7 days prior)*	X	X
After cycle 8 and / off-treatment	X	X

** Bone marrows after cycles 2, 4, 6 and 8 may be performed anytime in the week prior to the next cycle dose of Pembrolizumab.*

Peripheral Blood

Time-point	EDTA (4ml)	PAXgene (2.5ml)	CPT (8ml)	SST (3.5ml)	**Volume (ml)**
Cycle 1 Day 1	XXXXXX	X	X	X	38
Cycle 1 Day 8	XXXXXX	X	X	X	38
Cycle 1 Day 15	X	X	X	X	18
Cycle 2 Day 1	X	X	X	X	18
Cycle 3 Day 1	X	X	X	X	18
Cycle 4 Day 1	X	X	X	X	18
Cycle 5 Day 1	X	X	X	X	18
Cycle 6 Day 1	X	X	X	X	18
Cycle 7 Day 1	X	X	X	X	18
Cycle 8 Day 1	X	X	X	X	18
Cycle 9 Day 1	X	X	X	X	18
Off-Treatment	X	X	X	X	18
				Total Volume:	256

Other Tissue

Start of treatment: Buccal swab – non-invasive (cheek).
End of treatment: Skin punch biopsy (no greater than 3mm core).

Note: *Above represents points where biological research samples may be collected. Research sample collection at each of these time points is optional and may be modified at the discretion of the investigator on clinical and/or research grounds. Additional volume or time-points of clinical response or toxicity may be collected, with the total amount of not to exceed the institutional limits described in section 7.1. A record of all research samples collected will be kept.*

APPENDIX F: Current NCCN Guidelines for Relapsed/Refractory AML Treatment

(US national guidelines – updated frequently. Below is for general information only – please refer to latest guidelines at http://www.nccn.org/professionals/physician_gls/pdf/aml.pdf.)

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2016
Acute Myeloid Leukemia

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THERAPY FOR RELAPSE/REFRACTORY DISEASE[1]

Aggressive therapy for appropriate patients:
- **Cladribine + cytarabine + granulocyte colony-stimulating factor (G-CSF) ± mitoxantrone or idarubicin[1,2]**
- **HiDAC (if not received previously in treatment) ± anthracycline**
- **Fludarabine + cytarabine + G-CSF ± idarubicin[3,4]**
- **Etoposide + cytarabine ± mitoxantrone[5]**
- **Clofarabine ± cytarabine + G-CSF ± idarubicin[6,7]**

Less aggressive therapy:
- **Low-dose cytarabine**
- **Hypomethylating agents (5-azacytidine or decitabine)**
- **Hypomethylating agents (5-azacytidine or decitabine) + sorafenib for FLT3-ITD mutations[8]**

[1]Martin MG, Welch JS, Augustin K, et al. Cladribine in the treatment of acute myeloid leukemia: a single-institution experience. Clin Lymphoma Myeloma 2009;9(4):298-301.
[2]Wierzbowska A, Robak T, Pluta A, et al. Cladribine combined with high doses of arabinoside cytosine, mitoxantrone, and G-CSF (CLAG-M) is a highly effective salvage regimen in patients with refractory and relapsed acute myeloid leukemia of the poor risk: a final report of the Polish Adult Leukemia Group. Eur J Haematol 2008;80(2):115-126.
[3]Montillo M, Mirto S, Petti MC, et al. Fludarabine, cytarabine, and G-CSF (FLAG) for the treatment of poor risk acute myeloid leukemia. Am J Hematol 1998;58:105–109.
[4]Parker JE, Pagliuca A, Mijovic A, et al. Fludarabine, cytarabine, G-CSF and idarubicin (FLAG-IDA) for the treatment of poor-risk myelodysplastic syndromes and acute myeloid leukaemia. Br J Haematol 1997;99(4):939-944.
[5]Amadori S, Arcese W, Isacchi G, et al. Mitoxantrone, etoposide, and intermediate-dose cytarabine: an effective and tolerable regimen for the treatment of refractory acute myeloid leukemia. J Clin Oncol 1991;9(7):1210-1214.
[6]Becker PS, Kantarjian HM, Appelbaum FR, et al. Clofarabine with high dose cytarabine and granulocyte colony-stimulating factor (G-CSF) priming for relapsed and refractory acute myeloid leukaemia. Br J Haematol 2011;155:182-189.
[7]Faderl S, Ferrajoli A, Wierda W, et al. Clofarabine combinations as acute myeloid leukemia salvage therapy. Cancer 2008;113:2090-2096.
[8]Ravandi F, Alattar ML, Grunwald MR, et al. Phase 2 study of azacytidine plus sorafenib in patients with acute myeloid leukemia and *FLT-3* internal tandem duplication mutation. Blood 2013;121:4655-4662.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

APPENDIX G: Scheduled Clinical Assessments

Trial Period:	**Screening Phase**	**Induction Phase Treatment** *(Assessment at end of cycle…)*								**Post-Induction**	
	Up to 28 days prior to start of treatment	1	2	3	4	5	6	7	8	SFV / Off-study Visits	Contin. Phase (q3mo)
Pre-screening and Consents	X										
Inclusion/Exclusion Criteria	X										
Demographics and Full Medical History	X										
Interval Medical History		X	X	X	X	X	X	X	X	X	X
Focused Physical Exam		X	X	X	X	X	X	X	X	X	X
ECOG Performance Status	Baseline	X	X	X	X	X	X	X	X	X	X
Structured Review of Adverse Events	Baseline	X	X	X	X	X	X	X	X	X	X
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X
Trial Treatment Administration Log		X	X	X	X	X	X	X	X		
Therapy Response Assessment	X		X		X		X		X	X	X
Bone Marrow Examination* *(flow cytometry, morphology)*	X		X		X		X		X		
Cytogenetics (bone marrow)	X		If + @BL		If + @BL		If + @BL		X		
Pregnancy Test - – Urine or Serum β-HCG	W/in 72 hours of first dose.	X	X	X	X	X	X	X	X	X	X
Acute Care, Mineral and Hepatic Panels	W/in 14 days of first dose	X	X	X	X	X	X	X	X	X	X
Total protein, CK, Uric Acid, LDH, PT, PTT, Fibrinogen, CBC w/ diff, Reticulocyte, Type&Screen.	X	X	X	X	X	X	X	X	X	X	X
CMV IgG, IgM and PCR, HIV serology, Hepatitis serologies,	X										
Thyroid Function (T3, FT4 and TSH), EKG, HLA typing, Urinalysis	X										
Peripheral Blood Flow Cytometry (T/B/NK and AML)	X		X		X		X		X		

"If + @ BL" = Cytogenetics will be checked after cycles 2, 4 and 6 only if abnormal at baseline.

** = an additional bone marrow examination assessment will be performed on cycle 1 day 8 (+/- 3 days).*