THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

An Open-label Phase Ib/II Study of Avelumab in Combination with 5-azacytidine (Vidaza) for the Treatment of Patients with Refractory/Relapsed Acute Myeloid Leukemia

2016-0444

Core Protocol Information

Short Title	Avelumab (antiPDL1) and azacytidine in AML
Study Chair:	Naval Daver
Additional Contact:	Dana E. Brown Vicky H. Zoeller Leukemia Protocol Review Group
Additional Memo Recipients:	**Recipients List** **OPR Recipients** (for OPR use only) None **Study Staff Recipients** None
Department:	Leukemia
Phone:	713-794-4392
Unit:	428
Study Manager:	Cora M. Cheung
Full Title:	An Open-label Phase Ib/II Study of Avelumab in Combination with 5-azacytidine (Vidaza) for the Treatment of Patients with Refractory/Relapsed Acute Myeloid Leukemia
Public Description:	N/a
Protocol Type:	Standard Protocol
Protocol Phase:	Phase I/Phase II
Version Status:	Terminated 10/03/2019
Version:	15
Document Status:	Saved as "Final"
Submitted by:	Dana E. Brown--8/13/2019 10:24:00 AM
OPR Action:	Accepted by: Elaine Kramer -- 8/22/2019 9:13:02 AM

Which Committee will review this protocol?

(●) The Clinical Research Committee - (CRC)

Protocol Body

Avelumab and 5_azacytidine in AML_IST_(Final) 7.27.2018.doc

An Open-label Phase Ib/II Study of Avelumab in Combination with 5-azacytidine (Vidaza) for the Treatment of Patients with Refractory/ Relapsed Acute Myeloid Leukemia

Principal Investigator: Naval Daver, M.D.

Co-PI: Dr Jorge Cortes, M.D.

Sponsor: University of Texas, MD Anderson Cancer Center

IND number: 131967

University of Texas

MD Anderson Cancer Center

Leukemia Department, Unit

428

1.1 OBJECTIVES

1.2 Primary Objectives

Part a. Phase IB
To determine the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of avelumab in combination with 5-azacytidine in patients with refractory/ relapsed acute myeloid leukemia (AML).

Part b. Phase II
To determine the overall response rate (ORR) defined as complete remission (CR/complete remission with incomplete platelet recovery (CRp/complete remission with incomplete count recovery (CRi)/partial remission (PR)/hematologic improvement (HI)/morphologic leukemia free state (MLFS) of avelumab in combination with 5-azacytidine in patients with refractory/ relapsed AML.

1.3 Secondary Objectives:

1. To determine the number of patients who achieve > 50% reduction in blasts on therapy with this combinaiton.

2. To determine the duration of response, disease-free survival (DFS), and overall survival

3. (OS) of patients with refractory/ relapsed AML treated with this combination.

1.4 Exploratory Objectives:

1. To study immunological and molecular features at baseline and at predefined time-points on-therapy with avelumab and azacytidine in the peripheral blood and bone marrow to include (a) quantify immune ligand expression by the AML blasts and AML stromal components (MDSCs and MSCs) including 4-1BBL, ICOSL, PD-L1, PD-L2, OX-40L, CD137L and (b) determine the quantitative expression of positive and negative co-stimulatory molecules on individual T-lymphocyte subsets including 4-1BB, CTLA-4, ICOS, PD-1, OX40, LAG-3 and TIM-3, and (c) identify the immunophenotype of tumor-infiltrating T-lymphocytes (TILs) pre- and post-therapy with the combination: CD8+, CD4+ effector, or CD4+ regulatory.

2. To develop a micro-array based gene expression profile (GEP) predictor of response to anti-PDL1 and epigenetic therapy in AML.

3. To determine the correlation of responses to the combination with baseline cytogenetic and molecular abnormalities.

2.1 BACKGROUND

2.1.1 Acute myeloid leukemia

Approximately, 30-40% of adults with AML fail to achieve CR with 1 or 2 cycles of induction chemotherapy, and are deemed primary refractory. The outcomes of patients with acute myeloid leukemia (AML) who are refractory to induction therapy are dismal, with low

response rates to salvage chemotherapy and poor long-term survival [1-3]. We have previously reported a dismal median OS of 3.8 months for patients with AML who are refractory to HiDAC-containing induction therapy (defined as ≥ 1gm/m^2 cytarabine per dose)[2]. Salvage therapy in such patient populations yielded a response rate of 18% and median response duration of 9 months.

These results emphasize the need to explore alternate salvage regimens for patients with relapsed/refractory AML. The development of novel and effective anti-AML agents and/or combinations is crucial to improving the outcome of AML.

2.1.2 Relapsed/Refractory AML:

Therapy for AML has improved only modestly over the last 4 decades. Traditional induction chemotherapy produces cure rates of 30% in adults with AML[4-6]. Patients with relapsed AML have an overall survival of 1 to 4 months with current salvage therapy[2,7]. We have previously reported a dismal median OS of 3.8 months for patients with AML who are refractory to HiDAC-containing induction therapy (defined as ≥ 1gm/m2 cytarabine per dose). Salvage therapy in such patient populations yielded a response rate of 18% and median OS of 4.5 months. The preliminary data from investigators in our group[8] and from others[9,10] suggest that immune therapies may be a crucial third modality in combination with cytotoxic and/or targeted molecular therapy to produce deeper and/or more durable responses in AML and MDS.

These results emphasize the need to explore alternate salvage regimens for patients with relapsed/refractory AML. The development of novel and effective anti-AML agents and/or combinations is crucial to improving the outcome of AML.

2.1.3 Role of PD-1/PD-L1 interactions in AML

It is well known that negative regulatory mechanisms within the solid tumor microenvironment inhibit antitumor T-cell function. Proteins produced by the tumor cells often inhibit the anti-tumor activity of the microenvironment by altering immune cells contained within the microenvironment and by recruitment of immune-suppressor cells to that microenvironment. One inhibitory mechanism is up-regulation of programmed death-ligand 1 (PD-L1) expressed on tumor or stromal cells, which binds to programmed death-1 (PD-1) on activated T cells[11-14]. PD-1/PD-L1 engagement results in diminished antitumor response by producing a state of exhaustion for tumor-infiltrating cytotoxic T-lymphocytes (CTLs). PD-L1 expression is associated with poor prognosis in many cancers, including those of the lung, stomach, colon, breast, cervix, ovary, renal cell, and liver, as well as in adult T-cell leukemia, glioma, and melanoma[15-17]. Interaction between PD-L1 and PD-1 plays an important role in controlling immune responses and is involved in peripheral tolerance, autoimmunity, allergy, infection, and antitumor immunity[15,18].

The PD-1/PD-L1 pathway plays a major role in immune evasion and cytotoxic T-cell exhaustion in hematologic malignancies including AML and MDS[9,19,20]. Garcia-Manero et al identified ≥ 2-fold expression of PD-L1 in 34% of patients with AML or MDS[20]. Chen et al found increased expression of PD-L1 at AML progression, which was an independent negative prognostic factor for French-American-British type M5 AML[21]. Similarly, Zhou et al have demonstrated that AML progression in a murine model is associated with elevated PD-1

expression on CTLs and increased T-regulatory cells at the tumor site[10]. Both these mechanism decrease CTL activity at the tumor site. Elevated PD-1 expression independently dampens the anti-leukemic effect of CTLs. T-regulatory cells (Tregs) further suppress CTL activity and this suppression depends on PD-1 expression by Tregs and PD-L1 expression by antigen-presenting cells. Anti-PD-L1 monoclonal antibody treatment increased the proliferation and function of CTLs at tumor sites by reducing the interaction between PD1 and PDL1 resulting in decreased Treg-mediated suppression of CTLs. The enhanced CTL activity resulted in reduced AML tumor burden, and resulted in long-term murine survivors.

2.1.4 PD1, PDL1, OX40 and other costim receptor expression in AML (Data from MDACC)

The expression of co-stimulatory (costim) receptors/ligands in the BM and peripheral blood PB in patients with AML has not been clearly defined. To define the immune landscape of AML we performed 17-color multi-parametric flow-cytometry (MFC) on BM aspirates from 36 untreated AML and 39 relapsed AML between March, 2015 and January 2016 to assess the expression of costim ligands (4-1BBL, B7-1, B7-2, ICOSL, PDL-1, PDL-2, OX40L) on leukemic blasts and the costim receptors (4-1BB, CTLA-4, ICOS, PD-1, OX40, GITR, LAG-3, TIM-3) on T cell subsets: CD4 T effector cells [Teff]: $CD3^{+}CD4^{+}CD127^{lo/+}Foxp3^{-}$, CD4 T regulatory cells [Treg]: $CD3^{+}CD4^{+}CD127^{-}Foxp3^{+}$, and CD8 T cells: $CD3^{+}CD8^{+}$. Eight healthy human BMs were used as control. See figure 1 for T-cell population distributions. Blasts and T-cells were evaluated at the same time-point. OX40 and PD-1 TILs were significantly higher in untreated AML BM as well as relapsed AML BM as compared to healthy donor BM (Figure 2 and 3). AML patients had significantly increased OX40+ TILs as compared to healthy control marrow across all three lymphocyte subsets including CD4 effector, T regs, cytotoxic CD8+ cells. Similarly AML patients had significantly increased PD1+ TILS as compared to healthy control BMs across all three lymphocyte subsets. Furthermore, PD1+ and OX40+ TILs were significantly higher in relapsed AML BM as compared to newly diagnosed untreated AML (Daver N, Sharma P et al., AACR April 2016, poster attached). There were no noteworthy differences in ligand expression patterns between relapsed AML and new AML.

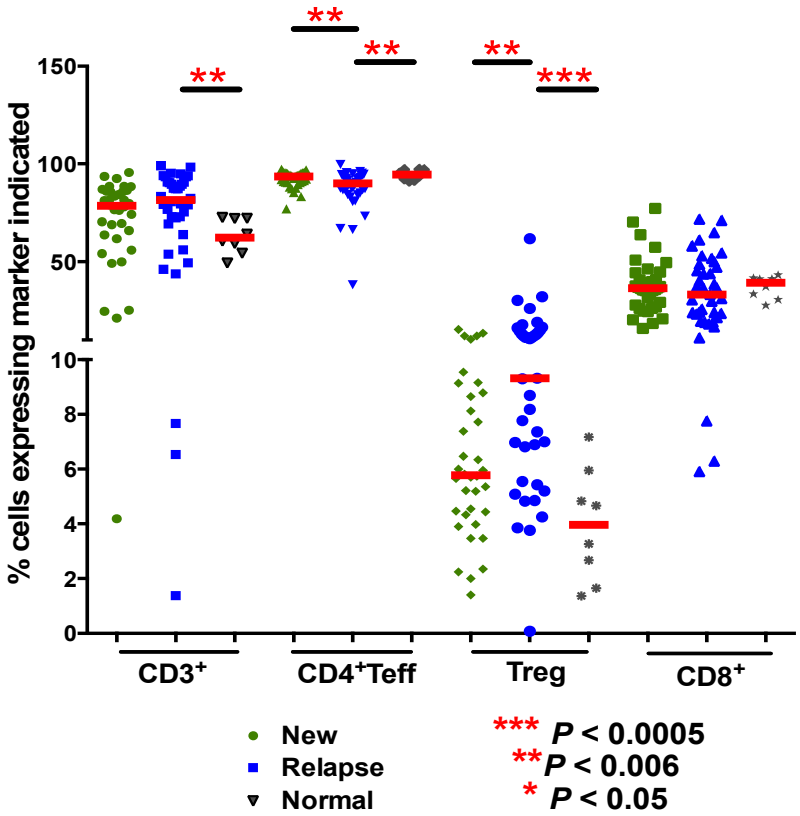


Figure 2: PD1 receptor in AML pts (n=75) vs healthy donors (n=8)

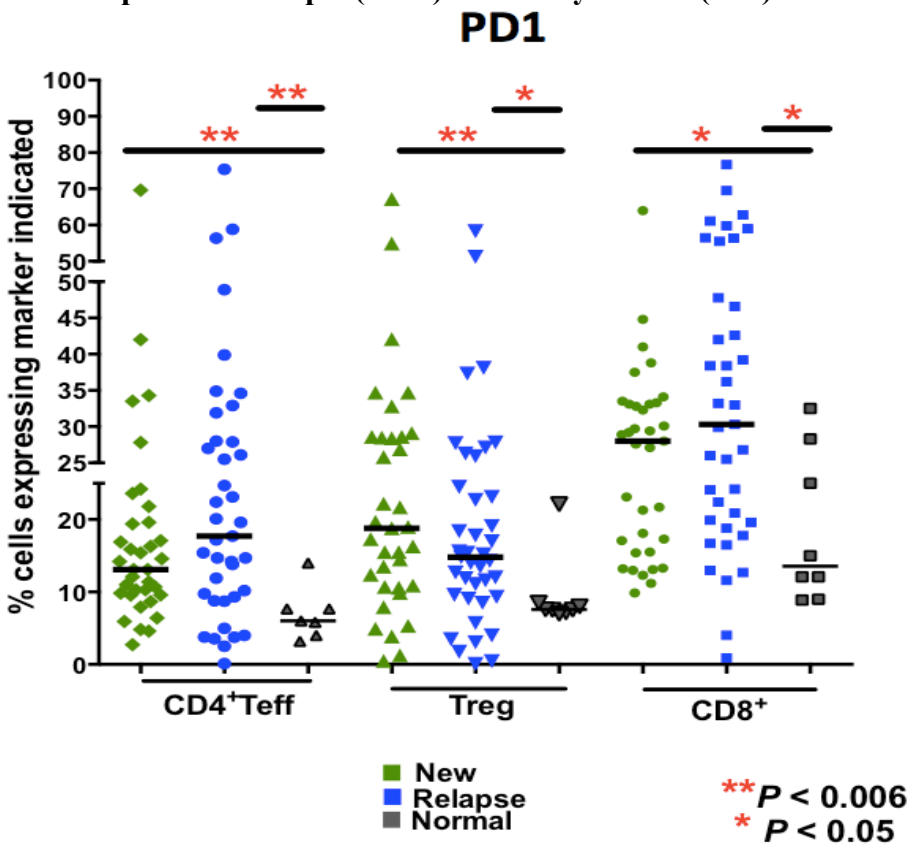
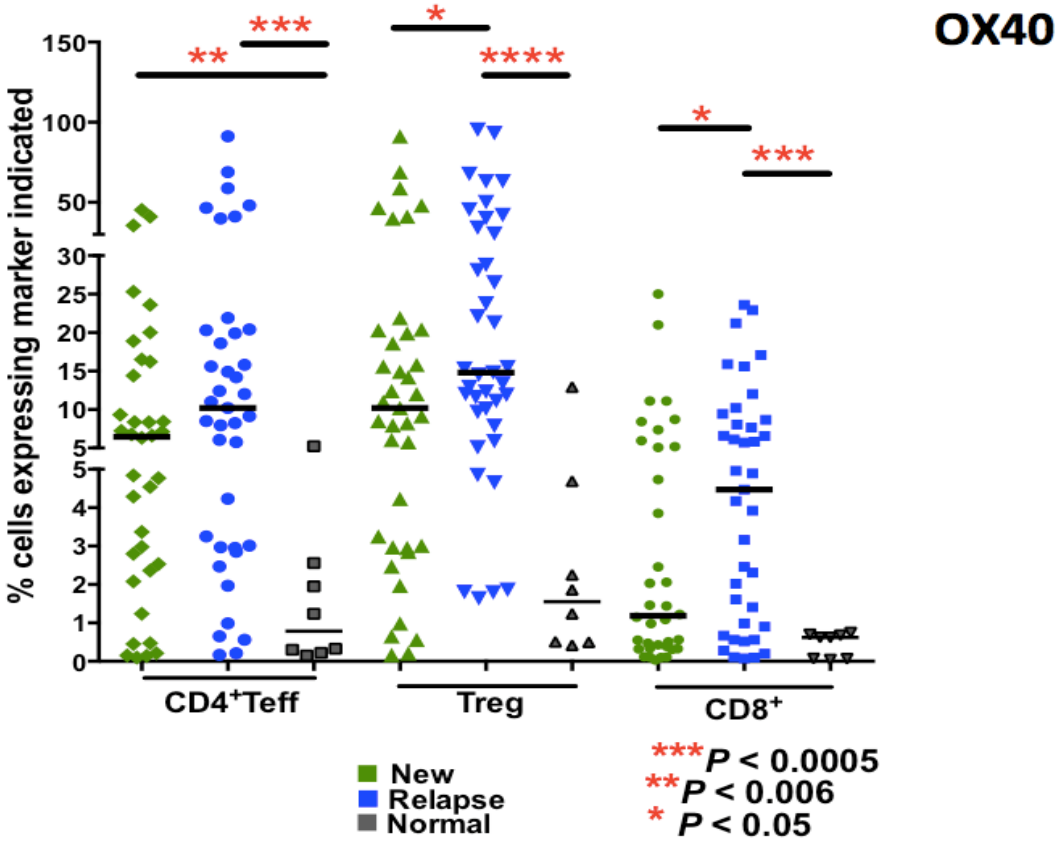


Figure 3: OX40 receptor in AML pts (n=75) vs healthy donors (n=8)



individual pts. The expression of costim receptors and ligands differed significantly between BM and PB from the same time-point in the same pt. A larger sample size is needed to confirm these data and find additional associations and this is currently underway at our institution. These data have been presented at AACR (Daver N, Sharma P et al. "Defining the Immune Landscape in AML", AACR April 2016) and further support the evaluation of anti-PD1/PDL1 based therapies in AML.

Dr Garcia-Manero from our group analyzed 124 patients with MDS/AML and noted that patients with lower PD-L1 expression had a non-significant trend toward better OS (31.5 versus 16.2 months, P=0.24)[8]. Patients with a greater degree of up regulation of these checkpoint molecules had a higher propensity of resistance to epigenetic therapy and a trend to inferior survival. Bone marrow samples from healthy donors were used as control for these studies. Another confirmation of the negative impact of PDL1 overexpression in patients with AML was obtained by interrogating the NEJM TCGA data by cBioportal. Patients with AML who had increased PDL1 mRNA had an inferior OS.

2.1.5 Role of DNA methyltransferase (DNMT) inhibitor (decitabine) in immune regulation

These findings in the baseline BM samples in AML patients (especially in relapsed AML) support our planned clinical trials of PD-L1 checkpoint blockade with avelumab in combination with decitabine in patients with AML. However, the clinical exigency of AML requires that chemotherapy and/or targeted therapy must be used as well, so it is necessary to consider how these may affect immune parameters. Dr. Garcia-Manero found that gene expression of PD-L1, PD-L2, and PD-1 were up regulated (≥2-fold) in >50% of 61 evaluable MDS/AML patients during their first course of DNMT inhibitor therapy[8]. There was a trend toward increased expression of all 3 genes in DNMT-inhibitor resistant patients compared with sensitive patients, suggesting up-regulation of immune makers as a mechanism of resistance to DNMT-inhibitor. These findings were confirmed in AML cell lines where decitabine (but not cytarabine at any concentration) induced dose-dependent up-regulation of PD-1 and PD-L1. These data suggest that concomitant inhibition of the PD-1/PD-L1 axis and potentially modulation of other checkpoint pathways may abrogate or overcome resistance to DNMT-inhibitors (basis of this clinical trial). Furthermore, recent data shows that decitabine increases NK-cell activating receptor ligands (NKG2DL) and enhanced ADCC when administered in combination with CD33-targeting antibody with preserved FCγIIIa domain (Vasu et al, Blood May 2016 and Daver N, Ravandi F et al, Blood May 2016). In addition to azacytidine's impact on T-cells costimulatory receptor and blast ligand expression we hope to further enhance the synergy by promoting ADCC by combining an epigenetic agent (azacytidine) with the ADCC inducing agent avelumab (also has a preserved FC domain to engage and activate NK cells).

2.1.6 Avelumab

Because of the known role of programmed death ligand 1 (PD-L1) in the suppression of T cell responses and the strong correlation between PD-L1 expression and prognosis in cancer, the blockade of the PD-L1/programmed death 1 (PD-1) interaction presents a highly promising strategy for cancer immunotherapy.

Avelumab binds PD-L1 and blocks the interaction between PD-L1 and PD-1. This removes the suppressive effects of PD-L1 on anti-tumor CD8+ T cells, resulting in the restoration of cytotoxic T cell response.

The active pharmaceutical ingredient in avelumab drug product is a fully human antibody (calculated molecular weight of 143832 Dalton) of the immunoglobulin G (IgG) 1 isotype that specifically targets and blocks PD-L1, the ligand for PD-1.

Nonclinical pharmacology

The nonclinical pharmacology studies have shown that avelumab functionally enhances T cell activation in vitro and significantly inhibits the growth of PD-L1 expressing tumors in vivo.

Avelumab binds to human PD-L1 with a high affinity of 0.7 nM and not to any other B7 family proteins, and competitively blocks the interaction of PD-L1 with PD-1. The in vitro study results have shown that by binding to PD-L1, avelumab effectively enhances T cell activation as measured by interleukin (IL)-2 or interferon-gamma (IFN-□□ production. In addition, as a fully human IgG1 antibody, avelumab has the potential to trigger the antibody-dependent cell-mediated cytotoxicity(ADCC) against target cells expressing PD-L1.

As a monotherapy, avelumab has demonstrated anti-tumor activity against murine MC38 colon carcinoma tumors that are characterized by a high level of PD-L1 expression. A dose-dependent trend was observed, and 400 μg per dose (20 mg/kg, approximately) was identified as the optimally effective dose when given every third day for 3 total doses.

The in vivo anti-tumor effects were found to be primarily mediated by CD8+ T cells as evidenced by the observation that in vivo depletion of this cell type completely abrogated the anti-tumor efficacy of avelumab. The contribution of ADCC as a potential mechanism of anti-tumor activity was further demonstrated in vivo using a deglycosylated version of avelumab to abrogate fragment crystalline (Fc) receptor binding or via the systemic depletion of natural killer (NK) cells. In both settings, loss of in vivo ADCC potential significantly reduced the anti-tumor activity.

The combination of avelumab with commonly used cancer treatments, such as cytotoxic agents and radiation therapy, resulted in an improved anti-tumor activity. Chemotherapy with combination therapy (with folinic acid, 5-fluorouracil, and oxaliplatin [FOLFOX], and radiation therapy showed the better tumor growth inhibition. In particular, radiation therapy was found to be a highly synergistic combination with avelumab capable of causing complete regression ofestablished tumors probably through generating anti-tumor immune memory.

Various immunomonitoring assays were incorporated into the in vivo studies. Treatment with avelumab resulted in a consistent increase in the percentage of CD8+PD-1+ T cells and an increased frequency of CD8+ T cells with an effector memory (TEM) phenotype as determined by flow cytometry. Furthermore, these changes correlated with the anti-tumor effect. Increases in tumor antigen-specific T cell responses, as measured by enzyme-linked immunosorbent spot and pentamer immunoassays, were evident following treatment with avelumab and these responses were enhanced when combined with FOLFOX or radiation. Hence, increases in CD8+PD-1+ T cells, CD8+ TEM cells, and antigen-specific T cell responses, may be leveraged as pharmacodynamics (PD) biomarkers with translational relevance to the clinical setting.

Nonclinical pharmacokinetics and metabolism

As expected for a monoclonal antibody (MoAb) binding to a cellular target, avelumab demonstrated pronounced non-linear pharmacokinetic (PK) characteristics in mice and monkeys in single dose studies at doses below 20 mg/kg, suggesting a combination of first order catabolic clearance and saturable target-mediated clearance. Toxicokinetic data from repeated dose toxicity studies in mice, rats, and monkeys indicated that the PK of avelumab was linear within the dose range of 20 to 140 mg/kg, suggesting that the target mediated clearance could be saturated when higher doses than 20 mg/kg are administered. Similar terminal half-lives (t1/2) of approximately 60 to 70 hours were observed in toxicity studies in mice and monkeys.

A PK/ PD study in C57BL/6 mice was used to correlate receptor occupancy data of avelumab in blood with drug concentrations. A plasma concentration of 58.5 µg/mL was calculated as required for 95% target occupancy (TO) in this model.

Avelumab is immunogenic in mice, rats, and monkeys with a lower incidence of anti-drug antibodies (ADAs) at higher doses. The latter is probably due to interference of free avelumab with the immunogenicity assay (drug interference). In animals, the generated ADAs seem to have the potential to increase the clearance of the avelumab. As the fully human avelumab represents a foreign protein to the immune system of animals, the observed immunogenicity of avelumab in rodents and non-human primates is not deemed predictive for an immune response to avelumab in humans.

Nonclinical toxicology

The toxicological profile of avelumab was evaluated in repeat-dose toxicity studies of 4-week duration with once weekly Iv bolus injection/infusion of avelumab in mice, rats, and cynomolgus monkeys. A repeat-dose toxicity study with intermittent once weekly Iv infusion of avelumab over 13 weeks followed by an 8-week recovery period in cynomolgus monkeys was also conducted. In addition, in vitro cytokine release assays (CRA) in human and cynomolgus monkey whole blood and peripheral blood mononuclear cells (PBMCs) followed by an optimized CRA in phytohemagglutinin (PHA) pre-stimulated PBMCs from 16 human volunteers was completed. Tissue cross reactivity (TCR) studies in normal human and cynomolgus monkey tissues have also been was performed.

On the basis of the binding affinity data, the cynomolgus monkey and the mouse were selected as relevant species for the nonclinical safety testing of avelumab. Due to severe hypersensitivity reactions after repeated administration of avelumab in mice and the low binding affinity in rats, rodent species are not considered appropriate for nonclinical safety testing of avelumab and therefore, a single species approach (cynomolgus monkey only) is applied.

In cynomolgus monkeys neither in the pilot 4-week Iv repeat-dose toxicity study nor in the pivotal 13-week study, clinical signs of hypersensitivity have been seen after repeated treatment with avelumab at dose levels of 20, 60, and 140 mg/kg, respectively. For the pilot 4-week study as well as for the pivotal 13-week Iv repeat-dose toxicity study, a no observed adverse effect level (NOAEL) of 140 mg/kg for systemic toxicity was established.

Initial CRA in human and cynomolgus monkey whole blood and PBMCs revealed no clear-cut evidence for release of pro-inflammatory cytokines. However, a subsequent, optimized CRA demonstrated evidence of potential cytokine release in PHA pre-stimulated PBMCs.

Clinical safety

Avelumab is currently in clinical development across Phases I, II, and III. This Investigator's Brochure includes safety data from the following 4 clinical trials:

- EMR 100070-001: A Phase I, open-label, multiple ascending dose trial to investigate the safety, tolerability, pharmacokinetics, biological and clinical activity of avelumab in subjects with metastatic or locally advanced solid tumors and expansion to selected indications

- EMR 100070-002: A Phase I trial to investigate the tolerability, safety, pharmacokinetics, biological and clinical activity of avelumab in Japanese subjects with metastatic or locally advanced solid tumors, with expansion part in Asian subjects with gastric cancer

- EMR 100070-003: A Phase II, single arm, open-label, multicenter trial to investigate the clinical activity and safety of avelumab in subjects with Merkel cell carcinoma (MCC)

- EMR 100070-004: A Phase III open-label, multicenter trial of avelumab versus docetaxel in subjects with non-small cell lung cancer that has progressed after a platinum-containing doublet

EMR 100070-001 is a Phase I, open-label, multiple ascending dose trial to investigate the safety, tolerability, PK, biological and clinical activity of avelumab in subjects with metastatic or locally advanced solid tumors and expansion to selected indications. This trial consists of 2 parts. In the dose escalation part, sequential cohorts of subjects were enrolled at progressively higher dose levels (ranging from 1.0, 3.0, 10.0, and 20.0 mg/kg once every 2 weeks) with a 3 + 3 algorithm design for determination of the maximum tolerated dose (MTD) of avelumab; in the treatment expansion phase, subjects in different tumor cohorts are being treated with 10 mg/kg of avelumab once every 2 weeks until, confirmed progression, unacceptable toxicity, or any reason for withdrawal occurs. More than 1500 subjects have been enrolled in the EMR 100070-001 trial. The 3 + 3 dose escalation algorithm to determine the MTD is complete and a dose of 10 mg/kg once every 2 weeks was determined for the tumor expansion cohorts on the basis of safety, PK, and PD observations. The treatment expansion part of the trial consists of 16 tumor treatment cohorts. As of 05 November 2015 (data cutoff for a pre-planned safety data review by the study Safety Monitoring Committee [SMC]), 53 subjects in the dose escalation part had received avelumab (4, 13, 15, and 21 subjects had received 1.0, 3.0, 10.0, and 20.0 mg/kg of avelumab, respectively) and 1300 subjects in the pooled expansion part had received 10 mg/kg avelumab and were followed up for at least 4 weeks.

The safety summary from the current Investigator's Brochure (IB FEB 2016 version 5; **Appendix C**) summarizes data from 1300 subjects treated in the pooled treatment expansion cohort from the ongoing Phase I Trial EMR 100070-001 (as of 05 November 2015). The pooled data included subjects treated in all tumor expansion cohorts, including non-small cell lung cancer (NSCLC), metastatic gastric cancer, breast cancer, colorectal cancer, castrate-resistant prostate cancer, adrenocortical carcinoma, melanoma, mesothelioma, urothelial

carcinoma, ovarian cancer, renal cell carcinoma, and squamous cell cancer of the head and neck. Safety data are also summarized for 52 subjects in the ongoing Phase I Trial EMR 100070-002 and for 88 subjects in the ongoing Phase II Trial EMR 100070-003 (as of 17 December 2015). For Trial EMR 100070-004, an overview of the serious adverse events (SAEs) is provided.

Most of the observed adverse events (AEs) were either in line with those expected in subjects with advanced solid tumors or with class effects of MoAb blocking the PD-1/PD-L1 axis. Infusion-related reactions including drug hypersensitivity reactions and immune-mediated adverse reactions have been identified as important risks for avelumab. Respective risk mitigation measures have been implemented in all ongoing clinical studies with avelumab

Clinical efficacy

The clinical efficacy information summarized in this Investigator's Brochure includes data from the NSCLC and ovarian cancer expansion cohorts of the ongoing Phase I Trial EMR 100070-001, and for 20 subjects in the gastric cancer expansion cohort of the ongoing Phase I Trial EMR 100070-002.

The NSCLC expansion cohort in the ongoing Phase I Trial EMR 100070-001 had a cutoff date of 15 January 2015, 6 months after start of avelumab treatment of the last subject in this expansion cohort (a total of 184 treated subjects). The objective response rate (ORR) based on confirmed and unconfirmed responses for subjects treated in the NSCLC expansion cohort was 14.1% (26 of 184 NSCLC subjects). Progression free survival (PFS) and overall survival (OS) were all evaluated for all NSCLC subjects treated in the expansion phase. As of 15 January 2015, the median PFS and OS for the NSCLC treatment expansion cohort were 11.6 weeks and 8.4 months, respectively.

The clinical activity of avelumab was also evaluated by subjects' tumor PD-L1 expression status in the NSCLC expansion cohort. An objective response was observed in 20 of 122 subjects (16.4%) who were PD-L1 positive (defined as having at least 1% PD-L1 positive tumor cells) compared with 2 of 20 subjects (10.0%) who were considered PD-L1 negative (defined as having less than 1% PD-L1 positive tumor cells). A longer median PFS (12.0 vs 5.9 weeks) and OS (8.9 vs 4.6 months) were both observed in PD-L1 positive compared with PD-L1 negative subjects.

The ovarian cancer expansion cohort had a data cutoff of 13 February 2015, approximately 13 weeks after the start of avelumab treatment on the last subject who was included in this pre-planned interim analysis on this expansion cohort. The ORR based on confirmed and unconfirmed responses for subjects treated in the ovarian cancer expansion cohort was 10.7% (8 of 75 subjects). The median PFS for the ovarian cancer expansion cohort was 11.4 weeks (95% confidence interval (CI): 6.3 to 12.0 weeks).

The preliminary efficacy data for the ongoing Phase I Trial EMR 100070-002 are based on a data cutoff of 11 March 2015. As of the data cutoff, 3 of 20 subjects responded to trial treatment (all responses were partial responses [PRs] and all responses were confirmed responses), and the best overall response (BOR) was 15.0% (95% CI: 3.2% to 37.9%). The

median PFS of this group was 11.9 weeks (95% CI: 6.0 to 12.3 weeks).

Further details can be found in the avelumab Investigators Brochure (**Appendix C**), which contains comprehensive pharmacology, toxicology, pharmacokinetics, pharmacodynamics, metabolism, preclinical, and clinical efficacy and safety data information

2.1.7 5-azacytidine (Vidaza):

5-azacytidine, an analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and deoxyribonucleic acid (DNA) synthesis and metabolism. Since the early 1970s, 5-azacytidine has been investigated primarily in the US for the treatment of acute leukemia. Clinical studies have focused mainly on patients with disease refractory to conventional chemotherapy. Results of these investigations demonstrated activity of 5-azacytidine in the treatment of AML. Clinical studies subsequently evaluated the effects of 5-azacytidine in a variety of other malignant and hematologic disorders, including solid tumors, hemoglobinopathies (e.g., thalassemia and sickle cell anemia), and MDS. In 1984, the Cancer and Leukemia Group B (CALGB) began a series of clinical studies with 5-azacytidine in patients with MDS. These studies, in addition to other supportive data, led to the approval of Vidaza® (5-azacytidine) in May 2004 for the treatment of MDS.

5-azacytidine inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT).[22-24] Increased methylation of DNA (hypermethylation) may result in the silencing of critical genes responsible for cell growth control and differentiation. Hypermethylation of CpG islands spanning the promoter regions of tumor suppressor genes is commonly associated with cancers.[25] It is now widely recognized that hypermethylation of DNA is frequently associated with myelodysplastic syndromes and other cancers,[26-28] such as renal,[29] melanoma,[30] breast,[31] colorectal,[32] non-small cell lung[33] and hematologic malignancies.[34] 5-azacytidine is believed to exert its antineoplastic effects through hypomethylation and cytotoxicity on abnormal hematopoietic cells in the bone marrow.[35-39] Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation.[25,40,41] The cytotoxic effects of 5-azacytidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.[35,42-44]

The cytotoxicity of 5-azacytidine is proportional to dose and exposure time.[35,36] Although the mechanisms of cytotoxicity are complex and multifaceted, there is general agreement that incorporation of 5-azacytidine into DNA and ribonucleic acid (RNA), and inhibition of protein synthesis, are critically important.[45] Cytotoxicity is greatest in cells that are proliferating (S phase) and metabolically active.[35] Cytotoxic effects may also be mediated through induction of the DNA damage response pathways.[44] Nonproliferating cells are relatively insensitive to 5-azacytidine.[35]

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys.[46] Most of the studies were performed during the 1970s and early 1980s according to existing guidelines and standards in place during that period. The preclinical studies identified the bone marrow, liver, kidneys, and lymphoid tissues (spleen, lymph nodes, and thymus) as the main target organs of toxicity for 5-azacytidine.[46] In single-dose studies, the lethal dose of 5-azacytidine

after intravenous (IV) administration in mice, rats, and dogs was approximately 250 mg/m^2. Repeated daily dosing appears to increase the toxicity of 5-azacytidine.[46] The genotoxicity of 5-azacytidine is consistent with that of other nucleoside analogs that interact with nucleic acids.[46] Likewise, similar to other agents with cytostatic properties, 5-azacytidine was embryotoxic and reduced the reproductive performance in mice and rats.[46]

Limited 5-azacytidine pharmacokinetic data are currently available. Based on human plasma concentrations of total radioactivity (which represents parent drug plus circulating metabolites), 5-azacytidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing.[46] 5-azacytidine and/or its by-products are then rapidly cleared by the kidneys. The half-lives and percent radioactivity recovered in urine are similar following IV and SC routes of administration. The effects of renal or hepatic impairment, gender, age, or race on the pharmacokinetics of 5-azacytidine have not been studied.[46] A single dose (75 mg/m^2) SC versus IV crossover study in 6 MDS subjects[47] revealed an approximate bioavailability of 89% for the SC dose (range 52% to 128%) with mean half-lives of 0.69 hour and 0.36 hour after SC and IV administration, respectively. These results demonstrated that 5-azacytidine is rapidly and nearly completely absorbed after SC administration and that elimination is also rapid. The apparent SC clearance (167 L/h or 2791 mL/min) and systemic IV clearance (147 L/h) of 5-azacytidine exceeded the glomerular filtration rate (approximately 125 mL/min) and total renal blood flow (1200 mL/min) in healthy subjects. This indicates that non-renal elimination (e.g., metabolism, hydrolysis, and/or degradation) plays a role in the elimination of parent 5-azacytidine. In addition, 5-azacytidine 75 mg/m^2 was generally well-tolerated after single SC injection or IV infusion over 10 minutes.[47]

A number of studies have looked at different parenteral doses and schedules of 5-azacytidine, finding maximum tolerated doses of up to 500 mg/m^2 when administered weekly.[48]

During the two decades between the start of the CALGB studies and the approval of 5-azacytidine, a new understanding of MDS has developed, such as the World Health Organization (WHO) diagnostic criteria, the International Prognostic Scoring System (IPSS), and the International Working Group (IWG) response criteria. Silverman et al. reevaluated the combined data (N = 309) from 3 of the CALGB studies using the WHO classification system for MDS and AML and the IWG response criteria.[49] Using the IWG response criteria in MDS patients, response rates were between 40% and 70% in 5-azacytidine treated patients. Ten to 17% of patients achieved a complete remission; partial remission was rare; and 23% to 36% of patients had a hematologic improvement. In patients with AML (N = 103), 35% to 48% had hematologic improvement or better responses. The median survival time for 27 patients assigned to 5-azacytidine was 19.3 months compared with 12.9 months for the 25 patients assigned to observation.[49]

A randomized international Phase III trial (Study 5-azacytidine PH GL 2003 CL 001) for higher-risk MDS patients, classified by FAB as RAEB, RAEB-T, or CMML with 0-29% marrow blasts, with an IPSS of Intermediate -2 or High by central pathology/cytogenetic review was recently reported.[50] Patients were randomized to 5-azacytidine (75 mg/m^2/day x 7days in 28 day cycles) or conventional care regimens (CCR), where CCR was pre-selected by

the Investigator as best supportive care (transfusions, antibiotics, and G-CSF for neutropenic infection), low-dose cytarabine (20 mg/m^2/day x 14 days in 28 day cycles); or standard chemotherapy (conventional induction/consolidation). Patients were stratified by FAB/IPSS and randomized 1:1 to 5-azacytidine or CCR. This trial did not allow erythropoietin. Three hundred fifty eight patients (70% male) were randomized at 79 centers to 5-azacytidine (N=179) or CCR (N=179): best supportive care only (N=105, 59%), low-dose cytarabine (N=49, 27%), or standard chemotherapy (N=25, 14%). Median age was 69 years (range 38-88 years). The 5-azacytidine and CCR groups were comparable for baseline patient characteristics. At baseline, 95% of patients were higher risk: RAEB (58%), RAEB-T/WHO AML (34%), CMML (3%), and other (5%). By IPSS, 87% were higher risk: Intermediate -2 (40%), High (47%), and 13% Indeterminate/other. 5-azacytidine was administered for a median of 9 cycles; low-dose cytarabine for 4 cycles. Median follow-up for the survival analysis was 21.1 months. 5-azacytidine demonstrated statistically superior overall survival compared to CCR, with a median overall survival of 24.4 months vs. 15 months for CCR (stratified log-rank p=0.0001, hazard ration 0.58). Two-year survival approximately doubled in the 5-azacytidine arms compared to CCR: 51% vs. 26% (p<0.0001). 5-azacytidine was well tolerated with safety data consistent with previous reports.

Further details can be found in the 5-azacytidine drug information (**Appendix D**), which contains comprehensive pharmacology, toxicology, pharmacokinetics, pharmacodynamics, metabolism, preclinical, and clinical efficacy and safety data information.[46]

2.1.8 Rationale for study

The PD-1/PD-L1 pathway plays a major role in immune evasion and cytotoxic T-cell exhaustion in AML[9,10,21]. PD-L1 expression in AML samples is increased at the time of disease progression or after exposure to IFN-gamma[21,51]. Overexpression of PD-L1 is an independent negative prognostic factor in AML[21]. Hypomethylating agents may alter immune regulation[52]. Yang et al have recently demonstrated that hypomethylating therapy leads to up regulation of PD-L1, PD-1, and PD-L2 gene expression[20]. Patients resistant to hypomethylating therapy had higher increments in gene expression suggesting that PD-1 up regulation may promote resistance to hypomethylating agents. Demethylation of the PD-L1 gene by chronic viral infection results in PD-L1 up-regulation and CTL exhaustion[53]. Similarly, exposure to azacytidine up regulates PD-L1, PD-1 and PD-L2 by demethylation of the PD-L1 locus[20]. These data suggest that blockage of PD-L1 by avelumab may improve response and abrogate resistance to hypomethylating agents. Furthermore, recent data shows that azacytidine increases NK-cell activating receptor ligands (NKG2DL) and enhanced ADCC when administered in combination with CD33-targeting antibody with preserved FCγIIIa domain (Vasu et al, Blood May 2016 and Daver N, Ravandi F et al, Blood May 2016). In addition to azacytidine's impact on T-cells costimulatory receptor and blast ligand expression we hope to promote ADCC by combining an epigenetic agent (azacytidine) with an agent that has preserved Fc receptor to engage NK cells (avelumab). Enhanced ADCC may be a second mechanism of antileukemic synergy when azacytidine and avelumab are administered in combination.

In this initial trial, we propose to explore these concept in patients with refractory/ relapsed AML with both clinical and focused correlative analysis. We propose to introduce PD-1

blockade early, when there are still leukemia cells that can prime the immune competent cells for eventual eradication. If the combination proves to be well tolerated and results in responses as expected, we would expand the use of this approach in the frontline setting for elderly AML and high-risk MDS (10-20% blast) patients who are not candidates for or refuse standard cytotoxic therapy either as an extension of this study or as a separate study.

3.0 STUDY DESIGN

- This will be a phase II, single-arm, open-label, non-randomized study with a safety lead-in phase IB.

- Patients will receive 5-azacytidine subcutaneously or intravenously daily for 7 days of each treatment cycle. For the lead-in phase IB portion, the length of the cycle will be at least 28 days to evaluate DLT. Subsequently, cycles will be repeated approximately every 28 days (+/-5 days), and therapy will be continued until clinically significant disease progression or documentation of unacceptable toxicity.

- Patients will receive therapy with avelumab IV infusion on Day 1 and day 14 (+/-3 days) of each 5-azacyidine cycle for first 4 cycles or until CR (whichever occurs earlier) followed by a maintenance regimen (one dose of avelumab on day 1 of each cycle of 5-azacytdine). Patients who have evidence of budding relapse or seem to have clinical benefit may continue or revert back to receiving avelumab on Day 1 and day 14 (+/-3 days) of each cycle of 5-azacytidine. Patients may receive avelumab more or less frequently during the maintenance regimen at the discretion of the treating physician only after discussion with the PI.

- The historical experience in relapsed/refractory AML patient population is that historical responses (CR/CRi/PR/HI with >50% blast reduction) with single agent hypomethylator therapy (5-azacytidine or decitabine) or with hypomethylator combinations (azacytidine+revlimid, decitabine+myelotarg, azacytidine+vorinostat, decitabine+valproate) investigated at MDACC over the last 10 years are 10% (Daver N, Kantarjian H et al., European Hematology Association Conference, June 2016). In many instances (e.g., patients with relapsed/refractory AML beyond first salvage) no standard therapy is available. Thus, an overall response rate (CR/CRi/PR) of 30% will be considered significant

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

4.1.1 Patients with AML who are refractory (up to salvage 2) or relapsed (up to 2nd relapse). For patients with prior MDS or chronic myelomonocytic leukemia (CMML) or MPN who transformed to AML, therapy received for MDS, CMML, or MPN is NOT considered as prior therapy for AML.

4.1.2 Prior therapy with hydroxyurea, chemotherapy, biological or targeted therapy (e.g.

FLT3 inhibitors, other kinase inhibitors), or hematopoietic growth factors is allowed.

4.1.3 Age >/=18 years

4.1.4 Eastern Cooperative Oncology Group (ECOG) Performance Status </=2

4.1.5 Adequate organ function: total bilirubin </= 1.5 times upper limit of normal (x ULN) (</= 3 x ULN if considered to be due to leukemic involvement or Gilbert's syndrome); aspartate aminotransferase or alanine aminotransferase </= 2.5 x ULN (</= 5.0 x ULN if considered to be due to leukemic involvement)

4.1.6 Adequate renal function defined by an estimated creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula (or local institutional standard method)

4.1.7 Patients must provide written informed consent.

4.1.8 In the absence of rapidly progressing disease, the interval from prior treatment to time of initiation of 5-azacytidine and avelumab will be at least 14 days OR at least 5 half-lives for cytotoxic/noncytotoxic agents, whichever is longer. The toxicity from prior therapy should have resolved to Grade </= 1, however alopecia and sensory neuropathy Grade </=2 is acceptable. The half-life for the therapy in question will be based on published pharmacokinetic literature (abstracts, manuscripts, investigator brochure's, or drug-administration manuals) and will be documented in the protocol eligibility document. Since the effect of both avelumab and 5-azacytidine may be delayed, use of hydroxyurea for patients with rapidly proliferative disease is allowed before the start of study therapy and will not require a washout. Concurrent therapy for CNS prophylaxis or continuation of therapy for controlled CNS disease is permitted. Patients with CNS disease or leukemic brain metastasis must have been treated locally and be clinically stable for at least 2 weeks prior to enrollment and have no ongoing neurological symptoms that are related to the CNS disease (sequelae that are a consequence of the treatment of the CNS disease are acceptable).

4.1.9 Females must be surgically or biologically sterile or postmenopausal (amenorrheic for at least 12 months) or if of childbearing potential, must have a negative serum or urine pregnancy test within 72 hours before the start of the treatment.

4.1.10 Women of childbearing potential must agree to use an adequate method of contraception during the study and until 3 months after the last treatment. Males must be surgically or biologically sterile or agree to use an adequate method of contraception during the study until 3 months after the last treatment.

Adequate methods of contraception include:

- Total abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- Female sterilization (have had surgical bilateral oophorectomy with or without

hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment

- Male sterilization (at least 6 months prior to screening). For female patients on the study, the vasectomized male partner should be the sole partner for that patient
- Combination of any of the two following (a+b or a+c or b+c)
  a. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception
  b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
  c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository

  In case of use of oral contraception, women should have been stable on the same pill before taking study treatment.

  Note: Oral contraceptives are allowed but should be used in conjunction with a barrier method of contraception due to unknown effect of drug-drug interaction.

  Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

4.2 Exclusion Criteria

4.2.1 Patients with a known allergy or hypersensitivity to avelumab, 5-azacytidine, or any of their components. Known severe hypersensitivity reactions to monoclonal antibodies (Grade ≥ 3 NCI-CTCAE v 4.03), any history of anaphylaxis, or uncontrolled asthma (that is, 3 or more features of partially controlled asthma).

4.2.2 Patients with a known history of severe interstitial lung disease or severe pneumonitis or active pneumonitis/pneumonia or pulmonary pathology that is not well controlled in the opinion of the treating physician and/or PI.

4.2.3 Patients who have previously been treated with avelumab (or another PD1/PDL1 inhibitor) in combination with 5-azacytidine will be excluded.

4.2.4 Persisting toxicity related to prior therapy of Grade >1 NCI-CTCAE v 4.03; however, alopecia and sensory neuropathy Grade ≤ 2 is acceptable

4.2.5 Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent:

a. Subjects with diabetes type I, vitiligo, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible

b. Subjects requiring hormone replacement with corticosteroids are eligible if the steroids are administered only for the purpose of hormonal replacement and at doses ≤ 10 mg or 10 mg equivalent prednisone per day
c. Administration of steroids through a route known to result in a minimal systemic exposure (topical, intranasal, intro-ocular, or inhalation) are acceptable

4.2.6 Patients with organ allografts (such as renal transplant) are excluded

4.2.7 Patients who are <90 days post allogeneic stem cell transplant will be excluded. Patients beyond 90 days post-allogeneic stem cell transplant with uncontrolled active GVHD > grade 1 will be excluded. Patients who are on a stable dose of immunosuppressive therapy (tacrolimus, cyclosporine, or other) for > 2 weeks will be eligible but those with recent increase in the immunosuppressive medication dose within last 2 weeks to control GVHD will not be included. Note: Subjects may be using systemic corticosteroids or topical or inhaled corticosteroids post allogeneic stem cell transplant (inclusion based on post stem cell transplant activity and tolerability of checkpoint inhibitor by Matthew D, et al., ASH 2015 Annual Conference abstract # 860). Patients requiring >/= 1 mg/kg prednisone for GVHD management at the time of screening will not be eligible until the prednisone can be weaned to <1 mg/kg. Such patients should be monitored for at least 14 days and if no flare of GVHD requiring re-escalation of steroids or additional interventions for the GVHD they will be eligible.

4.2.8 Patients with symptomatic CNS leukemia or patients with poorly controlled CNS leukemia.

4.2.9 Active and uncontrolled disease/(active uncontrolled infection, uncontrolled hypertension despite adequate medical therapy, active and uncontrolled congestive heart failure NYHA class III/IV, clinically significant and uncontrolled arrhythmia) as judged by the treating physician.

4.2.10 Patients with known Human Immunodeficiency Virus seropositivity will be excluded.

4.2.11 Known to be positive for hepatitis B by surface antigen expression. Known to have active hepatitis C infection (positive by polymerase chain reaction or on antiviral therapy for hepatitis C within the last 6 months)

4.2.12 Any other medical, psychological, or social condition that may interfere with study participation or compliance, or compromise patient safety in the opinion of the investigator

4.2.13 All other significant diseases (for example, inflammatory bowel disease, uncontrolled asthma), which, in the opinion of the Investigator, might impair the subject's tolerance of trial treatment

4.2.14 Patients unwilling or unable to comply with the protocol.

4.2.15 Pregnant or breastfeeding

4.2.16 Known alcohol or drug abuse within the last 1 year

4.2.17 Vaccination within 4 weeks of the first dose of avelumab and while on trial is prohibited except for administration of inactivated vaccines

4.2.18 Acute promyelocytic leukemia (APL).

4.2.19 Subject has a history of other malignancies prior to study entry, with the exception of:

a. Adequately treated in situ carcinoma of the cervix uteri or carcinoma in situ of breast;
b. Basal cell carcinoma of the skin or localized squamous cell carcinoma of the skin;
c. Previous malignancy confirmed and surgically resected (or treated with other modalities) with curative intent or completed definitive therapy (chemotherapy, radiation, others) for the malignancy at least 1 year prior to the date of screening.

5 TREATMENT PLAN

5.1 General

All patients will be registered through CORe. We will first treat 6 patients at dose level -1.

5.2 Schedule

The Investigator is responsible for completing the cohort summary template and submitting to the IND office Medical Monitor for review and approval prior to advancing subjects to the next protocol specified cohort/dose level. A copy of the cohort summary should be placed in the Investigator's Regulatory Binder under "sponsor correspondence". This should be submitted after the first six patients.

5.2.1 Patients will be treated according to the following schedule:

- 5-azacytidine will be administered subcutaneously (SQ) or intravenously (IV) for 7 days of every cycle with daily schedule of 7 consecutive days (Days 1-7) or for days 5-2-2 which is 5 consecutive weekdays (Days 1-5) with rest on the 2 weekend days (Days 6 and 7) and then dosing the first two weekdays of the next week (Days 8 and 9) of each 28-day cycle as determined by treating physician. Alternative dosing schedules such as 4-2-3 which is 4 consecutive weekdays (Days 1-4) with rest on the 2 weekend days (Days 5 and 6) and then dosing the first three weekdays of the next week (Days 7, 8, and 9) of each 28-day cycle or other schedules allowing the 2 day weekend interruption may be applied as long as we make every attempt to give the 7 days of azacytidine as consecutively as possible. This must be clearly documented in the medical record as determined by treating physician after approval from the PI. Both SQ

and IV forms of administration are FDA approved and considered interchangeable. Patients may start receiving 5-azacytidine by one route and changed to the other, and also administration schedules from one schedule and changed to the other at any time as needed based on patient and/or physician preference.

- Avelumab will be administered as an approximately 60 minute (+/- 30 minutes) IV infusion on Day 1 and day 14 (+/-3 days) of each 5-azacyidine cycle for the first 4 cycles or until CR (whichever occurs earlier) followed by a maintenance regimen (one dose of avelumab on day 1 of each cycle of 5-azacytdine). Patients who have evidence of budding relapse or seem to have clinical benefit may continue or revert back to receiving avelumab on Day 1 and day 14 (+/-3 days) of each cycle of 5-azacytidine. Patients may receive avelumab more or less frequently during the maintenance regimen only after discussion with the PI. Avelumab will be administered every cycle with no interruptions unless there are adverse events as described in Section 5.3.

- 5-azacytidine at a dose of 75 mg/m^2 on days 1-7 or days 5-2-2 has been shown to be safe in combination with multiple agents including lenalidomide[60], bortezomib[60], and vorinostat[61, 62]. We have proposed the starting dose level -1 of 5-azacytidine 75 mg/m^2 x 7 days and avelumab 3.0 mg/kg on days 1 and 14 of each cycle. The lead-in phase is to ensure that the combination is well tolerated with no unexpected side effects and to identify the MTD of the combination. Avelumab infusion should begin right after the administration of 5-azacytidine on day 1, as described above, whenever possible. The dosing calculations should be based on the body weight. All doses should be rounded to the nearest milligram. There will be no dose reductions (only interruptions when indicated) allowed for avelumab on this trial.

- Avelumab administration: Subjects will receive avelumab by IV infusion following pretreatment with H1 blockers and acetaminophen on day 1 and 14 of each cycle. Premedication with an antihistamine and with paracetamol (acetaminophen) (for example, 25 50 mg diphenhydramine and 500-650 mg paracetamol [acetaminophen] IV or oral equivalent) approximately 30 to 60 minutes prior to each dose of avelumab is mandatory. This regimen may be modified based on local treatment standards and guidelines as appropriate provided it does not include systemic corticosteroids.

5.2.1.1 The starting dose will be dose level -1(Table 1).

Table 1. Dose levels of 5-azacytidine and avelumab during the lead-in phase (phase IB)

Dose level	5-azacytidine (mg/m^2/d, Days 1-7)	Avelumab (mg/kg, day 1 and 14)
-3	25	3.0
-2	50	3.0
-1	75	3.0 (starting dose)

0	**75**	**10.0 (target dose)**

5.2.1.2 The goal of the lead-in phase IB is to identify the dose at which <2/6 patients experience DLT. During the lead-in phase the doses of 5- azacytidine and avelumab may not be reduced if 2 or more patients experience DLT at any given dose. After the lead-in phase IB is completed and the MTD dose of avelumab is established the dose of avelumab may not be reduced in the phase II portion of this study. For potential avelumab related AEs only dose interruptions will be permitted. Dose reductions or escalations of 5-azacytidine are permitted in the phase II portion of the study and dose reductions or escalations of 5-azacytidine beyond those mentioned in Table 1 or different to the doses specified should be discussed with the PI and documentation of the justification recorded in the chart. The escalation will in no circumstance exceed the established MTD for this trial.

5.2.1.3 The dose and /or schedule of administration is subject to modification pending information from ongoing clinical trials of avelumab as a single agent and in combination with other drugs.

DLT is defined as clinically significant non-hematologic adverse event or abnormal laboratory value assessed as unrelated to disease progression, intercurrent illness, or concomitant medications and occurring during the first cycle on study that meets any of the following criteria:

- CTCAE Grade 3 AST (SGOT) or ALT (SGPT) for >/= 7 days or grade 3 hyperbiliribunemia for >/= 7 days
- CTCAE Grade 4 AST (SGOT) or ALT (SGPT) of any duration
- All other clinically significant non-hematological adverse event that is Grade 3 or 4 according to the NCI common terminology criteria version 4.0 with the following exceptions:
- Grade 3 or 4 nausea, vomiting and diarrhea will be considered DLT only if not controlled by optimal therapy.
- Grade 3 biochemical abnormalities (e.g., lipase or bilirubin elevation) will only be considered DLT if accompanied by clinical consequences. Grade 3 or 4 electrolyte abnormalities will only be considered DLT if possibly related to study drug and not corrected by optimal replacement therapy.
- Any treatment-related death;
- Grade 4 neutopenia lasting ≥ 42 days from start of cycle in absence of evidence

of active AML.

5.2.1.4 We will first treat 6 patients at dose level -1 in the lead-in phase IB. If DLT occurs in >/=2/6 patients, this dose level would exceed the MTD, and 6 patients will be treated at the next lower dose level (i.e. dose level -2). If DLT occurs in < 2/6 patients, this dose level would not exceed the MTD, and 6 patients will be treated at the next higher dose level (i.e. dose level 0). The dose de-escalation (Table 1) will continue in cohorts of 6 until we reach the maximum dose level at which <2/6 patients experience a DLT in the first 28 days. The maximum dose level at which 0-1/6 patients experience a DLT in the first 28 days of treatment will be the MTD in this study and will be used to treat an additional 40 patients in the phase II portion of the study. If >/=2/6 patients experience DLT at dose level -3, the study will be revised to consider additional lower dose levels (based on potential synergistic toxicity).

5.2.1.5 If DLT is observed in the first 28 days of treatment in 0 or 1/6 at dose level 0, then dose level 0 will be used for the phase II portion of the study.

5.2.1.6 Patients must receive a total of 7 doses of azacytidine and 2 doses of avelumab during the first 28 days on trial (i.e. during cycle 1) to be evaluated for DLT. Patients may have up to a total of 7 days interruption in the azacytidine dosing during the first 28 days and still be evaluable for DLT if the interruption is for complications or hospitilizations NOT related to the azacytidine or the avelumab. Patients who receive less than 7 doses of azacytidine and/or less than 2 doses of avelumab during the first 28 days on trial will NOT be evaluable for DLT. Patients not evaluable for DLT will be replaced. These patients may continue therapy on trial after discussion with the PI if they are having clinical benefit or if in the best interest of the patient and the reasons for continuation and potential benefit/risk profile for the patient must be clearly documented in the medical records.

5.2.1.7 Once MTD is defined, the RP2D will be selected based on efficacy and safety result from the phase I in discussion with the PI and the sponsor. The Investigator is responsible for completing the cohort summary template prior to advancing subjects to the phase II portion. Any patients still on study from the phase I portion at a dose lower than RP2D can be dose escalated up to RP2D. However, the dose for any patient may never exceed the RP2D.

5.2.1.8 Once MTD is defined, the RP2D will be selected based on efficacy and safety result from the phase I in discussion with the PI and the sponsor. The Investigator is responsible for completing the cohort summary template prior to advancing subjects to the phase II portion. Any patients still on study from the phase I portion at a dose lower than RP2D can be dose escalated up to RP2D. However, the dose for any patient may never exceed the RP2D.

5.2.2 One cycle of therapy is defined as 28 days. Patients will receive one cycle of therapy every 28 days (+/- 5 days).

5.2.2.1 In the phase II portion of the study (once the MTD dose has been defined) cycles may be started early (but not earlier than day 23) for patients with active disease if judged in the best interest of the patient.
For the lead-in cohort, DLT defining period is 28 days. As such, cycle 2 should not be started before 28 days for the patients being treated on the lead-in cohort.

5.2.2.2 Subsequent cycles may be delayed for recovery of toxicity or other medical conditions (e.g. infections). Delays in start of subsequent cycles greater than 8 weeks will be acceptable only for patients who are deriving clinical benefit and after discussion with the principal investigator of potential risk/benefit ratio and clear documentation in the medical record of the reason for continuation and perceived benefit to the patient from continuation of this therapy.

5.2.2.3 In instances where one drug has to be discontinued transiently because of safety, the administration of the other drug may continue as scheduled.

5.2.2.4 Azacitidine and avelumab will be held during phase II of the study, for grade 4 neutropenia or grade 4 thrombocytopenia lasting ≥ 42 days from the start of each cycle, in the absence of evidence of active AML. If prolonged grade 4 neutropenia (more than 42 days) WITH evidence of a hypocellular marrow (marrow cellularity less than 5% without evidence of leukemia) is observed, 5-azacytadine and avelumab will be discontinued. Delays in start of subsequent cycles greater than 42 days will be acceptable only for patients who are deriving clinical benefit and after discussion with the principal investigator of potential risk/benefit ratio.
If the peripheral counts do not recover (ANC $<0.5 \times 10^9$/L and/or platelets $<25 \times 10^9$/L) but there is evidence of residual leukemia by morphology or flow-cytometry in the bone marrow, subsequent cycles can be administered at the discretion of the treating physician not earlier than 23 days after the prior cycle in the phase II portion of the study. For the lead-in cohort, DLT defining period is 28 days. As such, cycle 2 should not be started before 28 days for the patients being treated on the lead-in cohort.

5.2.2.5 For patients who discontinue therapy, the reason for treatment discontinuation will be captured.

5.3 Dose Adjustments

5.3.1 Avelumab and 5-azacytidine dose adjustments for hematological drug-related adverse events (AE):

Dose reduction/interruption/discontinuation decisions should be based on the

CTCAE version 4.03 (**Appendix E**) and the guidelines provided below. Patients with acute leukemia's usually present with abnormal peripheral blood counts at the time therapy is started and myelosuppression is an expected event during the course of therapy for acute leukemia. Thus, no dose adjustments or treatment interruptions for myelosuppression will be planned for the first 4 cycles and/or in the presence of residual leukemia. After that, treatment interruptions and dose adjustments may be considered according to the following guidelines only when there is no evidence of active leukemia (e.g., only if <5% blasts in the bone marrow or cytopenias not considered to be related to leukemia).

- Patients with a response (no marrow evidence of leukemia) and pre-cycle counts of neutrophils >1x10^9/L and platelets >50 x10^9/L who have sustained low counts of neutrophils <0.5 x10^9/L or a platelet count <20 x 10^9/L for more than 2 consecutive weeks in the current cycle, may have the treatment with 5-azacytidine interrupted at the discretion of the treating physician after discussing with the PI until neutrophils recover to ≥1 x10^9/L and platelets to ≥50 x10^9/L.

- If there are persistent peripheral blood blasts, or the bone marrow shows >5% blasts or evidence of residual leukemia, treatment may be continued regardless of neutrophil and platelet count with supportive care as needed. Dose-interruptions of 5- azacytidine in these patients should be considered on an individual case-by-case basis and discussed with the PI.

- Investigators should, whenever possible, determine which medication is causing the toxicity and interrupt or dose reduce 5-azacytidine and/or interrupt avelumab, as applicable. No dose reductions (only dose interruptions as needed) are permitted for avelumab in the phase II portion after RP2D has been established. Avelumab has not ben associated with neutropenia and/or thrombocytopenia in prior studies and should generally not be interrupted or discontinued for myelosuppression.

5.3.2 5-azacytidine dose adjustments for non-hematologic drug- related AEs

Investigators should, whenever possible, determine which medication is causing the toxicity and interrupt or dose reduce 5-azacytidine and/or interrupt avelumab, as applicable. No dose reductions (only interruptions) are permitted for avelumab in the phase II portion after RP2D has been established.

Dose reductions of azacytidine to be implemented in the phase II portion of the study will be as follows: dose level -1 of azacytidine: 50 mg/m2, dose level -2: 37.5 mg/m2, dose level -3: 25 mg/m2.

Table 2 Dose adjustments of azacytidine for non-hematologic drug-related AEs, clinically significant in the opinion of the investigator

Grade	Occurrence	Dose modification
1 or 2	Any time	No dose reduction
3 or 4 (Persistent grade 2: Consider similar dose adjustments if persistent and not responding to optimal management in the opinion of PI and treating physician)	1st and 2nd time	Hold 5-azacytidine. Resume 5-azacytidine at prior dose if recovery to ≤ Grade 1 occurs within 14 days. If toxicity persists for 15-28 days, hold therapy and resume 5-azacytidine at prior dose if recovery to ≤ Grade 1 OR resume 5-azacytidine at ONE dose level below current dose if recovery to ≤ Grade 2. Dose re-escalation to prior dose of 5-azacytidine is permitted in accordance with the dose-escalation guidelines in section 5.3.6.
	3rd. and 4th time	Hold 5-azacytidine. Follow until toxicity ≤ Grade 2. Resume 5-azacytidine at ONE dose level below current dose. Dose re-escalation of 5-azacytidine to prior dose is permitted in accordance with the dose-escalation guidelines in section 5.3.6..
	5th time	Discontinue therapy

5.3.3 Avelumab dose delay/interruption for immune-oncology drug-related AEs, clinically significant in the opinion of the investigator

Avelumab administration should be delayed for the following drug-related AEs, clinically significant in the opinion of the investigator:

- Any Grade >/=2 non-skin AE, except that
  - Grade 2 fatigue or laboratory abnormalities do not require delay, however
    - Patients with ALT or AST > 3 and up to 5 x ULN or total bilirubin greater > 2.0 mg/dL and up to 3 x ULN or creatinine >2.0 mg/dL and up to 3 x ULN should have avelumab treatment withheld.

Avelumab administration should be delayed for the following drug-related AEs, clinically significant in the opinion of the investigator:

- Any Grade 3 skin AE, or Grade 3 laboratory abnormality, with the following exceptions:
  - Grade 3 lymphopenia or leukopenia does not require dose delay.
  - However, patients with ALT or AST >5 x ULN or total bilirubin greater > 3 x ULN or creatinine >3 x ULN should have avelumab treatment interrupted.
- Any AE, laboratory abnormality, or intercurrent illness, which in the judgment of the investigator, warrants delaying the dose of study medication.
- Avelumab dose reductions are not permitted in this study (only dose delays when indicated).

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline or at least </= grade 1 before treatment is resumed
- Drug-related endocrinopathies adequately controlled may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the next scheduled time point will be delayed until dosing resumes. Patients who receive combination therapy in whom continuation of 5-azacytidine is considered to be inadequate, or inappropriate (e.g., because of pancytopenia) can discontinue 5-azacytidine and continue with avelumab only.

If treatment is delayed > 8 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

5.3.4 Avelumab Discontinuation Criteria

The following ADRs require permanent treatment discontinuation of avelumab:

---**Any Grade 4 AEs require treatment discontinuation with avelumab** except for single laboratory values out of normal range that are unlikely related to study treatment as assessed by the Investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management
---**Any Grade 3 AEs require treatment discontinuation with avelumab except for any of the following:**

- Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management

- Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1

- Single laboratory values out of normal range (excluding Grade ≥ 3 liver function test increase) that are unlikely related to study treatment according to the Investigator, do not have any clinical correlate, and resolve to Grade ≤ 1 within 7 days with adequate medical management

- Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor

- Change in ECOG PS to ≥ 3 that does not resolve to ≤ 2 within 14 days (infusions should not be given on the following cycle, if the ECOG PS is ≥ 3 on the day of study drug administration)

Any Grade 2 AEs should be managed as follows:

- If a Grade 2 AE resolves to Grade ≤ 1 by the last day of the current cycle, treatment may continue.

- If a Grade 2 AE does not resolve to Grade ≤ 1 by the last day of the current cycle, infusions should not be given on the following cycle. If at the end of the following cycle the event has not resolved to Grade 1, the subject should permanently discontinue treatment with a avelumab AE (except for hormone insufficiencies, that can be managed by replacement therapy; for these hormone insufficiencies, up to 2 subsequent doses may be omitted).

- Upon the second occurrence of the same Grade 2 AE (except for hormone insufficiencies that can be managed by replacement therapy) in the same subject, treatment with avelumab has to be permanently discontinued.

- Infusion-related reactions, hypersensitivity reactions (Grades 1 to 4), and tumor lysis syndrome should be handled according to guidelines provided.

• Any dosing interruption lasting > 8 weeks with the following exceptions:
  o Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the PI must be consulted. Tumor/Leukemia assessments should continue as per protocol even if dosing is interrupted.

  o Dosing interruptions > 8 weeks that occur for non-drug-related reasons may be allowed if approved by the PI. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the PI must be consulted. Tumor/Leukemia assessments should continue as per protocol even if dosing is interrupted.

  o Delays in start of subsequent cycles greater than 8 weeks will be acceptable only for patients who are deriving clinical benefit and after discussion with the PI of potential risk/benefit ratio and documentation of this discussion in the patents medical record.

5.3.5 Detailed management algorithms for immune-oncology drug-related adverse events (including gastrointestinal, renal, pulmonary, hepatic, endocrine, skin and neurological) are provided in Table 3 below. These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the PI and IND Office in specific cases.

Since inhibition of PD-L1 stimulates the immune system, immune-related AEs (irAEs) may occur. Treatment of irAEs is mainly dependent upon severity (NCI-CTCAE grade):

Grade 1 to 2: treat symptomatically or with moderate dose steroids, more frequent monitoring

Grade 1 to 2 (persistent): manage similar to high grade AE (Grade 3 to 4)

Grade 3 to 4: treat with high dose corticosteroids

Table 3. Management of Immune-Related Adverse Events

Gastrointestinal irAEs		
Severity of Diarrhea / Colitis (NCI-CTCAE v4.03)	**Management**	**Follow-up**
Grade 1 Diarrhea: < 4 stools/day over Baseline Colitis: asymptomatic	Continue avelumab therapy Symptomatic treatment (for example, loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2 or 3/4
Grade 2 Diarrhea: 4 to 6 stools per day over Baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Delay avelumab therapy Symptomatic treatment	If improves to Grade 1: Resume avelumab therapy If persists > 5 to 7 days or recur: 0.5 to 1.0 mg/kg/day methylprednisolone or equivalent When symptoms improve to Grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy per protocol. If worsens or persists > 3 to 5 days with oral steroids: Treat as Grade 3 to 4
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 hrs; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Discontinue avelumab therapy per protocol 1.0 to 2.0 mg/kg/day methylprednisolone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves: Continue steroids until Grade 1, then taper over at least 1 month If persists > 3 to 5 days, or recurs after improvement: Add infliximab 5 mg/kg (if no contraindication), Note: Infliximab should not be used in cases of perforation or sepsis

Dermatological irAEs		
Grade of Rash (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 to 2 Covering ≤ 30% body surface area	Symptomatic therapy (for example, antihistamines, topical steroids) Continue avelumab therapy	If persists > 1 to 2 weeks or recurs: Consider skin biopsy Delay avelumab therapy Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy If worsens: Treat as Grade 3 to 4
Grade 3 to 4 Covering > 30% body surface area; life threatening consequences	Delay or discontinue avelumab therapy Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent	If improves to Grade 1: Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections Resume avelumab therapy
Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 Radiographic changes only	Consider delay of avelumab therapy Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	Re-image at least every 3 weeks If worsens: Treat as Grade 2 or Grade 3 to 4
Grade 2 Mild to moderate new symptoms	Delay avelumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily, consider hospitalization 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent Consider bronchoscopy, lung biopsy	Re-image every 1 to 3 days If improves: When symptoms return to near Baseline, taper steroids over at least 1 month and then resume avelumab therapy and consider prophylactic antibiotics If not improving after 2 weeks or worsening: Treat as Grade 3 to 4

Grade 3 to 4 Severe new symptoms; New / worsening hypoxia; life-threatening	Discontinue avelumab therapy Hospitalize Pulmonary and Infectious Disease consults 2 to 4 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to Baseline: Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: Add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)
Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and / or total bilirubin > ULN to 1.5 x ULN	Continue avelumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3 to 4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and / or total bilirubin > 1.5 to ≤ 3 x ULN	Delay avelumab therapy Increase frequency of monitoring to every 3 days	If returns to Baseline: Resume routine monitoring, resume avelumab therapy If elevations persist > 5 to 7 days or worsen: 0.5 to 1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to Grade 1 or Baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy
Grade 3 to 4 AST or ALT > 5 x ULN and / or total bilirubin > 3 x ULN	Discontinue avelumab therapy Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist	If returns to Grade 2: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds: Add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines

	Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	
Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue avelumab therapy If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathies	Evaluate endocrine function Consider pituitary scan Symptomatic with abnormal lab / pituitary scan: Delay avelumab therapy 1 to 2 mg/kg/day methylprednisolone IV or by mouth equivalent Initiate appropriate hormone therapy No abnormal lab / pituitary MRI scan but symptoms persist: Repeat labs in 1 to 3 weeks / MRI in 1 month	If improves (with or without hormone replacement): Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections Resume avelumab therapy Subjects with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Suspicion of adrenal crisis (for example, severe dehydration, hypotension, shock out of proportion to current illness)	Delay or discontinue avelumab therapy Rule out sepsis Stress dose of IV steroids with mineralocorticoid activity IV fluids Consult endocrinologist If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathies	

ADL = activities of daily living; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CT = computed tomography; irAE = immune-related adverse event; IV=intravenous; LFT = liver function test; LLN = lower limit of normal; MRI = magnetic resonance imaging; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; T4 = free thyroxine; TSH = thyroid-stimulating hormone; ULN = upper limit of normal.

5.3.6 Intra-patient dose re-escalation for patients who have had dose-reductions due to hematological or non-hematological toxicity:

Once the MTD/RP2D has been established in the lead-in, the avelumab dose

will not be reduced or escalated during the phase II portion. Intra-patient dose re-escalation of 5-azacytidine (in accordance with the dosing schema in table 1) will be permitted provided:

- Patient has completed >/=1 cycle at their current dose level
- Patient has not experienced any grade 3 or higher non-hematologic drug-related toxicity, and
- Patient has not experienced drug-related hematologic DLT, and
- At least 6 patients have been treated at the next higher dose level and followed for at least 28 days without experiencing DLT.
- The dose may be escalated by one dose level per cycle (per table 1) provided such dose level does not exceed the established MTD/RP2D, whichever is lower. Dose level 0 is the maximum dose to be evaluated on this study. No dose escalation beyond dose level 0 will be permitted at any time on this study.

5.3.7 Modifications of dose schedules other than the above will be allowed within the following guidelines:

5.3.7.1 Dose adjustments by more than 1 dose level at a time (e.g., from 5-azacytidine 75 mg/m2 to 25 mg/m2) can be considered when judged in the best interest of the patient (e.g. severe myelosuppression) when toxicity has resolved. The reason for this reduction will be discussed with the PI or Co-PI and documented in the medical record.

5.3.7.2 A patient who has had a dose reduction because of any of the reasons mentioned above may have their dose escalated provided the patient has remained free of toxicity requiring dose adjustments as defined above for at least 1 month. Escalation will be made by 1 dose-level increment only, and not more frequent than every month.

5.3.7.3 Treatment interruptions and dose modifications other than the ones mentioned above can be considered after discussion with the PI and proper documentation of the rationale. Dose adjustment/delay of only one of the agents is permissible if the toxicity is most likely judged to be related to one of the agents by the investigator (e.g., in patients with autoimmune thyroiditis or autoimmune hepatitis this would be likely secondary to avelumab, in patients with cytopenia's this would be likely secondary to 5-azacytidine).

5.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

1. Clinically significant progressive disease, or

2. Intercurrent illness that prevents further administration of either treatment agent, or

3. Patient request, or

4. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or

5. Unacceptable toxicity that in the opinion of the investigator makes it unsafe to continue therapy.

5.4.1 It is planned that up to a total of 24 cycles of therapy will be administered for patients deriving benefit from this regimen. Continuation of therapy for patients completing 24 cycles of therapy may be considered on a case- by-case basis after discussion with the principal investigator and IND Office.

5.4.2. A minimum of 1 full course (defined as the administration of 5- azacytidine for 7 days and one dose of avelumab) will be required for a patient to be considered as having received an adequate trial to evaluate efficacy. All patients receiving at least one dose of any of the two drugs will be considered evaluable for toxicity.

5.5 Supportive Care:

Supportive care measures including blood products, infection prophylaxis and growth factors will be administered according to institutional and MDACC Leukemia Department guidelines.

Management Algorithms for Treatment of Avelumab Related Adverse Events

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Avelumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents such as avelumab may mitigate severe toxicity. Avelumab has a known safety profile however, a general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non- inflammatory etiologies should be considered and appropriately treated. Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events, and avelumab is no exception. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicities. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Management Algorithms have been developed to assist investigators in assessing and managing the following groups of adverse events: Endocrinopathies, Gastrointestinal, Hepatic, Neurological Pulmonary, Renal and Skin. These algorithms are found in the "Avelumab Investigator Brochure" (Appendix C) and "Table 3. Management of immune related adverse events" of this protocol. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

Finally, consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is highly recommended.

5.6 Concomitant Medications

Consistent with subject safety and comfort, administration of any prescription or over-the-counter drug products other than study medication should be minimized during the study period. Subjects should be discouraged from use of street drugs, herbal remedies, self-prescribed drugs, tobacco products, or excessive alcohol during the clinical study.

If considered necessary for the subject's wellbeing, drugs for concomitant medical conditions or for symptom management may be given at the discretion of the investigator. The investigator's decision to authorize the use of any drug other than study drug should take into account subject safety, the medical need, the potential for drug interactions, the possibility for masking symptoms of a more significant underlying event, and whether use of the drug will compromise the outcome or integrity of the study.

Subjects should be instructed about the importance of the need to inform the clinic staff of the use of any drugs or remedies (whether prescribed, over-the- counter, or illicit) before and during the course of the study.

Recommendations with regard to specific types of concomitant therapies, supportive care; diet and other interventions are as follows:

Concomitant medications are recommended as prophylaxis for nausea, vomiting, and infections, and are allowed for managing myelosuppression as shown in Table 3. Myelosuppression is expected in patients with AML due to underlying disease, as well as due to chemotherapy (such as 5-azacytidine), or both. Most patients have neutropenia, thrombocytopenia, or both at study entry. Significant or life-threatening myelosuppression may be managed with growth factor support and blood transfusion according to institutional standard of care, American Society of Clinical Oncology (ASCO) Practice Guidelines, and/or NCCN Practice Guidelines.

Infections secondary to myelosuppression are common in patients with AML, and may be related to underlying disease, chemotherapy, or both. Therefore, the use of prophylactic antibiotics, antifungal agents, and antiviral agents is recommended according to institutional standards.

Since the effect of both avelumab and 5-azacytidine may be delayed, patients with high WBC counts may receive hydroxyurea prior to study entry. Hydroxyurea is allowed before the start of study therapy and during the study treatment. Hydroxyurea use would be recorded in the case report form (CRF). Concurrent therapy for CNS prophylaxis or continuation of therapy for controlled CNS disease is permitted. With the exception of these agents, concomitant systemic chemotherapy or radiation therapy is not permitted. Subjects are not allowed to participate concurrently in any other therapeutic clinical study.

Subjects may be receiving systemic corticosteroids (daily doses ≤10 mg of prednisone or equivalent if indicated for adrenal replacement or antiemetic therapy), topical, or inhaled corticosteroids at study enrollment. They may receive systemic, topical, inhaled, or enteric corticosteroids while on study without limitation if they develop conditions that require corticosteroid therapy; such subjects are not required to discontinue study participation.

All ongoing medications and therapies (including herbal products, nutritional supplements, and nontraditional medications) at screening will be considered prior medications. Concomitant medication data will not be collected or entered into the case report form other than hydroxyurea as mentioned above; however, the subject's medication record will contain a list of concomitant medications. If a prohibited medication is inadvertently administered/ taken by the patient, the patient may remain on study as long as the prohibited medication is discontinued as soon as feasible. If a prohibited medication is considered essential for the patient well being, continuation on study with concomitant administration of such medication(s) will need to be discussed with and approved by the principal investigator and medical monitor

Table 4: Instructions for the use of concomitant medications and therapies

Category of Use	Medication	Comment on Use	Restriction on Use
Recommended	Prophylactic antibiotics, antifungal agents, and antiviral agents	Strongly encouraged	None
	Antiemetic agents	According to standard of care at MDACC	None
Allowed	Oral allopurinol or rasburicase	At investigators discretion	None
	Leukapheresis	According to standard of care at MDACC	Before induction 1 day 1 only
	Red blood cell	None	None

Category of Use	Medication	Comment on Use	Restriction on Use
	transfusion		
	Platelet transfusion	None	None
	White blood cell transfusion	At investigators discretion according to standard of care at MDACC	None
	Myeloid growth factors or platelet growth factor	At investigators discretion according to standard of care at MDACC	None
	Erythropoietin or darbepoetin	At investigators discretion according to standard of care at MDACC	None
	Any other medication for supportive care	At investigators discretion according to standard of care at MDACC	None

6.0 STUDY MEDICATIONS

6.1 Avelumab (Anti-PD1)

Avelumab is a fully human, monoclonal antibody of the IgG1 isotype that specifically targets and blocks the ligand (PD-L1) for PD-1 and has a preserved Fc domain. Avelumab is a sterile solution intended for intravenous (IV) infusion. Avelumab will be available as a sterile, clear, colorless, and non-pyrogenic solution for intravenous infusion. Each single-use vial contains 200 mg of avelumab, formulated as a 20 mg/mL preservative free acetated buffered solution at pH 5.2 in presence of Polysorbate 20 and Mannitol. It will supplied in Type I glass vials filled with 10 mL of liquid (200mg/vial), closed with a rubber septum and sealed with an aluminium flip off seal. The vials are not subject specific although there will be specific vial assignments by subject distributed by the Pharmacy in order to track drug usage and re-supply.

6.1.1 Dose Calculation of Avelumab

The determined dose is based on the patients bodyweight at each visit.

# of assigned vials for a patient = (Current weight x dose level)/ 200 (mg/vial)

For example, a subject weighing 70 kg who is scheduled receive a dose of 10 mg/kg would require 700 mg/ 200 (mg/vial) = 3.5 vials i.e. 4 vials have to be used for this patient.

6.1.2 Preparation and Dispensing of Avelumab

The product storage manager should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by the Investigator Brochure for avelumab (Appendix C). If concerns regarding the quality or appearance of the study drug arise, do not dispense the study drug and contact the supporting company (Pfizer) immediately. To prepare the dilutions, subsequent preparation steps must be accomplished by adequate trained personnel to guarantee the sterility of the product to be injected. Preferred method is to prepare the solution under laminar flow box using aseptic techniques.

Investigational product documentation must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (e.g. required diluents, administration sets).

Avelumab vials must be stored in the original packaging at a refrigerated conditions 2°C to 8°C until use with a temperature log maintained daily and should be protected from light. Avelumab stored at room temperature 15-25°C or higher temperatures for extended periods of time might be subject to degradation. Avelumab must not be frozen. Recommended safety measures for preparation and handling of avelumab include laboratory coats and gloves.

For details on prepared drug storage and use time of avelumab under room temperature/light and refrigeration, please refer to the Avelumab Investigator Brochure section (Appendix C) for “Recommended Storage and Use Conditions”. Care must be taken to assure sterility of the prepared solution, as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between avelumab and polyolefin bags have been observed.

6.1.3 Administration of avelumab

Patients will receive avelumab as an approximately 60 minute (+/- 30 minutes) IV infusion on Day 1 and day 14 (+/-3 days) of a treatment cycle every 28 days for 4 cycles or until CR (whichever occurs earlier) followed by a maintenance regimen (one dose of avelumab on day 1 of each cycle of 5-azacytidine). Dosing calculations should be based on the body weight assessed at the start of each cycle as described above. All doses should be rounded to the nearest milligram. The screening body weight may be used for dosing of cycle 1.

Prior to the preparation of dilution for final infusion, allow each vial to equilibrate to room temperature (15-25 C) preferably for 30-50 minutes. Rough shaking of the

solution must be avoided. The administration must be performed by adequately trained personnel. For application in clinical trials, avelumab must be diluted with a saline solution (Sodium Chloride for injection). The prepared avelumab dilution for infusion is connected to the infusion set equipped with a PES-inline filter and a 22G IV standard catheter. Alternatively, a permanent venous catheter or implantable PORT may be used. Prior to infusion the assembly is primed with the diluent. The administration is to be conducted in approximately 60 minutes by IV infusion. A constant infusion rate is achieved by using a microprocessor-controlled infusion pump. Variations in infusion times due to minor differences in IV bag overfill/under fill and institutional procedure on flushing chemotherapy lines will not result in protocol deviation. All infusion times are considered approximate. It is recommended (nut not mandatory) that immediately following the infusion of avelumab, using the same tubing, 25-100 mL normal saline are flushed and infused at the same rate to clear the infusion set of residual drug.

6.1.4 Patient Monitoring During Infusion

During the first infusion patient vital signs should be monitored prior to dosing, approximately 15 minutes after initiation of the infusion (then approximately every 15-20 minutes as indicated), at approximately 60 and 120 minutes after completion of the infusion, or longer if indicated, until the vital signs normalize or return to baseline. For subsequent infusions, vital signs should be collected prior to dosing, approximately every 30 minutes during dosing, and approximately 1 hour post dosing. There is a +/- 30-minute window allowed for all time points that vital signs are to be monitored.

6.1.5 Treatment of avelumab Related Infusion Reactions

Since avelumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgia's, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 5 days to the Pfizer Medical Monitor and reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 4.03) guidelines.

Treatment recommendations for avelumab related infusion reactions, including severe hypersensitivity reactions and tumor lysis syndrome (TLS) are provided below and may be modified based on MD Anderson treatment standards and guidelines, as appropriate:

A. Avelumab Infusion Related Reactions

Symptoms

- Fever
- Chills
- Rigors

- Diaphoresis
- Headache

Management

Table 5. Treatment Modification for Symptoms of Infusion-Related Reactions

NCI-CTCAE Grade	Treatment Modification for Study Drug
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the study drug infusion rate by 50% and monitor closely for any worsening. The total infusion time for study drug should not exceed 120 minutes.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	Stop study drug infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased to at least Grade 1 in severity, and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop the study drug infusion immediately and disconnect infusion tubing from the subject. Subjects have to be withdrawn immediately from study drug treatment and must not receive any further study drug treatment.

IV = intravenous; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; NSAIDs = nonsteroidal anti-inflammatory drugs.

Once the avelumab infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. If the subject has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, the event will be reviewed by and discussed with the PI, and if felt to be in the best interest of the pt, and the pt is receiving benefit, the pt may continue on study and receive avelumab at the 50% lower infusion rate for all subsequent cycles.

If a subject experiences a Grade 3 or 4 infusion-related reaction at any time, the subject must discontinue study drug.

B. Severe Hypersensitivity Reactions and Flu-Like Symptoms

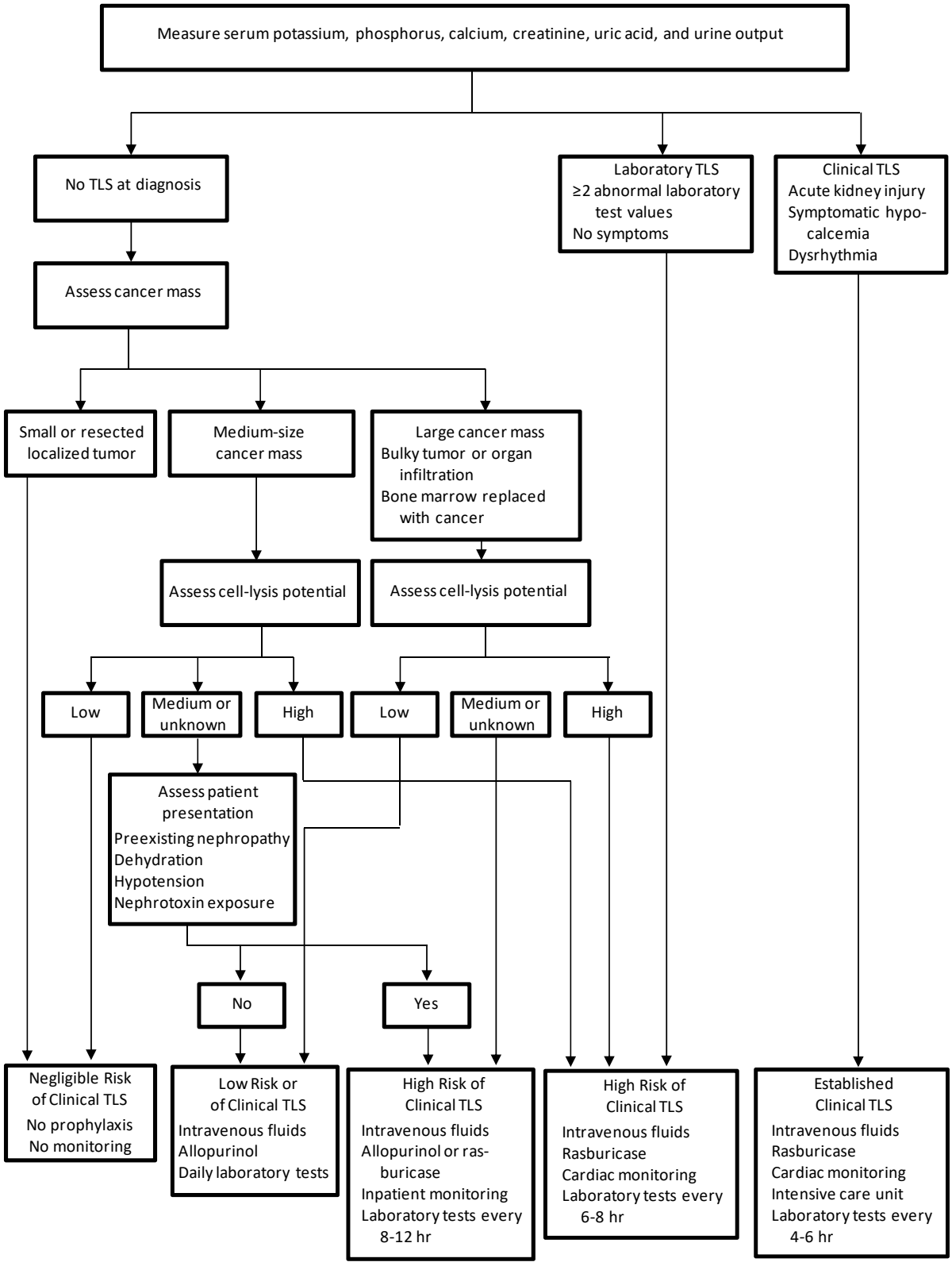
If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice. Subjects should be instructed to report any delayed reactions to the Investigator immediately.

For prophylaxis of flu-like symptoms, 25 mg of indomethacin or comparable nonsteroidal anti-inflammatory drug (NSAID) dose (for example, ibuprofen 600 mg, naproxen sodium 500 mg) may be administered 2 hours before and 8 hours after the start of each dose of avelumab IV infusion. Alternative treatments for fever (for example, paracetamol) may be given to subjects at the discretion of the Investigator.

C. Tumor Lysis Syndrome

In addition, since avelumab can induce antibody-dependent cell-mediated cytotoxicity, there is a potential risk of tumor lysis syndrome. Should this occur, subjects should be treated per the local guidelines and the management algorithm below (Howard 2011).

Assessment and Initial Management of Tumor Lysis Syndrome



TLS = tumor lysis syndrome

For further details regarding dose-calculation of avelumab, preparation and dispensing of avelumab, administration of avelumab, patient monitoring during infusion and treatment of avelumab related infusion reactions please see the Avelumab dosing procedure manual (Appendix H).

6.2 Azacytidine:

5-azacytidine will be commercially obtained. Refer to azacitidine prescribing information (appendix D) in addition to institutional standards for preparation and administration azacitidine.

6.3 Variations in infusion times of avelumab or 5-azacytidine due to minor differences in IV bag overfill/under fill and institutional procedure on flushing chemotherapy lines will not result in protocol deviation. All infusion times are considered approximate.

6.4 Unused or expired avelumab and 5-azacytidine will be safely disposed according to MD Anderson pharmacy standard guidelines.

7.0 PATIENT EVALUATION

Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory assessments completed outside of schedule, occasional missed required research samples such as correlative assays.

7.1 Pre-Treatment Evaluation

All pretreatment studies should be obtained within 14 days of entry into the trial, unless otherwise stated.

7.1.1 A complete history and physical, documentation of all measurable disease, concomitant medications and performance status.

7.1.2 CBC, platelet count, differential (differential can be omitted if WBC is $\leq 0.5 \times 10^9$/L).

7.1.3 Creatinine, total bilirubin, ALT or AST, sodium, potassium, chloride, glucose, uric acid, bicarbonate, BUN, calcium, magnesium, alk phos, direct bili.

7.1.4 Pregnancy test (urine or plasma) in females of childbearing potential should be performed within 72 hours before initiation of protocol therapy.

7.1.5 Bone marrow biopsy/aspirate during the last 28 days preceding study initiation. Cytogenetics will be obtained prior to therapy (results from prior analysis can be used for this purpose).

7.1.6 Pretreatment optional correlative studies (see below)

7.1.7 Electrocardiogram (EKG)

7.1.8 Routine urinalysis

7.1.9 Chest X-ray

7.1.10 TSH, free T3, free T4, free & total cortisol
7.1.11 Respiratory PCR panel

7.2 Evaluation During Treatment

7.2.1 Physical exam at the start of each cycle (± 4 days) and documentation of all concomitant medications.

7.2.2 CBC, platelet count, differential twice weekly (±4 days) for the first 3 cycles, then every 2-4 weeks (differential can be omitted if WBC is $\leq 0.5 \times 10^9/L$)

7.2.3 Creatinine, total bilirubin, ALT, or AST, calcium, magnesium, glucose, BUN, uric acid, fractionated bilirubin, alkaline phosphatase, sodium, potassium, chloride, bicarbonate twice weekly (±4 days) for the first 3 cycles, then every 2-4 weeks.

7.2.4 TSH, free T3, free T4, and free & total cortisol once monthly (±4 days) for the first 3 cycles, then every 3 months (±4 days).

7.2.5 Bone marrow aspiration on day 28 (+/- 7 days), then every 1-4 cycles. Bone marrow tests can be ordered more frequently if mandated by development of peripheral blood counts. No repeat bone marrow is necessary if nonresponse or progressive disease can be unequivocally diagnosed from peripheral blood tests or, in patients with a WBC < 0.3 if the bone marrow test is considered noncontributory by the investigator at any time point.

7.2.6 Concomitant medication data will not be collected or entered into the case report form except for concomitant hydroxyurea; however, the subject's medication record will contain a list of concomitant medications.

Correlative Studies relating to immunologic response (Optional):
Tumor tissue, blood samples and bone marrow aspirate will be collected from patients under Z20160444 and will be sent to Dr. Marina Konopleva's lab at MD Anderson for research

purposes (described below). Patients will be consented separately on this IRB approved consent for collection of additional material. Correlative laboratory studies will be conducted under this clinical trial protocol as described below.

7.2.7 Patients may participate in the clinical study protocol irrespective of whether they choose to participate in the correlative studies. In tumor tissues, immunohistochemical studies will be performed to evaluate tumor and immunological cell markers such as CD4 and CD8 T cells. In peripheral blood, we will also evaluate tumor and immune cell populations including but not limited to CD4 and CD8 T cells in pre and post therapy samples.

Proposed correlative studies to be performed:

- Standard of Care tests done on all AML patients at MDACC: To analyze for the presence of common somatic mutations, we routinely perform a 53-gene/28-gene panel with validated, next-generation platform sequencing on DNA extracted from bone marrow samples in the CLIA-certified molecular laboratory at MD Anderson Cancer Center at pre-treatment and end of study (if available) assessment for all AML patients at MDACC. Multiparametric flow-cytometry for minimal residual disease (MRD) assessment in AML by validated 15 color flow cytometry, cytogenetics, FISH and molecular markers (if mutated at baseline) at baseline and on treatment to assess for MRD will be done routinely at MDACC Hematopathology lab as standard of care. These data will automatically be available for all patients and will allow allow exploration of correlations between specific somatic mutations and/or cytogenetic aberrations and response in AML patients.

- Multi-stain immunohistochemistry and validated immune relevant multiparametric flow-cytometry on bone marrow and peripheral blood at the above mentioned time-points on all patients to: (a) identify the immunophenotype of tumor-infiltrating T-lymphocytes (TIL): CD8+, CD4+ effector, or CD4+ regulatory, (b) determine the quantitative expression of immune ligands on AML blasts, MSCs and (c) determine the quantitative expression of positive and negative co-stimulatory molecules on TILs pre- and post-therapy with the combination. For the analysis of the co-stimulatory ligands on leukemic blasts (defined as CD13+HLADR+CD33+CD38+), we have developed the “AML tumor ligand panel”: 4-1BBL, B7-1, B7-2, ICOSL, PDL-1, PDL-2, OX40L, CD40, CD27L, and CD137L. For the analysis of co-stimulatory molecules on T cells (CD4 T effector cells defined as CD3+CD4+Foxp3- cells; CD4 T regulatory cells defined as CD3+CD4+CD127-Foxp3+ cells, and CD8 T cells defined as CD3+CD8+) we have developed the “AML lymphocyte panel”: 4-1BB, CTLA-4, CD28, ICOS, PD-1, OX40, CD40L, LAG-3 and TIM-3. These studies may enable identification of prognostic and

predictive markers and enable the identification of other immune checkpoints of significance for future checkpoint based therapeutic approaches in AML. These studies may be performed in the Department of Leukemia and Immunotherapy platform using validated MFC (or Cytof panels that we are currently validating) or through Pfizer commercial vendors depending on the more financially viable option.

Peripheral blood (up to 45 mL within 24 hours: about 3 tablespoons) will be collected in patients who consent to the correlative studies for testing of biomarkers at the following time points:
-Baseline (prior to 5-azacytidine dose), on day 14 (prior to avelumab dose #2), and on day 28 (+/- 5) on cycle 1 (done at MD Anderson).
-In cycle 2 blood sample will be obtained on day 14 (prior to Avelumab dose) when possible.
-In cycle 4, 8, and 12 blood samples will be obtained on day 1 (prior to 5-azacytidine dose) and day 14 (prior to avelumab dose) when possible.
All these tests can be +/- 5 days.
-Samples will be collected at progression whenever possible.

Bone marrow samples will be collected in patients who consent to the correlative studies for testing of biomarkers at baseline, at day 28 (+/-5 days), at day 28 (+/- 5 days) of cycles 3, 7, and 11, and at progression (if possible).

All correlative samples are optional.

Missed samples for correlative studies will not constitute protocol deviations.

7.2.8 For patients that remain on study with no significant toxicity for more than 6 months, subsequent evaluations during study may be modified after discussion with the principal investigator. These include a decrease in frequency of bone marrow aspirations to every 6-12 months (or as clinically indicated), correlative studies to every 6-12 months (or suspension of sample collection for correlative studies), other laboratory tests to once every cycle.

7.2.9 ALL treatments with avelumab must be administered at the MDACC outpatient clinic. The first cycle of azacytidine must be administered at the MDACC outpatient clinic. Subsequently, patients will have the option of receiving 5-azacytidine injections or infusions at the MDACC outpatient clinic or local ambulatory treatment center. We do not intend for the subjects to receive avelumab at any time at an outside physician's office. During the first cycle all the laboratory

evaluations will be done at MDACC. Subsequently, the patient may have the laboratory work done at a local clinic and the results reported and filed by the MDACC research nurse for the study. The laboratory work done at the local clinic will be forwarded to the patient's attending physician at MDACC or PI of the study, who will sign off on the labs to verify that the results have been reviewed.

7.2.10 EKG beginning Cycle 2 on Day 1 (+/_5 days) for subsequent cycles.

7.2.11 Urinalysis beginning Cycle 2 on Day 1 (+/- 5 days) for subsequent cycles.

Outside Physician Participation During Treatment

1. MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician. This will be documented in the patient record.
2. A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (**Appendix F**).
3. Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it. Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.
4. A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.
5. Documentation to be provided by the local physician will include progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.
6. The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
7. All follow-up visits will be performed at MDACC.
8. Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.

7.2.12 Patients with an objective response at completion of active study treatment will be followed for survival at MD Anderson Cancer Center (MDACC) every 3 to 6 months for up to 5 years after completion of active treatment and while still on study. If the patient

is unable to return to MDACC the follow-up visits may be conducted via telephone. Every 3-6 months for up to 5 years after last dose of study drug patient will have a physical exam and blood (about 1-2 teaspoons) will be drawn for routine testing. If the patient is unable to return to MD Anderson, they may be called by a member of the research staff and asked how they are feeling.

Table 6. The study assessment schema is shown in tabular form below

Study Period/Cycle	Screening*	Treatment																		End of Study (EOS)[h,i]	Long-Term Follow-Up
		Cycle 1						Cycle 2						Cycle 3					Subsequent Cycles[k]		
Cycle Day		1	8	14	15	22	28	1	8	14	15	22	28	1	8	14	15	22	1	30 Days After Last Dose	Every 3-6 months
Study Day	-14 to 0	1	8		15	22		29	36		43	50		57	64	70	71	78	—		
Complete history	X	X						X						X					X		
Physical examination[a]	X	X						X						X					X	X	
Vital Signs**		X		X				X		X				X		X			X		
Performance status	X	X						X						X					X	X	
Document all measurable disease (if present)	X																				
Concomitant medications[a]	X	X						X						X					X		X
ECG[L]	X							X						X					X		
CBC with differential[b]	X	X	X		X	X		X	X		X	X		X	X		X	X	X	X	
Sodium, Potassium, Chloride, glucose, uric acid, bicarbonate, BUN, Creatinine, total bilirubin, direct bilirubin, calcium, magnesium, ALT, or AST, alk phos[b]	X	X	X		X	X		X	X		X	X		X	X		X	X	X	X	
Routine Urinalysis	X	X						X						X					X		
TSH, free T3, free T4[c], free & total cortisol		X						X						X					X		
Pregnancy test[d]	X																				
Bone marrow aspirate/ biopsy[e]	X						X												X		

Correlative	Screening	Cycle 1				Cycle 2				Cycle 3				Subsequent cycles	EOS
Optional correlatives on blood[f]	X			Day 14	Day 21-28			Day 14		No		No		Day 1 and 14 of cycles 4, 8, 12	If possible
Chest X-ray	X														
Respiratory PCR panel	X														
Optional correlatives on bone marrow[g]	X				C#1 Day 28				C#2 Day 28				C#3Day 28	Day 28 of cycle 6, 12	If possible

[*] Samples collected at screening do not need to be repeated on Day 1 of Cycle 1.
[**]During the first infusion patient vital signs should be monitored prior to dosing, approximately 15 minutes after initiation of the infusion (then approximately every 15-20 minutes as indicated), at approximately 60 and 120 minutes after completion of the infusion, or longer if indicated, until the vital signs normalize or return to baseline. For subsequent infusions, vital signs should be collected prior to dosing, approximately every 30 minutes during dosing, and approximately 1 hour post dosing.
[a]A complete physical examination and documentation of concomitant medications will be done on day 1 of each cycle (+/- 4 days).
[b]The specified labs must be done at least twice weekly (+/- 4 days) for the first 3 cycles, then every 2-4 weeks on subsequent cycles. The labs may be done more frequently than twice a week at the discretion of the treating physician and/or the PI.
[c]TSH, free T3, free T4 will be done at least once monthly (+/- 4 days) after the start of therapy for the first 3 cycles, then every 3 months (+/- 4 days) on subsequent cycles.
[d]Pregnancy test either urine or plasma should be done in women of childbearing potential 72 hours before initiation of protocol therapy.
[e]Bone marrow aspiration must be done within 28-days (+/- 7 days) of initiation of therapy, then Day 28 (+/- 7 days) every 1-4 cycles. Cytogenetics may be used from prior bone marrow analysis if these were not reported on the screening bone marrow.
[f]Correlative studies will be collected on peripheral blood at baseline (prior to 5-azacytidine dose), on day 14 (prior to avelumab), and between days 21 to 28 on cycle 1. Subsequently, peripheral blood will be obtained on days 1 and 14 of cycles 2, 4, 8, 12, and at progression if possible. All these tests can be +/- 5 days. Additional samples may be collected if the disease gets worse.
[g]Correlative studies will be collected on bone marrow at baseline, day 28 (+/-5 days), then on day 28 (+/- 5 days) of cycles 3, 7, 11 and at progression if possible.
[h]EOS visitis include a physical examination, CBC with differential and platelets and a limited chemistry profile (total bilirubin, serum creatinine, SGPT or SGOT). A bone marrow aspiration may be done if non-response or progressive disease cannot be unequivocally diagnosed from peripheral blood.
[i]Subject will be followed for toxicity for at least 30 days after the last protocol treatment. The 30-day follow-up visit (+ or – 3 days) will be scheduled as a

clinic visits for clinical evaluation and physical examinations. If the patient cannot make it to the MDACC clinic for this visit, the required follow up treatment procedures may be done with a local physician and the records forwarded to MDACC. The research nurse will contact the patient by telephone and get a verbal assessment of the patient's condition. The phone conversation will then be documented in the patient's charts.

[j] Every 3-6 months for up to 5 years after last dose of study drug patient will have a physical exam and blood (about 1-2 teaspoons) will be drawn for routine testing. If the patient is unable to return to MD Anderson, they may be called by a member of the research staff and asked how they are feeling.

[k] For patients that remain on study with no significant toxicity for more than 6 months, subsequent evaluations during study may be modified after discussion with the principal investigator. These include a decrease in frequency of bone marrow aspirations to every 6-12 months (or as clinically indicated), correlative studies to every 6-12 months (or suspension of sample collection for correlative studies), other laboratory tests to once every cycle.

[L] Perform at specified time-points and repeat as clinically indicated

Data regarding adverse events will be collected during the study. Protocol specific data will be entered into PDMS/CORe. PDMS/CORe will be used as the electronic case report form for this protocol.. While investigators do not need to report all adverse reactions, they must be recorded. The Principal Investigator will sign and date the AE log per each patient at the completion of each course. Following signature, the AE log will be used as source documentation for the adverse events for attribution.

Treatment may be discontinued for a variety of reasons, including patient withdrawal, investigator decision, and reasons specified by the protocol. Reasons for discontinuation of treatments are described in section 9.0.

8.0 CRITERIA FOR RESPONSE:

Response Criteria for AML

Responses will be assessed by the International Working Group for AML [67]. Responders are patients who obtain a CR, CRi, or PR, with or without cytogenetic response, hematologic improvements, and morphologic leukemia-free state.

Hematologic Improvement (HI): Hematologic response will be assessed by the MDS IWG response criteria (Cheson et al., Blood 2006)

- **Erythroid response (E)** (pretreatment Hgb <11 g/dL)
  Hgb increase by >/=1.5 g/dL

- **Platelet response (P)** (pretreatment platelets <100 x10^9/L)
  Absolute increase of >/=30 x 10^9/L for patients starting with > 20 x 10^9/L platelets
  Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%

- **Neutrophil response (N)** (pretreatment ANC <1.0 x10^9/L)
  At least 100% increase and an absolute increase > 0.5 x 10^9/L

- **Blast response (B)**
  >/=50% reduction in peripheral blood or bone marrow blasts but still >5%

8 DISCONTINUATION OF TREATMENT:

8.1 Discontinuation Criteria for Individual Patients

9.1.1 Patient Withdrawal

Patients may voluntarily withdraw consent to participate in the clinical study at any time and without giving any reason. Their withdrawal will not jeopardize their relationship with their healthcare providers or affect their future care. Patients may also choose to withdraw from study treatment, but agree to remain in the study for follow-up procedures.

9.1.2 Investigator Discontinuation of Patient

The investigator may exercise medical judgment to discontinue study treatment if clinically significant changes in clinical status or laboratory values are noted.

9.1.3 Criteria for Protocol-Defined Required Discontinuation of Treatment

The protocol requires discontinuation of study treatment for the following reasons:

1. Patient requests discontinuation.
2. Unacceptable toxicity that in the opinion of the investigator makes it unsafe to continue therapy.
3. Clinically significant progressive disease.
4. Investigator discretion.

9.1.4 Follow-Up at Treatment Discontinuation or Early Withdrawal

Patients who discontinue treatment for any reason should complete end-of-treatment procedures when possible. End of treatment procedures will include a physical examination, CBC with differential and platelets and a limited chemistry profile (total bilirubin, serum creatinine, SGPT or SGOT). A bone marrow aspiration may be recommended only if non-response or progressive disease cannot be unequivocally diagnosed from peripheral blood. Although treatment will be discontinued at that time, all patients who do not withdraw consent for follow-up, die, or become lost to follow-up, will remain on study for follow-up evaluations. Subject will be followed for toxicity for at least 30 days after the last protocol treatment. The 30-day follow-up visit (+ or – 3 days) will be scheduled as a clinic visits for clinical evaluation and physical examinations. The Subject will then be followed every 3-6 months for up to 5 years after the last dose of study drug. If the patient cannot make it to the MDACC clinic for this visit, the required follow up treatment procedures may be done with a local physician and the records forwarded to MDACC. The research nurse will contact the patient by telephone and get a verbal assessment of the patient's condition. The phone conversation will then be documented in the patient's charts.

9.2 Study Stopping Rules

The principal investigator and MDACC IND office have the right to terminate this clinical study at any time. The principal investigator and MDACC IND office, as appropriate, will be involved in any decisions regarding terminating the study, temporarily suspending enrollment, or stopping ongoing treatment with study treatment.

Reasons for terminating the clinical study or a study site's participation include, but

are not limited to, the following:

- The incidence or severity of an adverse reaction related to treatment in this study or other studies indicates a potential health hazard to patients
- Data recording is significantly inaccurate or incomplete
- Study site personnel are noncompliant with study procedures
- Pattern of noncompliance is observed

9.3 Protocol Violations and Deviations

Protocol violations are defined as significant departures from protocol-required processes or procedures that affect patient safety or benefit potential, or confound assessments of safety or clinical activity. A protocol deviation is a departure from the protocol that does not meet the above criteria. Protocol violations or deviations may be grouped into the following classes:

- Enrollment criteria
- Study activities (missed evaluations or visits) except for those allowed per protocol
- Noncompliance with dose or schedule, including dose calculation, administration, interruption, reduction, or delay; or discontinuation criteria
- Investigational product handling, including storage and accountability
- Informed consent and ethical issues

9 ADVERSE EVENT REPORTING

9.1 Adverse event is any untoward medical occurrence that may present during treatment with a pharmaceutical product but which does not necessarily have a causal relationship with this treatment.

Adverse drug reaction is a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function.

Assessing causal connections between agents and disease is fundamental to the understanding of adverse drug reactions. In general, a drug may be considered a contributory cause of an adverse event if, had the drug not been administered, 1) the event would not have happened at all, 2) the event would have occurred later than it actually did, or 3) the event would have been less severe.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

9.2 Serious Adverse Event Reporting (SAE)

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

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

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

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24**

hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

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

10.5 Investigator Communications with Pfizer

All Serious Adverse Events must be reported to Pfizer Clinical Trial Department

- All SAEs, whether related or unrelated to avelumab and all pregnancies must be reported to Pfizer (by the investigator or designee) within 24 hours.
- All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:
- **SAEs may be submitted directly via email.**

SAE Email Address: **USA.AEReporting@pfizer.com**
SAE Fax Number: **1-866-997-8322**

11.0 STATISTICAL CONSIDERATIONS

This will be a single arm, single center, open label study of avelumab in combination with 5-azacytidine in patients with refractory, relapsed or untreated AML.

11.1 Sample Size

Part a. Lead-in phase IB
The primary objective of the lead-in phase is to determine the MTD and DLT of avelumab in combination with 5-azacytidine in patients with refractory/ relapsed AML during the first cycle (28 days). 12 - 18 participants will be accrued into lead-in part of the study.

Once safety of the MTD has been established, any patients still on study at a dose lower than MTD can be dose escalated up to MTD.

Part b. Phase II

The primary objective of the phase II is to determine the efficacy of avelumab in combination with 5-azacytidine in patients with refractory/relapsed or untreated AML. The efficacy of the combination will be measured by the overall response rate (ORR), defined as CR (complete remission) + CRp (complete remission with incomplete platelet recovery) + CRi (complete remission with incomplete count recovery) + PR (partial response) + morphologic leukemia fre state (MLFS)+HI within 3 months of treatment initiation among adult patients with refractory/ relapsed AML. ORR and toxicity will be monitored simultaneously using the Bayesian approach of Thall, Simon, Estey (1995, 1996) and the extension by Thall and Sung (1998).

Up to 40 additional patients (excluding patients treated at the MTD from the lead-in part) will be recruited for the Phase II part.

Total accrual: 12-18 patients from lead-in + 40 Phase II patients (N= 52 - 58 patients)

11.2 Statistical Design

Part a. Lead-in phase IB
The MTD is defined as the highest dose level with </= 1 out of 6 patients experience a DLT during the first 28 days of treatment. DLT is defined in section 5.2.2. The dosing schema for the combination treatment during the lead in phase is shown in Table 1 in section 5.2.1.
A Toxicity Summary will be submitted to the IND Office Medical Monitor, after the first three evaluable patients have completed their first cycle, and every three evaluable patients thereafter, prior to dose modification, expansion, or phase II initiation.

Part b. Phase II

An Efficacy/Toxicity Summary will be submitted every five evaluable patients during Phase II.

The historical experience in relapsed/refractory AML patient population is that historical responses (CR/CRp/CRi/PR/HI/MLFS with >50% blast reduction) with single agent hypomethylator therapy (5-azacytidine or decitabine) or with hypomethylator combinations (azacytidine+revlimid, decitabine+myelotarg, azacytidine+vorinostat, decitabine+valproate) investigated at MDACC over the last 10 years are 10% (Daver N, Kantarjian H et al., European Hematology Association Conference, June 2016). The target ORR with the experimental treatment is 30%. This regimen of the combination treatment will be considered worthy of further investigation if it elicits an increase in ORR to 30% with acceptable toxicity. A >30% drug-related grade 3/4 toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions above, were constructed that meet the following two conditions,

1) Stop if Prob{ p(ORR,E)<p(ORR,H) + 0.20 | data> 0.975, or

2) Stop if Prob{ p(TOX,E)>0.30| data}>0.975,

where P(ORR, E) and P(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 2.5%) that ORR rate of the combination treatment is greater than the ORR rate of standard treatment by 20.0%. The second condition will stop the study early if excessive therapy-related grade 3/4 toxicity (>30%) is highly probable (i.e., probability >97.5%) for the combination treatment. Monitoring for toxicity and futility will not begin until 5 patients have been evaluated, and cohort size for future evaluations is 5.

The monitoring rule for the toxicity rate, based on these assumptions and monitoring conditions above is found in Table 7. **For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.**

Table 7. Stop accrual if the number of drug-related grade 3/4 toxicities is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	5	10	15	20	25	30	35	40
# patients with toxicities	4-5	7-10	9-15	11-20	13-25	15-30	17-35	Always stop

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 8. **For example, accrual will cease if no patients experience an overall response within 3 months of initiation of therapy in the first 10 patients treated.**

Table 8. Stop accrual if the number with overall response is less than or equal to indicated (i.e., # patients with overall response) among the number of patients evaluated

# patients evaluated	5-10	15	20	25	30	35	40

# patients with overall response	0	0-1	0-2	0-3	0-4	0-5	Always stop

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 9). In order to utilize the software for the design, a beta (0.2, 1.8) was assumed for the experimental treatment response prior distribution and a constant rate of 10% for the standard treatment, respectively. For response stopping criterion, the shif parameter delta was 0.20. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity constant rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the combination treatment was 30% and the true toxicity rate was 30% was 25%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 45% when the true ORR was 30% and 95% when true ORR rate was 10%.

Table 9. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.10	0.9294
	0.20	0.5119
	0.30	0.2029
	0.40	0.0817
	0.50	0.0319
0.20	0.10	0.9300
	0.20	0.5156
	0.30	0.2090
	0.40	0.0887
	0.50	0.0393
0.30	0.10	0.9339
	0.20	0.5429
	0.30	**0.2535**
	0.40	0.1400
	0.50	0.0934
0.40	0.10	0.9512

Table 9. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
	0.20	0.6623
	0.30	0.4486
	0.40	0.3648
	0.50	0.3303
0.50	0.10	0.9803
	0.20	0.8637
	0.30	0.7774
	0.40	0.7436
	0.50	0.7297

Statistical Analysis Plan

All patients who received any dose of the study agent will be included in the analysis for efficacy and safety. Demographic/clinical characteristics (including duration of response) and safety data of the patients will be summarized using descriptive statistics such as mean, standard deviation, median and range. For the primary efficacy analysis, we will estimate the ORR for the combination treatment, along with the 95% credible interval. For the efficacy, a sample size of 40 patients ensures a posterior 95% credible interval for ORR of (0.17, 0.43), if the trial is not terminated early, under the assumption of a 30% ORR. Patients who drop out of the study before completing all the cycles will be treated as "failures" for the primary analysis. ORR during the study period will also be presented with the 95% confidence credible. The association between ORR and patient's clinical characteristics will be examined by Wilcoxon's rank sum test or Fisher's exact test, as appropriate. Toxicity type, severity and attribution will be summarized for each patient using frequency tables.

The distribution of time-to-event endpoints (DFS and OS) including overall survival and progression free survival will be estimated using the method of Kaplan and Meier. Comparisons of time-to-event endpoints by important subgroups will be made using the log-rank tests. Paired t-tests will be used to determine the immunological and molecular changes in the peripheral blood and bone marrow from baseline to the time of response, and to the time of disease progression. Correlation analysis (such as logistic regression analysis) will be conducted to determine the relationship between induction of hypomethylation / DNA damage and clinical response.

A Toxicity Summary will be submitted to the IND Office Medical Monitor, after the first three patients have completed their first cycle of study therapy or administration is

discontinued, whichever comes first, and every three evaluable patients thereafter during the Lead-in Phase IB.

An Efficacy/Toxicity Summary will be submitted every five evaluable patients during **Phase II**.

Statistical analysis of biomarker data: Descriptive statistics including plots, mean, median and standard deviations will be used to summarize data. For continuous outcomes, t-test and ANOVA will be used to compare outcome measures across patient characteristics. Dunnett's and Tukey's test that properly adjust for multiplicity in multiple tests will be implemented. Pairwise comparisons will be performed using pre- and post-therapy samples from each patient . The chi-square test or Fisher's exact test will be used to test the association between two categorical variables such as disease state and performance status. Both univariate and multivariate logistic regressions will be performed to model prognostic factors.

12.0 PROTOCOL ADMINISTRATION

This study will be monitored by the MD Anderson IND Office and a protocol-specific monitoring plan will be followed.

Protocol amendments

Changes to the protocol will be made only when protocol amendments have been signed by the principal investigator and approved by Pfizer and the IRB of the study center.

Archival of data

MD Anderson-IND study must retain all records *indefinitely*.

13.0 REFERENCES

1. Estey EH. Treatment of relapsed and refractory acute myelogenous leukemia. *Leukemia*. 2000;14(3):476-479.
2. Ravandi F, Cortes J, Faderl S, et al. Characteristics and outcome of patients with acute myeloid leukemia refractory to 1 cycle of high-dose cytarabine-based induction chemotherapy. *Blood*. 2010;116(26):5818-5823; quiz 6153.
3. Stanisic S, Kalaycio M. Treatment of refractory and relapsed acute myelogenous leukemia. *Expert Rev Anticancer Ther*. 2002;2(3):287-295.
4. Kantarjian HM. Therapy for elderly patients with acute myeloid leukemia: a problem in search of solutions. *Cancer*. 2007;109(6):1007-1010.
5. Kantarjian H, O'Brien S, Cortes J, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. *Cancer*. 2006;106(5):1090-1098.

6. Buchner T, Berdel WE, Haferlach C, et al. Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: a study by the German Acute Myeloid Leukemia Cooperative Group. *J Clin Oncol*. 2009;27(1):61-69.
7. Giles F, O'Brien S, Cortes J, et al. Outcome of patients with acute myelogenous leukemia after second salvage therapy. *Cancer*. 2005;104(3):547-554.
8. Yang H, Bueso-Ramos C, Dinardo C, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia*. 2013.
9. Zhang L, Gajewski TF, Kline J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. *Blood*. 2009;114(8):1545-1552.
10. Zhou Q, Munger ME, Veenstra RG, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood*. 2011;117(17):4501-4510.
11. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med*. 1999;5(12):1365-1369.
12. Blank C, Brown I, Peterson AC, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. *Cancer Res*. 2004;64(3):1140-1145.
13. Curiel TJ, Wei S, Dong H, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med*. 2003;9(5):562-567.
14. Hirano F, Kaneko K, Tamura H, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res*. 2005;65(3):1089-1096.
15. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. *Cancer Immunol Immunother*. 2007;56(5):739-745.
16. Loos M, Giese NA, Kleeff J, et al. Clinical significance and regulation of the costimulatory molecule B7-H1 in pancreatic cancer. *Cancer Lett*. 2008;268(1):98-109.
17. Gao Q, Wang XY, Qiu SJ, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clin Cancer Res*. 2009;15(3):971-979.
18. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677-704.
19. Liu J, Hamrouni A, Wolowiec D, et al. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. *Blood*. 2007;110(1):296-304.
20. Yang W, Song Y, Lu YL, Sun JZ, Wang HW. Increased expression of programmed death (PD)-1 and its ligand PD-L1 correlates with impaired cell-mediated immunity in high-risk human papillomavirus-related cervical

intraepithelial neoplasia. *Immunology*. 2013;139(4):513-522.
21. Chen X, Liu S, Wang L, Zhang W, Ji Y, Ma X. Clinical significance of B7-H1 (PD-L1) expression in human acute leukemia. *Cancer Biol Ther*. 2008;7(5):622-627.
22. Jones PA, Taylor SM, Wilson VL. Inhibition of DNA methylation by 5-azacytidine. *Recent Results Cancer Res*. 1983;84:202-211.
23. Santi DV, Garrett CE, Barr PJ. On the mechanism of inhibition of DNA-cytosine methyltransferases by cytosine analogs. *Cell*. 1983;33:9-10.
24. Gabbara S, Bhagwat AS. The mechanism of inhibition of DNA (cytosine-5-)-methyltransferases by 5-azacytosine is likely to involve methyl transfer to the inhibitor. *Biochem J*. 1995;307:87-92.
25. Bird AP. The relationship of DNA methylation to cancer. *Cancer Surv*. 1996;28:87-101.
26. Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet*. 1999;21:163-167.
27. Karpf AR, Jones DA. Reactivating the expression of methylation silenced genes in human cancer. *Oncogene*. 2002;21(35):5496-5503.
28. Uchida T, Kinoshita T, Nagai H, et al. Hypermethylation of the $p15^{INK4B}$ gene in myelodysplastic syndromes. *Blood*. 1997;90(4):1403-1409.
29. Herman JG, Latif F, Weng Y, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci U S A*. 1994;91(21):9700-9704.
30. van der Velden PA, Metzelaar-Blok JA, Bergman W, et al. Promoter hypermethylation: a common cause of reduced p16(INK4a) expression in uveal melanoma. *Cancer Res*. 2001;61(13):5303-5306.
31. Dobrovic A, Simpfendorfer D. Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res*. 1997;57:3347-3350.
32. Hiltunen MO, Alhonen L, Koistinaho J, et al. Hypermethylation of the APC (Adenomatous Polyposis Colie) gene promoter region in human colorectal carcinoma. *Int J Cancer*. 1997;70:644-648.
33. Merlo A, Herman JG, Mao L, et al. 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat Med*. 1995;1(7):686-692.
34. Herman JG, Jen J, Merlo A, Baylin SB. Hypermethylation-associated inactivation indicates a tumor suppressor role for p15INK4B. *Cancer Res*. 1996;56(4):722-727.
35. Li LH, Olin EJ, Buskirk HH, Reineke LM. Cytotoxicity and mode of action of 5-azacytidine on L1210 leukemia. *Cancer Res*. 1970;30(Nov):2760-2769.
36. Li LH, Olin EJ, Fraser TJ, Bhuyan BK. Phase specificity of 5-azacytidine against mammalian cells in tissue culture. *Cancer Res*. 1970;30(Nov):2770-2775.
37. Silverman LR. Targeting hypomethylation of DNA to achieve cellular differentiation in myelodysplastic syndromes (MDS). *Oncologist*. 2001;6(suppl 5):8-14.
38. Jones PA, Taylor SM, Wilson V. DNA modification, differentiation, and

transformation. *The Journal of Experimental Zoology*. 1983;228(2):287-295.
39. Leone G, Teofili L, Voso MT, Lübbert M. DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias. *Haematologica*. 2002;87(12):1324-1341.
40. Silverman LR, Holland JF, Demakos EP, et al. Azacitidine (Aza C) in myelodysplastic syndromes (MDS), CALGB studies 8421 and 8921. Ann Hematol. Vol. 68; 1994:A12.
41. Robertson KD, Jones PA. DNA methylation: past, present and future directions. *Carcinogenesis*. 2000;21(3):461-467.
42. Plagemann PG, Behrens M, Abraham D. Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 mouse leukemia cells and their enhancement by preincubation with pyrazofurin. *Cancer Res*. 1978;38(8):2458-2466.
43. Juttermann R, Li E, Jaenisch R. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc Natl Acad Sci U S A*. 1994;91(25):11797-11801.
44. Ueno M, Katayama K, Yasoshima A, Nakayama H, Doi K. 5-Azacytidine (5AzC)-induced histopathological changes in the central nervous system of rat fetuses. *Exp Toxic Pathol*. 2002;54:91-96.
45. Glover AB, Leyland-Jones B, Chun HG, Davies B, Hoth DF. Azacitidine: 10 years later. *Cancer Treat Rep*. 1987;71(7-8):737-746.
46. Pharmion Corporation. Azacitidine Investigator's Brochure, Edition 5. Boulder, CO; 2007.
47. Marcucci G, Silverman L, Eller M, Lintz L, Beach CL. Bioavailability of azacitidine subcutaneous versus intravenous in patients with the myelodysplastic syndromes. *J Clin Pharmacol*. 2005;45(5):597-602.
48. Von Hoff DD, Slavik M, Muggia FM. 5-Azacytidine. A new anticancer drug with effectiveness in acute myelogenous leukemia. *Ann Intern Med*. 1976;85(2):237-245.
49. Silverman LR, McKenzie DR, Peterson BL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the cancer and leukemia group B. *J Clin Oncol*. 2006;24(24):3895-3903.
50. Fenaux P, Mufti G, Santini V, et al. Azacitidine (AZA) treatment prolongs overall survival (OS) in higher-risk MDS patients compared with conventional care regimens (CCR): Results of the AZA-001 phase III study. Blood. Vol. 110; 2007:250a.
51. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8(8):793-800.
52. Bontkes HJ, Ruben JM, Alhan C, Westers TM, Ossenkoppele GJ, van de Loosdrecht AA. Azacitidine differentially affects CD4(pos) T-cell polarization in vitro and in vivo in high risk myelodysplastic syndromes. *Leuk Res*. 2012;36(7):921-930.

53. Youngblood B, Oestreich KJ, Ha SJ, et al. Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T cells. *Immunity*. 2011;35(3):400-412.