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Protocol Page

Phase II combination of lirilumab and nivolumab with 5-azacitidine in patients with myelodysplastic syndromes (MDS)
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Study Chair:	Guillermo Garcia-Manero
Additional Contact:	Shannon L. Bailey Shunice Edwards-Harris
Department:	Leukemia
Phone:	713-745-3428
Unit:	428
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Phase II Combination of Lirilumab and Nivolumab with 5-azacitidine in Patients with Myelodysplastic Syndromes (MDS)

Guillermo Garcia-Manero MD
Department of Leukemia
MD Anderson Cancer Center

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Combination of Lirilumab and Nivolumab with 5-azacitidine in Patients with Myelodysplastic Syndromes (MDS)

1.0 Objectives

Primary: To determine the safety of nivolumab and lirilumab, as single agents or in combination and with 5-azacitidine, in patients with MDS.

Secondary:

1. To explore the clinical activity of nivolumab and lirilumab, as single agents or in combination and with 5-azacitidine, in patients with MDS including response, overall survival and progression free survival

2. To explore the biological activity of these compounds in patients with MDS.

2.0 Background and hypothesis

MDS consists of an heterogeneous group of myeloid malignancies characterized by bone marrow failure and increased risk of transformation to acute myelogenous leukemia (AML) [1]. In general, patients are classified using IPSS criteria[2], and more recently IPSS-R[3], into a lower risk (low, int-1) and higher risk (int-2, high risk) category. The standard of care for most patients with MDS, except for those with an alteration of chromosome 5, is a hypomethylating agent such as 5-azacitdine[1]. Although this class of agents has significant activity in MDS and has been shown to improve survival [1], duration of response is usually limited and the prognosis of patients with HMA failure is extremely poor[4]. Therefore, the need for new therapies and combinations for this group of patients.

Several groups have reported on the expression of Kir ligands and receptors in myeloid leukemias[5]. Our group has also reported up regulation of innate immunity signals and the PD1/PDL1 axis in MDS and the fact that expression is up regulated by treatment with a hypomethylating agent[6,7]. We therefore hypothesized that immune checkpoints maybe involved in resistance to HMA agents and therefore maybe clinically synergistic with HMAs ref. To test these concepts, we propose a study where different cohorts of patients with MDS are treated either with single agent immune checkpoint inhibitor (nivolumab) in combination with an antibody against KIR (lirilumab) and with 5-azacitidine. Data from this pilot trial will allow us to determine the toxicity profile of these agents in MDS and the potential for clinical activity that could then guide larger definitive clinical trials for more specific subsets of patients.

3.0 Nivolumab (BMS-936558, MDX1106)

Mechanism of Action Immune activation is tightly regulated by co-stimulatory (e.g. CD28 and ICOS) and co-inhibitory (e.g. CTLA-4 and PD-1) receptors expressed on T cells. Agonistic antibodies against co-stimulatory T cell receptors and blocking antibodies against co-inhibitory T cell surface receptors have both been shown to potentiate T cell activation for tumor cell killing. PD-1 is mainly expressed by activated CD4+ and CD8+ T cells, as well as antigen presenting cells (APCs). It has two ligands, PD-L1 and PD-L2, with distinct expression profiles. PD-L1 is expressed not only on APCs, but also on non- hematopoietic cells, including tumor cells. Expression of PD-L2 is largely restricted to APCs including macrophages and myeloid dendritic cells, as well as mast cells. The role of PD-1 as a negative regulator of T cells was best demonstrated by the finding that PD-1 deficient mice developed significant autoimmunity with high titers of autoantibodies. Subsequently, blocking antibodies against PD-1 were shown to activate immune responses that resulted in reduction of tumor metastasis and tumor growth in a number of experimental tumor models. Consistent with the immune inhibitory role of PD-1/PD-L1/2 signaling, forced expression of PD-L1 in

murine tumor cell lines allowed increased tumor growth in vivo, which was otherwise kept in check by T cells. The inciting effect of PD-L1 on tumor growth was reversed by blocking PD-L1 with antiPD-L1 antibodies.

Nivolumab (BMS-936558) is a fully human, IgG4 (kappa) isotype, monoclonal antibody that binds PD-1. Blockade of the PD-1 pathway by nivolumab was studied using the mixed lymphocyte reaction (MLR). PD-1 blockade resulted in a reproducible enhancement of both proliferation and IFN-γ release in the MLR. The effect of nivolumab on antigen-specific recall response was investigated using a CMV-restimulation assay with human peripheral blood mononuclear cells (PBMCs), and was evaluated by ELISA. These data indicated that nivolumab, versus an isotype-matched control antibody, augmented IFN-γ secretion from CMV-specific memory T cells in a dose-dependent manner. PD-1 blockade by nivolumab is therefore considered a promising immunotherapeutic strategy.

Summary of Safety Results from Nivolumab Program

For a complete review of clinical information, please refer to the nivolumab Investigator Brochure.

Summary of Safety

The monitoring of subject safety during and after a clinical study with nivolumab, including any special monitoring precautions, tests, or observations, and the proper means of recording and reporting adverse safety information [including adverse events (AEs) and abnormal laboratory values] will follow the procedures outlined in the specific study protocol.

The overall safety experience with nivolumab is based on experience in approximately 1500 subjects as either a monotherapy or in combination with other therapeutics. In general for monotherapy, the safety profile is similar across tumor types. The one exception is pulmonary inflammation AEs which may be numerically

greater in subjects with non-small cell lung cancer (NSCLC) possibly because in some cases it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. The most frequently reported treatment-related AE is fatigue, which is almost always low grade.

The safety profile is generally consistent across completed and ongoing clinical trials with no maximum tolerated dose (MTD) reached at any dose tested up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. Most related AEs are thought to be due to the effects of inflammatory cells on specific tissues. Most AEs were low-grade (Grade 1 to Grade 2) with relatively few related high-grade (Grade 3 to Grade 4) AEs. Most high-grade events were manageable with use of corticosteroids or hormone replacement therapy (endocrinopathies).

Clinical Safety in Advanced Malignancies (Nivolumab Monotherapy)

A total of 306 subjects with treatment-refractory malignancies have been treated in an ongoing, Phase 1 multidose study (MDX1106-03, CA209003). This is an ongoing phase I dose-escalation study of nivolumab monotherapy in patients with advanced cancers; 1, 3, or 10 mg/kg nivolumab and 0.1 and 0.3 mg/kg (included as part of Amendment 4) administered by IV Q2W; treatment up to 2 years. Results were published by Topalian et al. (NEJM 2012). The baseline disease diagnosis by treatment for MDX1106-03 is provided in Table 1. A review of the safety data by tumor type (RCC, NSCLC, mCRPC, CRC, and melanoma) did not show any clinically meaningful differences in the proportion of subjects with AEs noted across tumor type.

Table 1: Baseline Disease Diagnosis by Treatment - MDX1106-03

	No. of Subjects					
Nivolumab (mg/kg)	**0.1 mg/kg**	**0.3 mg/kg**	**1 mg/kg**	**3 mg/kg**	**10 mg/kg**	**TOTAL**
MDX1106-03, Total N	**17**	**18**	**86**	**54**	**131**	**306**

NSCLC	0	0	33	37	58	128
Melanoma						
RCC	0	0	18	0	16	34
mCRPC	0	0	0	0	17	17
CRC	0	0	0	0	19	19

Abbreviations: CRC: colorectal adenocarcinoma; mCRPC: metastatic castration-resistant prostate cancer; NSCLC: non-small cell lung cancer; RCC: renal cell carcinoma;
Source: Preliminary data, CA209003. Clinical data cut-off date 18 Mar-2013.

Adverse Events

There was no pattern in the incidence, severity, or causality of AEs related to the dose of nivolumab, between 1 and 10 mg/kg, in MDX1106-03. Of the 306 treated subjects in MDX1106-03, 303 (99.0%) subjects have at least 1 reported AE regardless of causality (Table 2). The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%). Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. **Treatment-related Grade 3-4 AEs were reported in 52 (17.0%) of subjects.** The most frequently reported treatment-related high grade AE was fatigue (6.5%).

Table 2: Summary of Adverse Events Reported in ≥15% of All Treated Subjects-MDX1106-03

Preferred Term	**No. of Subjects (%)**			
	AEs regardless of causality		**Treatment-related AEs**	
	Any Grade N=306	**Grade 3-4 N=306**	**Any Grade N=306**	**Grade 3-4 N=306**
Any AE	**303 (99)**	**127 (42)**	**230 (75)**	**52 (17)**
Fatigue	168 (55)	20 (7)	86 (28)	7 (2)
Decreased appetite	107 (35)	3 (1)	28 (9)	1(0.3)

Diarrhea	105 (34)	3 (1)	41 (13)	3 (1)
Nausea	92 (30)	9 (3)	27 (9)	2 (1)
Cough	90 (29)	4 (1)	11 (4)	1 (0.3)
Dyspnea	80 (26)	27 (9)	11 (4)	0
Constipation	78 (26)	2 (1)	5 (2)	0
Rash	74 (24)	0	45 (15)	0
Vomiting	70 (23)	7 (2)	10 (3)	1 (0.3)
Back pain	68 (22)	7 (2)	3 (1)	1 (0.3)
Arthralgia	63 (21)	4 (1)	15 (5)	0
Pyrexia	61 (20)	1 (0.3)	17 (6)	0
Headache	59 (19)	1 (0.3)	8 (3)	0
Edema peripheral	59 (19)	1 (0.3)	3 (1)	0
Dizziness	56 (18)	1 (0.3)	10 (3)	0
Pruritus	56 (18)	1 (0.3)	32 (11)	1 (0.3)
Weight decreased	48 (16)	1 (0.3)	11 (4)	0
Malignant neoplasm progression	4 (1)	1 (0.3)		

Select Adverse Events

Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressant's and/or endocrine replacement therapy) include: GI AEs, pulmonary AEs, renal AEs, hepatic AEs, skin AEs, and endocrinopathies. In addition, Select AEs include a category for infusion reactions. Each category is composed of a discrete set of preferred terms, including those of greatest clinical relevance. These Select AEs are considered events of interest based on the mechanism of action and were previously referred to as immune-related AEs or immune-mediated AEs. The frequencies of these events are summarized in investigator brochure (Appendix A). The 10-mg/kg cohort had numerically greater frequency of high-grade select AEs including the subcategories of endocrinopathies, GI, pulmonary, and infusion reactions.

Adverse Events Leading to Discontinuation

At least 1 treatment-related AE leading to discontinuation was reported in 32 (10.5%) of

the 306 treated subjects. Grade 3-4 treatment-related events were reported in 14 (4.6%) subjects. The frequency of treatment-related AEs leading to discontinuation was not associated with the dose of nivolumab. Pneumonitis was the most common treatment-related AE leading to discontinuation (8 subjects, 2.6%); pneumonitis reported in 3 (1.0%) subjects was Grade 3-4. Treatment-related AEs reported in at least 2 subjects included pneumonitis (8 subjects, 2.6%), colitis (3 subjects, 1.0%) and myalgia, hepatitis, hypersensitivity, and infusion-related reactions (each reported in 2 subjects, 0.7%). One event of Grade 5 sepsis was reported for 1 subject, a 62-year-old male treated with 1 mg/kg nivolumab.

Deaths

As of 18-Mar-2013, 195 deaths have been reported in MDX1106-03 during the course of the study or within 30 days of last dose of study drug. The majority of the deaths were considered secondary to disease progression and malignant disease.

Three subjects in MDX1106-03 died after developing pneumonitis. A 62-year-old male (MDX1106-03-1-699) with NSCLC (adenocarcinoma) in the 1 mg/kg treatment group and a 59-year old male (MDX1106-03-1-3582) with CRC in the 10 mg/kg treatment group both died due to Grade 5 sepsis after having developed Grade 4 pneumonitis. Sepsis and pneumonitis were considered related to study drug by the investigator in both of these cases. In addition, a 40-year- old female (MDX1106-03-5-710) with NSCLC (adenocarcinoma) in the 1 mg/kg treatment group died due to respiratory failure after having developed Grade 4 pneumonitis and tumor progression. In this case, respiratory failure and pneumonitis were considered related to study drug by the investigator.

Additional deaths reported as due to “other” in MDX1106-03 included:

- MDX1106-03-2-3436 (10 mg/kg): Ischemic cardiomyopathy
- MDX1106-03-4-3484 (10 mg/kg): Death due to abdominal pain caused by superior mesenteric vein thrombosis

- MDX1106-03-3-674 (1 mg/kg): Progressive lung cancer

Summary of Efficacy (Nivolumab monotherapy)

The clinical activity data presented below are from MDX1106-03 (nivolumab monotherapy).

Table 3: Objective Response Rate and Progression Free Survival 24 Weeks Rate in Melanoma Subjects - MDX1106-03

Dose (mg/kg)	N	ORR	PFSR at 24 weeks (%)
All Melanoma	**107**	**33 (31)**	**44**
0.1	17	6 (35)	39
0.3	18	5 (28)	33
1.0	35	11 (31)	51
3.0	17	7 (41)	55
10.0	20	4 (20)	35

Abbreviations: ORR: objective response rate, PFSR: progression-free survival rate
Source: Preliminary data, MDX1106-03. Clinical cut-off date: 18-Mar-201

Table 4: Objective Response Rate per RECIST 1.0 and Progression Free Survival 24 Weeks Rate by Histology in Non-small Cell Lung Cancer Subjects - MDX1106-03

Dose (mg/kg)	N	Histology	ORR No. of Subjects (%)	PFSR at 24 weeks (%)
All NSCLC	**129**	**NA**	**22 (17)**	**34**
1.0	15	SQ	0	36
	18	NSQ	1 (6)	19
3.0	18	SQ	4 (22)	45
	19	NSQ	5 (26)	42
10.0	21	SQ	5 (24)	45
	37	NSQ	7 (19)	25

Abbreviations: NA: not applicable, NSCLC: non-small cell lung cancer; NSQ: non-squamous, ORR: objective response rate; PFSR: progression free survival rate, SQ: squamous
Source: Preliminary data, MDX1106-03. Clinical cut-off date 18- Mar-2013.

4.0 Lirilumab (IPH2102/BMS-986015)

Lirilumab (BMS-986015, IPH2102) is a fully human IgG4 monoclonal antibody (mAb) that is specific for a subset of human KIRs. Lirilumab is a mAb that blocks the KIR/HLA interaction, and lowers the threshold for activation of NK cells without directly activating NK cells. Once activated, NK cells release preformed cytotoxic granules into the target cell leading to direct killing of cancer cells. The concurrent release of cytokines and chemokines also results in a micro-environmental milieu that recruits other immune cells.

Lirilumab is the second anti-KIR antibody. IPH2101 is the first anti-KIR antibody. IPH2101 and lirilumab have identical target specificities. The differences are that IPH2101 is a native IgG4 antibody produced from a hybridoma cell line, whereas lirilumab has a single amino acid mutation to stabilize the hinge region of the molecule and is produced by Chinese hamster ovary (CHO) cells. No additional clinical trials will be conducted with IPH2101. Lirilumab is being developed for treatment of patients with hematologic and solid tumor malignancies. Lirilumab is currently being tested in 4 clinical trials and will be used in all subsequent trials.

Evidence in support of NK cell involvement in the anti-tumor response comes from the hematopoietic stem cell transplant setting. Given the diversity of both KIR and HLA, it is not surprising that KIR on donor NK cells may not recognize host HLA, referred to as KIR mismatch. The finding that patients with acute myeloid leukemia (AML) transplanted with KIR-mismatched donor NK cells had lower relapse rates (3% versus 47%, $p < 0.01$) and reduced risk of relapse (relative risk: 0.48, 95% confidence interval (CI) [0.29, 0.78]) compared to patients transplanted with KIR-matched donor NK cells gave scientific support for the role of NK cells in the anti-tumor response[8].

Lirilumab binds specifically and with high affinity to a subset of KIR, namely KIR2DL-1, 2, and 3 and KIR2DS-1 and 2, thus preventing interaction between KIR and HLA-C.

Surface plasmon resonance analysis demonstrated that the mean (standard deviation [SD]) monovalent affinity of lirilumab for recombinant soluble KIR2DL1 was 2.04 □ 10-8 (0.31 □ 10-8) M and for KIR2DL3 was 3.01 x 10^{-10}($0.41x10^{-10}$) M.

The scientific rationale for the clinical development of lirilumab was based on the following findings:

1) Blockade of inhibitory KIR resulted in killing of AML blasts by KIR-mismatched NK cells but not by KIR-matched NK cells in vitro.
2) NOD/SCID mice infused with AML cells and NK cells died of disease within†60 days. In contrast, all mice treated with KIR blockade survived at Day 75 ($p < 0.01$)[9].

Lirilumab also is being developed in combination with the T-cell checkpoint inhibitors, ipilimumab and nivolumab. Nonclinical studies combining anti-Ly49 (5E6 F(ab')2), the murine functional homolog of lirilumab, with the mAbs specific for the murine versions of either cytotoxic T-lymphocyte antigen 4 (CTLA-4) or programmed cell death 1 (PD-1) demonstrated enhanced anti-tumor efficacy.

Lirilumab does not bind to NK cells from non-human primate or other species traditionally used for safety testing. Safety testing was performed in mice because Ly49C/I, the murine inhibitory receptors, are functionally homologous to human KIR. Mice treated with lirilumab at 10 mg/kg once weekly for 4 weeks, or the surrogate anti-Ly49 (5E6 F(ab')2) at up to 10 mg/kg twice weekly for 13 weeks, showed no signs of toxicity.

Lirilumab is being developed for immunotherapy in patients with various hematologic malignancies and solid tumors. A total of 100 subjects have been treated in 4 ongoing clinical trials assessing safety, pharmacokinetics, biomarker modulation, and clinical activity. The first (IPH2102-101) is a monotherapy, dose escalation, Phase 1 trial to determine the maximum tolerated dose (MTD). The second is a double-blind, placebo-

controlled, Phase 2 trial of lirilumab in patients with AML who are in complete remission but ineligible for allogeneic transplant. One-third of subjects in this study will be receiving placebo. The third (CA223001) and fourth (CA223002) are Phase 1 trials of lirilumab in combination with either the anti-PD1 antibody nivolumab or the anti-CTLA-4 antibody ipilimumab, respectively, to determine if coordinate modulation of the innate and adaptive immune systems results in greater clinical benefit.

As of 29-Jul-2013, the majority of adverse events (AEs) in these 4 ongoing trials were mild or moderate (Grade 1 or 2), self-limiting, and manageable. The most common related AEs were asthenia, fatigue, pruritus, infusion-related reaction, chills, and headache. Only 2 serious adverse events (SAEs) (abnormal liver function tests and iridocyclitis) that were related to study treatment were reported. Blockade of inhibitory KIR by lirilumab is thus a promising mechanism to promote killing of tumor cells by the innate immune system.

5.0 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 (eg, thalassemia and sickle cell anemia), and MDS. In 1984, the Cancer and Leukemia Group B (CALGB) began a series of clinical studies with 5-azacytidine in patients with MDS. These studies, in addition to other supportive data, led to the approval of Vidaza® (5-azacytidine) in May 2004 for the treatment of MDS.

5-azacytidine inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT). Increased methylation of DNA (hypermethylation) may result in the silencing of critical genes responsible for cell growth control and differentiation. Hypermethylation of CpG islands spanning the promoter regions of tumor suppressor genes is commonly associated with cancers. It is now widely recognized that hypermethylation of DNA is frequently associated with myelodysplastic syndromes and other cancers, such as renal, melanoma, breast, colorectal, non-small cell lung and hematologic malignancies. 5-azacytidine is believed to exert its antineoplastic effects through hypomethylation and cytotoxicity on abnormal hematopoietic cells in the bone marrow.[10-14] Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of 5-azacytidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms.

The cytotoxicity of 5-azacytidine is proportional to dose and exposure time. Although the mechanisms of cytotoxicity are complex and multifaceted, there is general agreement that incorporation of 5-azacytidine into DNA and ribonucleic acid (RNA), and inhibition of protein synthesis, are critically important. Cytotoxicity is greatest in cells that are proliferating (S phase) and metabolically active.[10] Cytotoxic effects may also be mediated through induction of the DNA damage response pathways. Nonproliferating cells are relatively insensitive to 5-azacytidine.

Toxicology studies have been conducted in mice, rats, dogs, and Rhesus monkeys. Most of the studies were performed during the 1970s and early 1980s according to existing guidelines and standards in place during that period. The preclinical studies identified the bone marrow, liver, kidneys, and lymphoid tissues (spleen, lymph nodes, and thymus) as the main target organs of toxicity for 5-azacytidine.[15] In single-dose studies, the lethal dose of 5-azacytidine after intravenous (IV) administration in mice, rats, and dogs was approximately 250 mg/m^2. Repeated daily dosing appears to increase the toxicity of 5-azacytidine. The genotoxicity of 5-azacytidine is consistent

with that of other nucleoside analogs that interact with nucleic acids. Likewise, similar to other agents with cytostatic properties, 5-azacytidine was embryotoxic and reduced the reproductive performance in mice and rats.

Limited 5-azacytidine pharmacokinetic data are currently available. Based on human plasma concentrations of total radioactivity (which represents parent drug plus circulating metabolites), 5-azacytidine is rapidly absorbed when given subcutaneously (SC), with maximum plasma concentrations found 0.5 to 2 hours after dosing.[15] 5-azacytidine and/or its by-products are then rapidly cleared by the kidneys. The half-lives and percent radioactivity recovered in urine are similar following IV and SC routes of administration. The effects of renal or hepatic impairment, gender, age, or race on the pharmacokinetics of 5-azacytidine have not been studied. A single dose (75 mg/m^2) SC versus IV crossover study in 6 MDS subjects revealed an approximate bioavailability of 89% for the SC dose (range 52% to 128%) with mean half-lives of 0.69 hour and 0.36 hour after SC and IV administration, respectively. These results demonstrated that 5-azacytidine is rapidly and nearly completely absorbed after SC administration and that elimination is also rapid. The apparent SC clearance (167 L/h or 2791 mL/min) and systemic IV clearance (147 L/h) of 5-azacytidine exceeded the glomerular filtration rate (approximately 125 mL/min) and total renal blood flow (1200 mL/min) in healthy subjects. This indicates that non-renal elimination (eg, metabolism, hydrolysis, and/or degradation) plays a role in the elimination of parent 5-azacytidine. In addition, 5-azacytidine 75 mg/m^2 was generally well-tolerated after single SC injection or IV infusion over 10 minutes.

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

During the two decades between the start of the CALGB studies and the approval of 5-azacytidine, a new understanding of MDS has developed, such as the World Health

Organization (WHO) diagnostic criteria, the International Prognostic Scoring System (IPSS), and the International Working Group (IWG) response criteria. Silverman et al. reevaluated the combined data (N = 309) from 3 of the CALGB studies using the WHO classification system for MDS and AML and the IWG response criteria.[16] 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.[16]

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.[17] Patients were randomized to 5-azacytidine (75 mg/m^2/day x 7days in 28 day cycles) or conventional care regimens (CCR), where CCR was pre-selected by the Investigator as best supportive care (transfusions, antibiotics, and G-CSF for neutropenic infection), low-dose cytarabine (20 mg/m^2/day x 14 days in 28 day cycles); or standard chemotherapy (conventional induction/consolidation). Patients were stratified by FAB/IPSS and randomized 1:1 to 5-azacytidine or CCR. This trial did not allow erythropoietin. Three hundred fifty eight patients (70% male) were randomized at 79 centers to 5-azacytidine (N=179) or CCR (N=179): best supportive care only (N=105, 59%), low-dose cytarabine (N=49, 27%), or standard chemotherapy (N=25, 14%). Median age was 69 years (range 38-88 years). The 5-azacytidine and CCR groups were comparable for baseline patient characteristics. At baseline, 95% of patients were higher risk: RAEB (58%), RAEB-T/WHO AML (34%), CMML (3%), and other (5%). By IPSS, 87% were higher risk: Intermediate -2 (40%), High (47%), and 13% Indeterminate/other. 5-azacytidine was administered for a median of 9 cycles; low-dose cytarabine for 4 cycles. Median follow-up for the survival analysis was 21.1 months. 5-azacytidine demonstrated statistically

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

Further details can be found in the 5-azacytidine Investigator's Brochure (Appendix B), which contains comprehensive pharmacology, toxicology, pharmacokinetics, pharmacodynamics, metabolism, preclinical, and clinical efficacy and safety data information.

6.0 Study Population

Inclusion Criteria

1. Patients with MDS (up to 20% blasts) of any risk. Patients with lower risk MDS (low and int-1 by IPSS) could have received prior non-hypomethylating agent therapy (ie growth factors or lenalidomide). Patients with higher risk MDS (int-2 or high by IPSS) should not have received prior therapy with a hypomethylating agent.

2. Age 18 years or older.

3. Adequate organ function: creatinine ≤2.5 x ULN; serum bilirubin ≤2.5 x ULN; AST and ALT ≤2.5 x ULN.

4. ECOG performance status ≤2

5. Females of childbearing potential must have a negative serum or urine beta human chorionic gonadotrophin (β-hCG) pregnancy test result within 24 hours prior to the first dose of treatment and must agree to use an effective

contraception to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug. Females of non- childbearing potential are those who are postmenopausal greater than 1 year or who have had a bilateral tubal ligation or hysterectomy.

6. Males who have partners of childbearing potential must agree to use an effective contraceptive method during the study and for 31 weeks after the last dose of nivolumab.

7. Patients or their legally authorized representative must provide written informed consent.

Exclusion Criteria

1. History of another primary invasive malignancy that has not been definitively treated or in remission for at least 2 years. Patients with non-melanoma skin cancers or with carcinomas in situ are eligible regardless of the time from diagnosis (including concomitant diagnoses).

2. Any major surgery, radiotherapy, chemotherapy, biologic therapy, immunotherapy, experimental therapy within 2 weeks prior to the first dose of the study drugs.

3. Patients with any other known concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes; cardiovascular disease including congestive heart failure NYHA Class III or IV, myocardial infarction within 6 months, and poorly controlled hypertension; chronic renal failure; or active uncontrolled infection) which, in the opinion of the investigator could compromise participation in the study.

4. Patients unwilling or unable to comply with the protocol.

5. Patients who are on high dose steroid (ie prednisone or equivalent more than 10 mg a day) or immune suppression medications.

6. Patients with autoimmune diseases (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], Systemic Lupus Erythematosus, autoimmune vasculitis [e.g., Wegener's Granulomatosis]).

7. Patients with a history of Inflammatory Bowel Disease such as Crohn's disease and ulcerative colitis

8. Patients known to be positive for hepatitis B surface antigen expression or with active hepatitis C infection (positive by polymerase chain reaction or on antiviral therapy for hepatitis C within the last 6 months) or with a history of HIV disease.

9. Current therapy with other systemic anti-neoplastic or anti-neoplastic investigational agents.

10. Females who are pregnant or lactating

11. Prior treatment with stem cell transplantation.

12. Prohibited Prior Treatments and/or Therapies:
    a. Prior therapy with an anti-KIR, anti-PD-1, or anti-PD-L1, antibody.
    b. Prior treatment regimens with any immune cell modulating antibody such as anti-CD137 and anti-OX40. However, prior anti-CTLA4 therapy is allowed if the last dose is 101 days or more from the first dose of study drug.
    c. Exposure to any other investigational drug within 2 weeks prior to the first dose of study drug (within 101 days for anti-CTLA4 therapy).

d. Any anti-cancer therapy (e.g., chemotherapy, biologics, vaccines, radiotherapy with curative intent, or hormonal treatment) within 2 weeks prior to the first dose of study drug administration (within 101 days for anti-CTLA4 therapy administration.
e. Use of non-oncology vaccines containing live virus for prevention of infectious diseases within 4 weeks prior to study drug. The use of the inactivated seasonal influenza vaccine (Fluzone®) is allowed.
f. Systemic corticosteroid at immunosuppressive doses (> 10 mg/day of prednisone or equivalent), must be discontinued at least 2 weeks prior to enrollment

7.0 Treatment Plan

We plan to study 2 different patient groups (lower risk MDS [defined as low and int-1 by IPSS] and higher risk [defined as int-2 and high risk by IPSS]) with 2 cohorts each. Each cohort will be composed of N=20 patients. Accrual to cohort will be sequential in each patient group. Accrual will be parallel for each patient group.

All treatments can vary +/- 3 days.

1. *Lower Risk MDS cohorts (Low and Int-1):*
    - Cohort 1: Lirilumab single agent Q4 wks.
    - Cohort 2: Lirilumab Q4 weeks+ Nivolumab, Q2 wks X 9 cycles then both Q4 wks.
2. *Higher Risk MDS cohorts (Int-2 and High):*
    - Cohort 3: 5-azacitidine IV, D1-7 of 28 day cycle, in combination with Lirilumab IV on D7

- Cohort 4: 5-azacitidine IV, D1-7 of 28 day cycle, in combination with Lirilumab IV Day 7 and Nivolumab IV D7 & D21 X 9 cycles then D7 only on Cycle 10 +

Specific cohorts can be closed based on ongoing experience with this trial or other relevant information**.**

Patients will receive study drug(s) according to the following treatment plan. Individual minor variations in the initiation of therapy, are acceptable as indicated by patient condition and physician judgment. These variations should be documented in the patient's medical record.

Dosing calculations for Nivolumab and 5-Azacitidine will be based actual body weight.

Single agent:

Lirilumab: Patient will receive lirilumab 3 mg/kg IV over approximately 60 minutes every 4 weeks. One cycle is defined as 4 weeks.

Combination:

Lirilumab and Nivolumab: Patient will receive Nivolumab 3 mg/kg IV over approximately 60 minutes every 2 weeks for 9 cycles. Lirilumab will be administered at 3 mg/kg IV over approximately 60 minutes once every 4 weeks. One cycle will be defined as 4 weeks.

Azacitidine+ lirilumab: Azacitidine 75 mg/m^2 IV daily x 7 days and lirilumab 3 mg/kg IV over approximately 60 minutes on day 7. A cycle will be considered 4 weeks.

Azacitidine+ lirilumab + Nivolumab: Azacitidine 75 mg/m^2 IV daily x 7 days followed on day 7 by nivolumab and lirilumab and nivolumab also on day 21. A cycle will be

considered 4 weeks. Doses of nivolumab and lirilumab are 3 mg/kg IV each over approximately 60 minutes.

Dose Adjustments

Based on the adverse effect profiles of the 3 agents, the following dose adjustments are recommended. Hematological toxicities will be attributed primarily to Azacitidine and preferentially will be dose reduced (Table 1). Specific non-hematological toxicities will be attributed primarily to NIVOLUMAB OR LIRILUMAB. No dose reductions of these two agents are allowed, see below.

Table 1: Dose Adjustments for Grade 3 – 4 Hematological Toxicity

	Azacitidine IV (x 7 days)
Level – 1	50 mg/m^2/day
Level – 2	25 mg/m^2/day

Nivolumab and Lirilumab Dose Modification

Nivolumab/ Lirilumab Dose reductions or dose escalations are not permitted.

Dose Delay Criteria:

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories.

Dose delay criteria apply for all drug-related adverse events

Nivolumab & Lirilumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
    - However, dose delays should occur for the following laboratory

abnormalities:
  - G2 Creatinine, or
  - G2 ALT, AST or total bilirubin
- Any Grade 3 skin, drug-related AE
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:
  - Grade 3 lymphopenia or leukopenia does not require dose delay.
  - Grade 3 AST, ALT or total bilirubin requires permanent discontinuation of nivolumab
- Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Criteria to Resume Treatment:

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 before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

Management Algorithms:

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab & Lirilumab are considered immuno-oncology agents in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity. Management algorithms have been developed to assist investigators in assessing and managing the following groups of AEs:

Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, Neurological.

For subjects expected to require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider recommendations provided in the algorithms. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

Discontinuation Criteria:

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment

- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction **<u>of any duration</u>** requires discontinuation

    - Grade 3 drug-related laboratory abnormalities do not require treatment

discontinuation except those noted below:

- Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation

- Grade 3 drug-related ALT, AST, or total bilirubin require discontinuation

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.

  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

- Any dosing interruption lasting > 6 weeks with the following exceptions:

  - 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 > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted

  - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as

per protocol even if dosing is interrupted

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

Supportive Care

Patients will receive a 5-HT3 antagonist, or any other antiemetics at the discretion of the treating physician, prior to each dose of Azacitidine to prevent nausea and emesis.

Routine supportive measures for leukemia patients such as analgesics, blood transfusions, and antimicrobials are permitted. Prophylactic antimicrobials may be prescribed at the discretion of the treating physician.

Patients aged 50 or older may receive their first course of therapy in the Protected Environment if preferred by the treating physician.

Hematopoietic colony stimulating factors may be used at the discretion of the treating physician to provide optimal patient care.

The administration of other anticancer therapies, other investigational agents, or erythropoetic agents is not permitted.

Hydroxyurea can be used in patients with rapidly proliferative disease at the discretion of the treating physician. That said, concomitant use of hydroxyurea with 5-azacitidine will not be recommended.

Treatment may continue until:

1. Clinically significant progressive disease, or

2. Intercurrent illness that prevents further administration of treatment, 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.

8.0 Study Medications

For treatment visits where both Lirilumab and Nivolumab are administered, Nivolumab will be administered first followed by Lirilumab within 30 minutes after completion of the Nivolumab.

8.1 Nivolumab (Anti-PD1)

Nivolumab is a fully human, IgG4 (kappa) isotype, monoclonal antibody that binds PD-1. Nivolumab will be supplied in vials of 100 mg (10 mg/mL) and packaged in an open-label fashion. Ten nivolumab vials (each 10 mL) will be packaged within a carton. 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.

PRODUCT INFORMATION TABLE:

Product Description:(Other names = MDX-1106, ONO-4538, anti-PD-1)

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/ Label Type	Secondary Packaging (Qty)/Label Type	Appearance	Storage Conditions (per label)

Nivolumab (BMS-936558-01)* Injection drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL	100 mg/Vial (10 mg/mL)	Carton of 5 or 10 vials	10-cc Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals.	Clear to opalescent, colorless to pale yellow liquid. May contain particles	**BMS-936558-01 Injection** must be stored at 2 to 8 degrees C (36 to 46 degrees F) and protected from light and freezing

*Nivolumab may be labeled as BMS-936558-01 Solution for Injection

8.1.1 Dose Calculation of nivolumab

Total dose should be calculated as in the following example:

Subject's actual body weight in kg x 3 mg = total dose in mg

Therefore, a subject weighing 70 kg who is to receive a dose of 3 mg/kg would be administered 210 mg of nivolumab (70 kg x 3 mg/kg = 210 mg).

8.1.2 Preparation and Dispensing of Nivolumab

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. If concerns regarding the quality or appearance of the study drug arise, do not dispense the study drug and contact the sponsor immediately.

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).

Nivolumab vials must be stored at a temperature of 2°C to 8°C and should be protected from light. If stored in a glass front refrigerator, vials should be stored in

the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

For details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the Investigator Brochure section 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 nivolumab and polyolefin bags have been observed.

Nivolumab is to be administered as a 60 minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be below 0.35 mg/ml. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

8.1.3 Administration of nivolumab

Patients will receive nivolumab as a 60 minute IV. 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.

Patients may be dosed no less than 12 days from the previous dose of drug.

The dosing calculations should be based on the actual body weight. If the subject’s weight on the day of dosing differs by > 10% from the weight used to calculate the original dose, the dose must be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed

8.1.4 Patient Monitoring During Infusion

Patient vital signs should be monitored prior to dosing, about 15 minutes after initiation of the infusion (then every 15-20 minutes as indicated), at 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, every 30 minutes during dosing, and 1 hour post dosing. All vital sign times will be +/- 10 mins.

8.1.5 Treatment of nivolumab Related Infusion Reactions

Since nivolumab 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, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to the BMS Medical
Monitor and reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 4.0) guidelines.

Treatment recommendations for nivolumab related infusion reactions are provided below and may be modified based on MD Anderson treatment standards and guidelines, as appropriate:

For Grade 1 symptoms (Mild reaction; infusion interruption not indicated; intervention not indicated):Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g. antihistamines,

non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours):

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen 325 to 1000 mg; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the case report form (CRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms [Severe reaction, Grade 3: prolonged (i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g. renal impairment, pulmonary infiltrates), Grade 4: life- threatening; pressor or ventilatory support indicated]:

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or

diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Institutional guidelines will be followed for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g. appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g. oral antihistamine or corticosteroids).

8.1.6 Concomitant medications

In general, the use of any concomitant medication/therapies deemed necessary for patient supportive care and safety are permitted. Other anticancer agents, specifically systemic chemotherapy, radiation therapy, or biologic response modifiers are not permitted during the study. Intrathecal chemotherapy is allowed if intended for prophylaxis. No other investigational drug is allowed during the study. Prophylaxis may be used for the prevention of infections or nausea and vomiting. Concomitant medications data will not be collected or entered into the case report form other than hydrea; however, the subject's medication record will contain a list of concomitant medications.

8.2 Lirilumab (BMS-986015)

Lirilumab injection has been developed to be used as an intravenous (IV) infusion for clinical studies. The drug product is a clear to opalescent, colorless liquid, which may contain few particles. It is a sterile, non-pyrogenic, single-use, preservative-free, ready-to-use solution containing 10 mg/mL lirilumab. The 50-mg/Vial and 100-mg/Vial presentations are contained in 6-cc and 10-cc Type I flint glass vials, respectively, closed with 20-mm stoppers, and sealed with aluminum seals. The formulation composition is identical for these two presentations. Each vial of drug product contains the labeled amount of lirilumab drug substance, sucrose, sodium phosphate monobasic, sodium phosphate dibasic, polysorbate 80, and water for

injection. Diluted sodium hydroxide and/or hydrochloric acid solution may be added to adjust the pH to 7.0. A sufficient overfill is included in each vial to account for vial, needle, and syringe holdup. The placebo for lirilumab injection is composed of 0.9% sodium chloride injection (normalsaline; NS).

Drug Product Preparation

Lirilumab injection is administered intravenously either undiluted (10 mg/mL) or diluted in NS to lirilumab concentrations as low as 0.5 mg/mL. The dosing solution is to be infused over 60 minutes through a non-di-2-ethylhexyl phthalate (DEHP) or DEHP IV infusion set with a 0.2-micron polyethersulfone (PES) in-line filter. The drug product is not to be administered as an IV push or bolus injection. Care must be taken to ensure the sterility of the prepared solutions as the drug product does not contain anti-microbial preservatives or bacteriostatic agents. The placebo is administered in a similar fashion.

Recommended Storage and Use Conditions

Lirilumab injection should be stored refrigerated at 2º-8ºC and protected from light and freezing. After dilution with NS or transfer from the original vial to an IV bag, the infusion solution must be administered within 4 hours, including the time for infusion, if stored at room temperature, 20º-25ºC/ambient light. If not dosed immediately, the infusion solution may be stored in a refrigerator, 2º-8ºC, for up to 24 hours, and a maximum of 4 hours, including the time for infusion, of the total 24 hours can be at room temperature, 20º-25ºC/ambient light

8.3 Azacitidine

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

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

Classification: Antimetabolite, DNA hypomethylating agent

CAS Registry Number: 320-67-2

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

Approximate Solubility: soluble (soluble in caustic soda)

Mode of Action:
Azacitidine is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms. Non-proliferating cells are relatively insensitive to azacitidine.

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

after SC administration is 41 ± 8 minutes.

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

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

Route of Administration: Intravenous

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

thoroughly wash with soap and water. If it comes into contact with mucous membranes, flush thoroughly with water.

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

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

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

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

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

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

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

Reconstitution Recommendations for Azacitidine for IV Administration

General Cautions:

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

Procedure:

1. Reconstitute each Azacitidine drug product vial with 10mL of Sterile Water for Injection, USP (SWFI).
2. Shake/roll the vial until all solids are dissolved as determined by visual inspection. Vigorous shaking is allowed if required to ensure complete dissolution. The reconstituted solution should be clear.
3. The concentration of the reconstituted drug product is 10 mg/mL Azacitidine.
4. Withdraw and dilute the required amount of reconstituted drug product in an appropriate container with either 0.9% Sodium Chloride Injection, USP, or Lactated Ringer's Injection, USP. The final volume of the diluted drug product should be no greater than 100mL.
5. If a patient requires more than one vial of Azacitidine, reconstitute each vial as described above and dilute all required, reconstituted Azacitidine into a single container with a total volume not to exceed 100mL. (Final concentration should be <10 mg/mL).

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

8.4 Study Drug Destruction

All expired or unused study drugs will be destroyed or returned as per institutional and investigational pharmacy standard operating procedures, with supplier agreement.

9.0 Patient Evaluation

Pre-Treatment Evaluation: All pretreatment studies should be obtained within 14 days (±3 days) of entry into the trial, unless otherwise stated.

1. A complete history and physical, including assessment of baseline adverse events.
2. CBC, platelet count, differential (differential can be omitted if WBC is ≤0.5 $x10^9$/L).
3. Creatinine, total bilirubin, ALT, AST, amylase, lipase, and TSH.
4. Pregnancy test (urine or plasma) in females of childbearing potential should be is required within 24 hrs of study enrollment or randomization, then every 6 weeks. After discontinuation from nivolumab these should be repeated at approximately 30 days and approximately 70 days [or more frequently if required by local standard].
5. Bone marrow aspirate during the last 14 days preceding study initiation. Cytogenetics will be obtained prior to therapy in patients known to have cytogenetic abnormalities at the time of diagnosis or relapse (results from prior analysis within 4 weeks can be used for this purpose).
6. Chest x-ray.
7. Pretreatment optional correlative studies. Every effort will be made to collect all samples at all time points for all patients; however, missing collection in

one or more of these time points in occasional patients will not be considered a protocol deviation/violation.

10.0 Evaluation during Treatment

All evaluations can vary +/- 3 days.

Complete history and physical and assessments for toxicity should be performed weekly for the first course, then once per course.

CBC, platelet count, differential, creatinine, albumin, SGPT, electrolytes, total bilirubin, and TSH weekly for the first course, then once per course at the discretion of the treating physician.

Bone marrow aspiration with cytogenetics (if abnormal at baseline) on Day 28 of Course 1 (+/- 3 days) and afterwards every 3 months or as indicated to document response or to decide on therapy administration. Once a response is obtained, bone marrow sampling can be repeated at the discretion of the treating physician to confirm remission status or document loss of response.

11.0 Criteria for Response

For MDS, the International Working Group criteria will be use to assess response (11).

Remission is calculated from date of first response until relapse. Survival is calculated from start of therapy until death from any cause.

12.0 Reporting of Adverse Events

Baseline events will be recorded in the medical history section of the case report form and will include the terminology event name, grade, and start date of the event.

Baseline events are any medical condition, symptom, or lab abnormality present before the informed consent is signed. If exact start date is unknown, month and year or year may be used as the start date of the baseline event.

The maximum grade of the adverse event will be captured per cycle.

Serious Adverse Event Reporting (SAE) Language for M. D. Anderson-sponsored IND Protocols

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 Sponsor, 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 sponsor's guidelines, and Institutional Review Board policy.

Investigator Communication with Supporting Companies:

Expedited reporting by investigator to BMS

Serious adverse events (SAE) are defined above. The investigator will inform BMS of any SAE within 24 hours of being aware of the event via email and/or fax.

- All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

  SAE Email Address: Worldwide.Safety@BMS.com

  SAE Fax Number: 609-818-3804

All pregnancies will also be reported.

Reporting of adverse event information following study completion

Collection of safety information following the end of investigational product administration is important in assisting in the identification of possible delayed toxicities or withdrawal effects. All SAEs must be collected which occur within 100 days of discontinuation of dosing or completion of the patient's participation in the study if the last scheduled visit occurs at a later time.

'Expectedness': AEs can be 'Unexpected' or 'Expected' for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR.

Attribution of the AE:

Definite – The AE *is clearly related* to the study treatment.

Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

- Physician or designee will assign attributions.
- Adverse events will be captured in CORe.
- CTC version 4.03 will be used for this trial.
- PDMS will be used as the case report form for this study.

Pregnancy Statement

All women of childbearing potential MUST have a negative pregnancy test within 24 hours prior to receiving the investigational product. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

During the course of the trial, all patients of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition, a missed or late menstrual period should be reported to the treating physician. If a female patient or the treating physician suspects that the female patient may be pregnant prior to administration of study drugs, the study drugs must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed the patient must not receive study medications and must be withdrawn from the study. Throughout the entire pregnancy, additional contact should be made with the patient, and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 4 weeks.

If a male patient is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus.

In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via maternal interviews, medical record abstraction, or a combination of these methods. All serious adverse event reports relating to the pregnancy, including spontaneous abortion, elective abortion and congenital anomalies, should be forwarded to the FDA & BMS.

13.0 Informed Consent

The investigator is responsible for obtaining written, informed consent(s) from each patient interested in participating in this study prior to conducting any study-related procedures. Written informed consent should be obtained after adequate, thorough and clear explanation of the aims, methods, objectives, and potential hazards of the study as well as any use of the patient's genetic information from the study. The investigator must use the most current IRB-approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the patient and the person obtaining consent. The investigational site must retain the original signed consent and provide a copy to the patient.

14.0 Correlative Studies

Correlative studies will be considered exploratory and will be performed whenever possible. Bone marrow and blood samples will only be collected on patients who consent to protocols: LAB01-473 and PA13-0291. If a patient's baseline correlative bone marrow sample is not collected for any reason, no additional correlative bone marrow or blood samples will be collected on the patient. Additionally, any samples that

are missed are not considered a protocol deviation.

Gene sequencing. Patients not consented through the Apollo program (PI Futreal) will be offered consenting under protocol LAB03-473 at baseline and their genomes will be sequenced using that platform. 5 cc of blood in a green tube will be required for this analysis. If the patient participates on LAB01-473, the following samples will be collected at the specified time points:

- Bone Marrow aspirate: will be baseline, C1D28, thereafter every 3 cycles, at time of best morphological response and at progression.
- Peripheral Blood: screening, prior to each dose of study drug during the first 3 cycles, at time of best morphological response, and at progression.

Immune assessment. Tumor tissue, blood samples and bone marrow aspirate will be collected on a separate IRB approved laboratory protocol (PA13-0291) for immune monitoring as previously published,[18-21] under the supervision of the Immunotherapy Platform. In tumor tissues, immunohistochemical studies will be performed to evaluate tumor and immunological cell markers such as CD4 and CD8 T cells. In peripheral blood, we will also evaluate tumor and immune cell populations including but not limited to CD4 and CD8 T cells in pre and post therapy samples.

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

- At screening
- Prior to each dose of study drug during the first 3 cycles. On days when both Nivolumab and Lirilumab are administered, a blood sample will only be collected prior to the Nivolumab dose.
- At time of best morphological response
- At progression

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

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

15.0 Statistical Considerations

This is a Phase II open-label study designed to determine the safety and efficacy of nivolumab and lirilumab, as single agents or in combination and with 5-azacitidine, in patients with myelodysplastic syndromes (MDS). Two different patient groups (lower risk MDS and High MDS) with 2 cohorts each will be study. Each cohort will be composed of N=20 patients. Accrual will be parallel for each patient group.
The primary efficacy outcome for each cohort is the overall response rate (ORR) defined as CR + PR + hematological improvement (HI). Overall response will be assessed after 6 cycles of treatment; cycle length is 28 days. A maximum of 20 total evaluable patients will be enrolled in each cohort (N=80).

Lower Risk MDS cohorts: cohorts (1 and 2): each cohort separately

The target ORR with the experimental treatment (i.e. lirilumab alone or in combination with nivolumab) is 70%, assuming the standard treatment ORR is 50%. The regimen of the experimental treatment will be considered worthy of further investigation if it elicits an increase in ORR to 70% with acceptable toxicity. A >30% therapy related non-hematological grade 3/4 toxicity rate is considered unacceptable. Thus, interim

monitoring rules, assuming the prior distributions above, were constructed that meet the following two conditions,

1) Stop if Prob{ p(ORR,E)<p(ORR,H) + 0.20 | data} > 0.95, or
2) Stop if Prob{ p(TOX,E)>0.30| data}>0.975,
3) where P(ORR, E) and P(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 5.0%) that ORR rate of the experimental 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 non-hematological grade 3/4 toxicity (>30.0%) is highly probable (i.e., probability >97.5%) for the experimental treatment. Monitoring for toxicity and futility will start when the first patient has 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 1. For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.

Table 1. Stop accrual if the number of drug-related non-hematological grade 3/4 toxicities is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	5	10	15	20
# patients with toxicities	4-5	7-10	9-15	Always stop with this many patients

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 2. For example, accrual will cease if 1 patient experience an overall response within 6 cycles in the first 5 patients treated.

Table 2. 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
# patients with overall response	1	4	7	Always stop with this many patients

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 3). In order to utilize the software for the design, a response constant rate of 50% and beta (1.0, 1.0) priors were assumed for

the standard treatment response distribution and experimental treatment response prior distribution, respectively. A delta 20% was assumed. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity constant rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the experimental treatment was 70% and the true toxicity rate was 30% was 12.4%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 72.6% when the true ORR was 50%, and 43.2% when true ORR rate was 70%.

Table 3. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with experimental treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.50	0.5590
	0.60	0.2739
	0.70	0.0849
	0.80	0.0137
	0.90	0.0010
0.20	0.50	0.5621
	0.60	0.2790
	0.70	0.0914
	0.80	0.0207
	0.90	0.0081
0.30	0.50	0.5779
	0.60	0.3050
	0.70	**0.1241**
	0.80	0.0560
	0.90	0.0439
0.40	0.50	0.6271
	0.60	0.3861
	0.70	0.2263
	0.80	0.1661
	0.90	0.1554
0.50	0.50	0.7261
	0.60	0.5490
	0.70	0.4316
	0.80	0.3874
	0.90	0.3795

High Risk MDS cohorts (3 and 4): each cohort separately

The target ORR with the experimental treatment (i.e. 5-azacitidine in combination with lirilumab, or combination of 5-azacitidine, Lirilumab and nivolumab) is 70%, assuming the standard treatment ORR is 50%. The regimen of the experimental treatment will be considered worthy of further investigation if it elicits an increase in ORR to 70% with acceptable toxicity. A >30% therapy related non-hematological grade 3/4 toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions above, were constructed that meet the following two conditions,

1) Stop if Prob{ p(ORR,E)<p(ORR,H) + 0.20 | data} > 0.95, or
2) Stop if Prob{ p(TOX,E)>0.30| data}>0.975,
3) where P(ORR, E) and P(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 5.0%) that ORR rate of the experimental 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 non-hematological grade 3/4 toxicity (>30.0%) is highly probable (i.e., probability >97.5%) for the experimental treatment. Monitoring for toxicity and futility will start when the first patient has 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 4. For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.

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

# patients evaluated	5	10	15	20
# patients with toxicities	4-5	7-10	9-15	Always stop with this many patients

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 5. For example, accrual will cease if 0 patients experience an overall response within 6 cycles in the first 5 patients treated.

Table 5. 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
# patients with overall response	1	4	7	Always stop with this many patients

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 6). In order to utilize the software for the design, a response constant rate of 50% and beta (1.0, 1.0) priors were assumed for the standard treatment response distribution and experimental treatment response prior distribution, respectively. A delta 20% was assumed. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity constant rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the experimental treatment was 70% and the true toxicity rate was 30% was 12.4%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 72.6% when the true ORR was 50%, and 43.2% when true ORR rate was 70%.

Table 6. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with experimental treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.50	0.5590
	0.60	0.2739
	0.70	0.0849
	0.80	0.0137
	0.90	0.0010
0.20	0.50	0.5621
	0.60	0.2790
	0.70	0.0914
	0.80	0.0207
	0.90	0.0081
0.30	0.50	0.5779
	0.60	0.3050

	0.70	**0.1241**
	0.80	0.0560
	0.90	0.0439
0.40	0.50	0.6271
	0.60	0.3861
	0.70	0.2263
	0.80	0.1661
	0.90	0.1554
0.50	0.50	0.7261
	0.60	0.5490
	0.70	0.4316
	0.80	0.3874
	0.90	0.3795

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 (i.e. including duration of response) and safety data of the patients will be summarized using descriptive statistics such as mean, standard deviation, median and range. For the primary efficacy analysis, we will estimate the ORR for the experimental treatments, along with the 95% credible intervals. 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 (PFS and OS) including overall survival and event 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.

16. Confidentiality Plan

Confidentiality will be maintained via codification of data collected and restriction of access to the data. In particular, to be authorized to use the PDMS database, M. D.

Anderson employees must have an institutional USERID assigned through the Information Services Security Department. State regulations for database computer access are followed. ALL database analysts regularly log and monitor the system access and log-in activity. All unsuccessful attempts to access the ALL database are logged to a file that is reviewed daily by the ALL database coordinator. If the user has updated patient data, they will be prompted for the password upon exiting the ALL database. If the password is not verified, an automated e-mail is sent to the ALL database coordinator, and a log is created. Users are required to change their password every three months. The passwords are stored in an encrypted format.

Unless specifically described and explained to the clinical trial participants in the informed consent document, results of the study will not be shared with the patients. The profiles will be obtained on coded samples with no patient identifiers, and confidentiality will be maintained. Results of the clinical trial will be entered in clinicaltrials.gov, but individual patient's results will remain confidential information.

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