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An Open Label Phase II Trial of Guadecitabine and Pembrolizumab in Platinum Resistant Recurrent Ovarian Cancer

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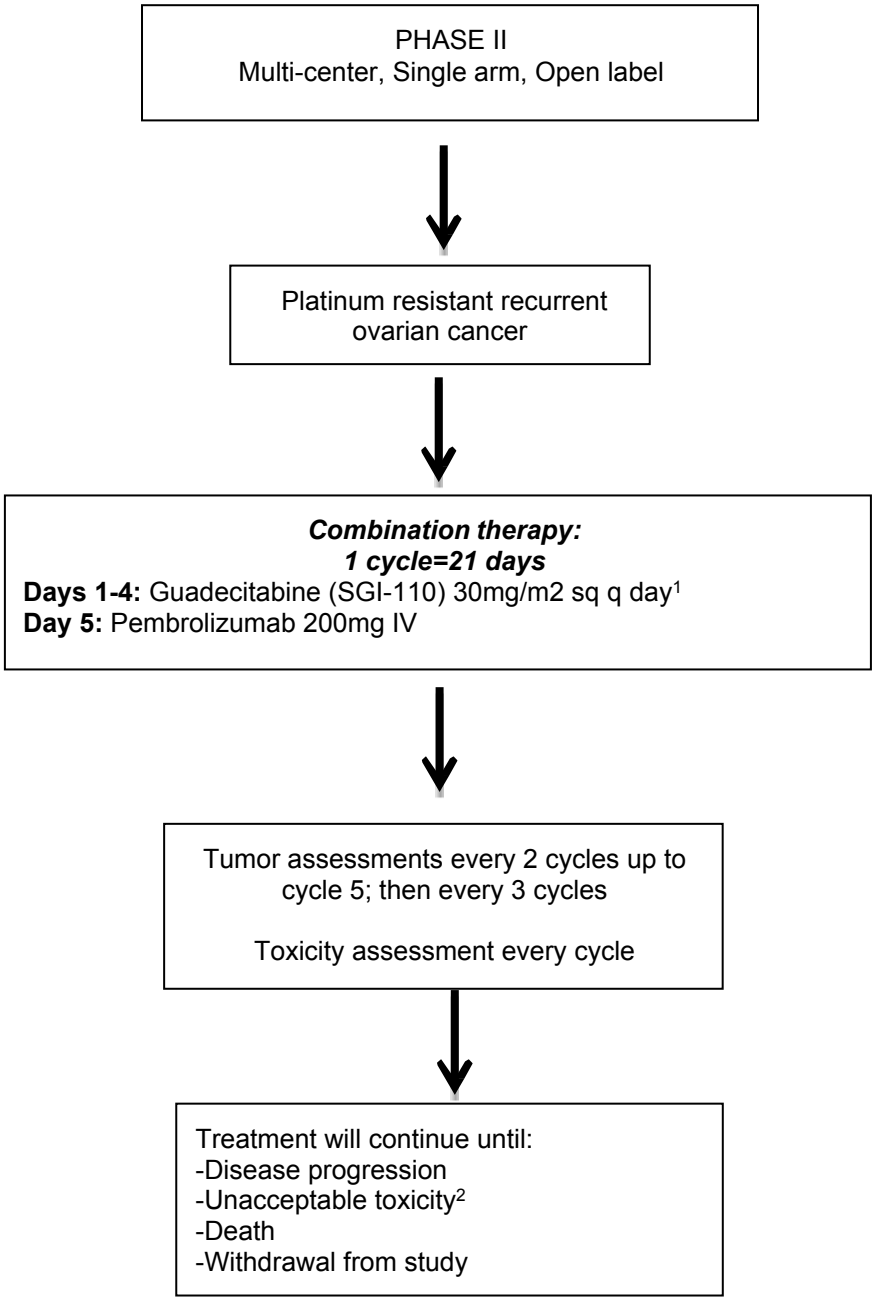
TABLE OF CONTENTS

LIST OF ABBREVIATIONS

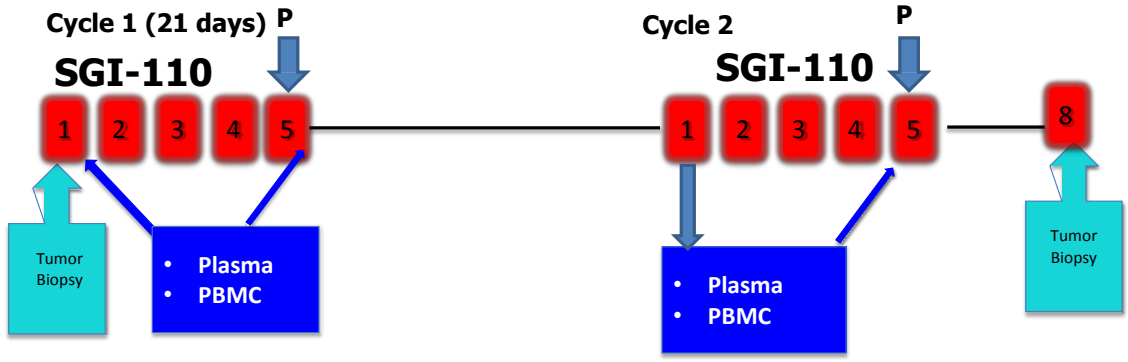
AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CBR	Clinical Benefit rate
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
ECI	Events of Clinical interest
H&PE	History & Physical Exam
IV (or iv)	Intravenously
irAE	Immune-related Adverse Events
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
OC	Ovarian Cancer
ORR	Overall Response Rate or Objective Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
PO (or p.o.)	Per os/by mouth/orally
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
SQ	Subcutaneous
WBC	White Blood Cells

STUDY SCHEMA



[1] Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles) at the discretion of the treating physician, if the patient has stable disease or response. If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days).

[2] If a patient is unable to tolerate the combination despite dose reduction, but has completed at least 2 cycles of combination therapy, the patient may be permitted to continue on single agent pembrolizumab (please see Section 4.4.1 for details and rationale).



Schematic of proposed analyses with the phase II trial (P=Pembrolizumab).
Tumor biopsies: RNA--tumor antigen expression, IHC for TILs subsets
Archival tumor IHC: PD-L1
Plasma: DNA for gene specific methylation (pyrosequencing)
PBMC: LINE1 methylation (pyrosequencing)

Note: Guadecitabine will be administered on Days 1-4 of 21-day cycles. Pembrolizumab will be administered on Day 5 only of 21-day cycles. During Cycle 2 only, subjects will have an additional study visit on Day 8 in order to complete blood collection and tumor biopsy for research purposes. *Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles) at the discretion of the treating physician, if the patient has stable disease or response. If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days).*

For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.

Please refer to Section 9.0 for more details about correlative analysis.
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STUDY SUMMARY

Title	An Open Label Phase II Trial of Guadecitabine and Pembrolizumab in Platinum Resistant Recurrent Ovarian Cancer
Short Title	Guadecitabine and pembrolizumab in platinum resistant recurrent ovarian cancer
Version	November 18, 2020 (Amendment 11)
Study Design	This will be an open label phase II trial with a safety run-in component testing the experimental combination of guadecitabine and pembrolizumab.
Study Center(s)	Lead site: Robert H. Lurie Comprehensive Cancer Center (RHLCCC) Affiliate site: University of Chicago
Objectives	**Primary Objective:** Measure Objective Response Rate (ORR) to guadecitabine and pembrolizumab in patients with recurrent platinum resistant ovarian cancer. **Secondary Objectives:** • Measure Progression Free Survival (PFS) and Clinical Benefit Rate (CBR) to the combination of guadecitabine and pembrolizumab. • Measure toxicity profiles to the combination of guadecitabine and pembrolizumab. Refer to Section 2.0 for Exploratory Objectives.
Sample Size	Maximum accrual limit: 46 Evaluable subjects: 35
ESTIMATED ENROLLMENT PERIOD	Estimated 24 months
ESTIMATED STUDY DURATION	Estimated 36 months

Diagnosis & Key Eligibility Criteria	**Inclusion Criteria:** 1. Histological or cytological evidence/confirmation of epithelial ovarian cancer, primary peritoneal carcinomatosis, or fallopian tube cancer. 2. Patients must have recurrent platinum-resistant disease, defined as progression < 6 months after completion of platinum-based chemotherapy or as persistent disease that remains after completing the most recent line of platinum-based therapy. The platinum-free interval should be calculated from the last administered dose of platinum therapy. 3. Measurable disease according to RECIST 1.1 ≤28 days prior to registration. 4. Prior therapy allowed: • At least one and no more than 3 platinum based chemotherapy regimens. • Up to 2 non-platinum, cytotoxic regimens. • There is no limit on use of prior biological therapies (hormonal or targeted therapy). • Prior immunotherapy is not allowed. 5. Demonstrate adequate organ function (see section 3.1). **Exclusion Criteria:** 1. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy ≤7 days prior to the first dose of trial treatment. 2. Hypersensitivity to pembrolizumab or any of its excipients. 3. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. 4. Has had a prior anti-cancer monoclonal antibody (mAb) ≤4 weeks prior to registration or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier. 5. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy ≤ 2 weeks prior to registration or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent. - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study. - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

Treatment Plan	Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Schedule of Events Calendar and Section 5.0. Trial treatment may be administered up to +28 days after the scheduled Day 1 of each cycle due to administrative reasons. All trial treatments will be administered on an outpatient basis. Guadecitabine 30mg/m^2 will be administered as a subcutaneous injection on Days 1-4 of every 21 day cycle. Pembrolizumab 200 mg will be administered as a 30 minute IV infusion on Day 5 of every 21 day cycle. *Note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles) at the discretion of the treating physician, if the patient has stable disease or response. If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days).* *For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.* To exclude prohibitive toxicity, a safety run-in cohort of the initial 6 patients will be done. All subjects in the safety run-in cohort will be observed <u>for at least 2 weeks after completion of cycle 1</u> for dose limiting toxicity (DLT) (Refer section 4.3)
Statistical Methodology	This is a non-randomized, open label phase II study. Study endpoints are defined in Section 6.0 We will utilize the optimum two stage design, and we anticipate enrolling 35 evaluable patients. This two-stage design, which will test the null hypothesis that ORR<=0.10 versus the alternative that ORR>=0.30 (where ORR is calculated via RECIST v1.1), has an expected sample size of 22.5 and a probability of early termination of 0.71 when ORR=0.10. If the drug combination is actually not effective, there is a 0.047 probability of concluding that it is (the target for this value was 0.050). If the drug is actually effective, there is a 0.098 probability of concluding that it is not (the target for this value was 0.100). After testing the drug on 18 patients in the first stage, consideration will be given to trial termination if 2 or fewer patients demonstrate clinical benefit (CR, PR, or SD for at least 3 months). If the trial goes on to the second stage, a total of **35 evaluable patients will be studied**. If the total number responding is less than or equal to 6, the combination is rejected. Subjects who are not evaluable for response will be replaced so that up to 46 subjects will be enrolled.

1.0 INTRODUCTION – BACKGROUND & RATIONALE

1.1 Ovarian Cancer Background

Ovarian cancer (OC) causes more deaths than any other female reproductive tract cancer [3,4]. The majority of women diagnosed with advanced-stage epithelial OC experience tumor recurrence associated with the development of chemo-resistance, and platinum-resistant OC is uniformly fatal [5]. Development of novel therapies for this deadly cancer is critically needed. The analysis of the ovarian cancer tumor genome revealed that molecular subtypes are difficult to define and target, the majority of tumors being characterized by complex genomic alterations with tens of mutations and chromosomal aberrations occurring per tumor. Targeted therapy, therefore, is unlikely to succeed in OC.

Immunotherapy has demonstrated efficacy in some cancers (melanoma, lung and renal cell carcinoma). Although preclinical evidence provides the rationale for investigating immunotherapy in OC, clinical trials testing immune approaches (vaccines, IL2, ipilimumab) have not been successful to date. New immunologic approaches targeting immune checkpoint pathways are showing promise in clinical development for other solid tumors [6]. Immune checkpoint pathways such as the programmed cell death protein-1 (PD-1) block T-cell activation as a physiologic response that keeps nascent T-cells in check and prevents immune responses against normal tissues. During tumorigenesis, cancer cells exploit this co-inhibitory pathway to avoid elimination by the adaptive immune system. One such mechanism is aberrant expression of PD ligands (PD-L) on tumor cells which leads to impaired antitumor immunity and immune evasion. In an analysis of OC specimens, increased PD-L1 expression was correlated with decreased intratumoral CD8(+) T lymphocytes and worse survival [7]. In addition dendritic cells expressing PD1 have been identified in the OC microenvironment in association with suppressed T cell activity and decreased infiltrating T cells in advanced ovarian tumors[8]. The expression of the T cell exhaustion marker PD1 correlated with decreased number of TILs in an OC xenograft model and PD-1/PD-L1 blockade restored anti-tumor immunity[9]. This evidence supports testing PD1-blockade in OC. The focus of this protocol is to develop a combination regimen that enhances the activity of PD1-targeted immunotherapy in OC.

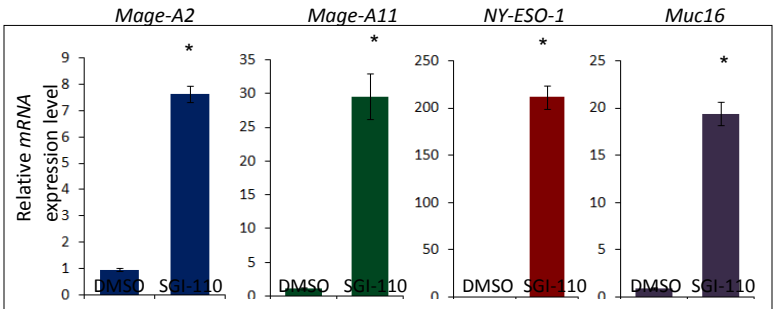

Figure 1. SGI-110-induced expression of tumor antigens. SKOV3 OC cells were treated with DMSO (control) or SGI-110 (100 nM) for 72 hours. Quantitative RT-PCR measured *mRNA* expression levels of *Mage-A2* and *–A11, NY-ESO-1* and *Muc 16*. Results are presented as fold change vs. control. * denote $p<0.05$

1.2 Intervention Background & Overview

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and

favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors [1,2].

1.2.1 Pembrolizumab (Keytruda) background

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

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.On September 4, 2014, the Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab(Keytruda), made by Merck Sharp & dohme Corp.) for the treatment of patients with unresectable or metastatic melanoma whose disease progressed following treatment with ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

In Oct. 2015, the U.S. Food and Drug Administration granted accelerated approval for Keytruda (pembrolizumab) to treat patients with advanced (metastatic) non-small cell lung cancer (NSCLC) whose disease has progressed after other treatments and with tumors that express a protein called PD-L1. Keytruda was approved for use with a companion diagnostic, the PD-L1 IHC 22C3 pharmDx test, the first test designed to detect PD-L1 expression in non-small cell lung tumors.

1.3 Guadecitabine (SGI-110) Background

Guadecitabine is a dinucleotide incorporating decitabine (5-aza-2'-deoxycytidine) and deoxyguanosine that act as pro-drugs of decitabine. Guadecitabine is resistant to modification by cytidine deaminase leading to longer half-life compared to decitabine and ensuring prolonged exposure to the active compound and sustained hypomethylating effect *in vivo*[16]. The FDA has granted an orphan drug designation for Astex Pharmaceuticals' investigational drug, guadecitabine (SGI-110), in the treatment of acute myeloid leukemia (AML). Guadecitabine is currently being evaluated in a large global Phase 3 study (ASTRAL-1) in treatment naïve AML patients who are unfit to receive, or unsuitable for, intensive induction chemotherapy. For complete information on guadecitabine please refer to the Investigator's Brochure (IB).

The active metabolite of guadecitabine (2'-deoxy-5-azacytidylyl-(3'→5')-2'-deoxyguanosine sodium salt), a dinucleotide, is decitabine. Guadecitabine is resistant to modification by cytidine deaminase, a common pathway of decitabine metabolism and deactivation [20,21]. The molecular weight of guadecitabine and decitabine are 580 Da and 228 Da, respectively. Therefore, the molar equivalent dose of 1 mg of decitabine is approximately 2.5 mg of guadecitabine. Guadecitabine's activity was demonstrated with the same preclinical pharmacodynamic assays used to demonstrate decitabine's efficacy e.g., re-expression of p15, p16, and MLH1 and induction of fetal hemoglobin, in vivo. In xenograft studies, guadecitabine demonstrates promising preclinical activity in both hematologic and solid tumors.

Guadecitabine was developed for subcutaneous administration. Guadecitabine is pharmacologically active both in vitro and in vivo in a variety of tumor cells and murine xenograft models when administered subcutaneously. Treatment is well tolerated via the subcutaneous route in murine xenografts. When administered subcutaneously to non-human primates, guadecitabine releases decitabine slowly compared to other species, possibly prolonging the effect over longer periods. Guadecitabine has been developed as a non-aqueous formulation to ensure formulation stability.

In vitro and in vivo evidence suggest that guadecitabine has a longer half-life than decitabine in the presence of cytidine deaminase. These promising observations suggest that guadecitabine has improved pharmaceutical properties and biological activities that expand on decitabine's current clinical utility. Pharmacokinetic studies in a recent clinical trial in patients with ovarian cancer testing 2 dose levels of guadecitabine (30mg/kg QD x 5 days) and 45mg/m2 QD x 5 days) shows that in vivo exposure to active decitabine is much longer with guadecitabine compared to the parent drug decitabine (Figure 3).

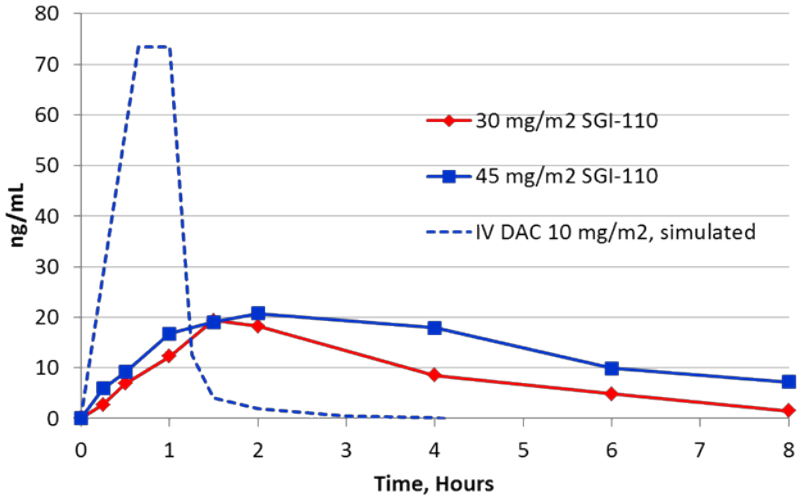

Figure 3: Decitabine exposure window after SQ injection of guadecitabine is prolonged compared to previously published data of decitabine IV infusion

1.3.1 Nonclinical Pharmacokinetics of Guadecitabine

The overall pharmacokinetic characteristics of guadecitabine are summarized as follows. The relative bioavailability of guadecitabine dosed subcutaneously to the rat is close to 100%. Circulating guadecitabine levels were very low in the mouse, rat and rabbit. However, higher levels were observed in the monkey post subcutaneous dosing. A rapid decline in systemic exposure of guadecitabine with an elimination plasma half-life (T1/2) in the range of 0.4-1 hours was observed in rat and monkey.

High levels of decitabine were observed after a subcutaneous dose of guadecitabine in the mouse, rat, and rabbit (maximum in rat, 54 µg/mL). Levels in the monkey (maximum 463 ng/mL) were substantially lower. A Rapid decline in systemic exposure of decitabine with an elimination plasma T1/2 of 3.7 hour in rat and 1 hour in monkey was observed.

In monkey, pharmacokinetic parameters were similar on Day 1 and Day 15 of a study in which they were dosed once weekly for three consecutive weeks, suggesting no significant accumulation of the parent or the active metabolite, decitabine.

The metabolic characteristics of guadecitabine are summarized as follows. Guadecitabine was more stable in human, dog and mouse and was less stable in rat and rabbit plasma. Incubation of guadecitabine with liver microsomes from mouse, rat, rabbit, dog and human showed little apparent metabolism of the compound. Incubation of guadecitabine with human hepatocytes also showed little apparent metabolism of the compound, based on disappearance of the parent. Guadecitabine does not significantly bind to human plasma proteins; the in vitro unbound fraction was estimated to be 91%. Guadecitabine has poor in vitro bidirectional permeability, which correlates well with its poor oral absorption in vivo. Guadecitabine shows no appreciable induction of CYP1A1/2, CYP2C9 and CYP3A4 in human hepatocytes. Guadecitabine does not have any CYP450 inhibitory effect on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.

1.3.2 Nonclinical Safety of Guadecitabine

Guadecitabine toxicity findings in rat and rabbit studies are similar to the non-clinical study findings of decitabine in New Drug Application (NDA) supporting Good Laboratory Practices (GLP) toxicology studies. Myelosuppression, decreases in thymus weight, and testicular atrophy (the main findings of the guadecitabine studies) were also observed as the main study findings in repeat dose toxicity studies with decitabine in mice, rats, rabbits, and dogs. As with guadecitabine, myelosuppression and thymus toxicities after decitabine administration were reversible during recovery periods, while testicular atrophy persisted. Myelosuppression, particularly neutropenia, has been reported as a dose-limiting toxicity for decitabine in human clinical studies. Signs of testicular toxicity have not been observed in any of the published clinical studies of decitabine to date [17,22].

No studies have been performed to evaluate the genotoxic, mutagenic, carcinogenic or reproductive and developmental toxicity of guadecitabine. Decitabine may have genotoxic potential; decitabine is mutagenic and in preclinical studies in mice and rats, decitabine was teratogenic, fetotoxic, and embryotoxic.

1.3.3 Guadecitabine Clinical Data

For the most updated clinical data, please refer to the most recent Investigator Brochure.

Guadecitabine was studied in a first-in-human, single-agent study (SGI-110-01). This study was a Phase 1/2, dose escalation, multicenter study of two subcutaneous regimens of guadecitabine in subjects with intermediate or high-risk myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML). This study had two parts: a Dose Escalation Segment and a Dose Expansion Segment. The study sought to evaluate the biological activity, preliminary safety, and efficacy of guadecitabine with two dosing schedules in intermediate to high risk MDS or relapsed or refractory AML subjects, while the Dose Expansion Segment further evaluated the safety and efficacy at the recommended dose. The study was based on a 3 + 3 design within each regimen. The recommended dose going forward was 60mg/m2 QD x 5 days, every cycle being 28 days [22].

Guadecitabine was also studied in a phase I leading into a randomized phase II study in combination with Carboplatin in patients with recurrent ovarian cancer (Study SGI-110-02). Two dose levels of guadecitabine were studied: 30mg/m2 QD x 5 days with Carboplatin AUC 4 and 45mg/m2 qd X 5 days with Carboplatin AUC 5. The second dose level was not tolerated, with neutropenic fever and thrombocytopenia being DLTs. The dose taken for further development in the randomized phase II portion of the trial was 30mg/m2 QD x 5 days with Carboplatin AUC, each cycle being 28 days[17].

1.3.4 Clinical Efficacy/Biological Activity/Safety of Guadecitabine

Based on PD assessment of global hypomethylation from the first 7 cohorts in Study SGI-110-01, the LINE-1 demethylation data show dose dependent hypomethylation induction in the daily schedule reaching a plateau at cohort 5 (60 mg/m2). The Biologically Effective Dose or BED, therefore, was established as 60 mg/m^2 SC daily x 5. The hypomethylation of the weekly schedule was inferior to the daily schedule and plateaued early (daily x 5) [22]. In the ovarian cancer SGI-110-02 study, LINE-1 methylation was measured at both dose levels (Figure 4). Effective LINE-1 hypomethylation (~-17%) was observed and was similar at both dose levels [17]. Therefore, in this

protocol, we will use the lower dose of 30mg/m2, which has been shown to induce hypomethylation and gene re-expression.

Of note is that the current protocol will use a shorter cycle duration (21 days, as compared to 28 days in previous studies). We will use the 21 day course to accommodate a regimen giving pembrolizumab q 3 weeks. We anticipate that the shorter course will be tolerated, given that 30mg/m^2 daily for 5 days given in combination with Carboplatin was tolerated.

SGI-110 Cohort (mg/m2 qd x5)	Max LINE-1 Demethylation
1 (45)	-17.4 ± 6.9% (n=6)
2 (30)	-19.5 ± 8.6 % (n=14)

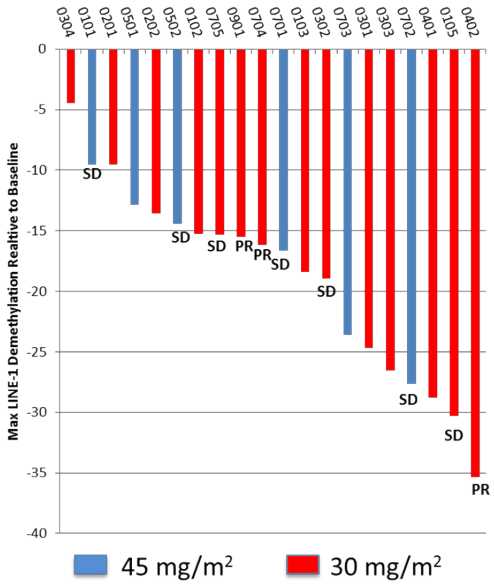

Figure 4 LINE-1 Demethylation in Blood DNA B) Individual Patients Max LINE-1 Demethylation

1.4 Rationale for the Current Study

We speculate that an important mechanism of immune evasion in OC is represented by epigenetic silencing of tumor antigens. We and others have shown that OC progression is accompanied by the establishment of stable and transcriptionally repressive epigenetic modifications [10, 11]. Some of these modifications include DNA methylation at promoters of genes encoding tumor antigens that serve as potent immunogenic stimuli in the anti-tumor response. DNA methylation is regulated by DNA methyl transferases (DNMT-1, -3a and -3b). Recent studies have shown that DNMT inhibitors (DNMTI) cause DNA hypomethylation and restore the expression of epigenetically silenced genes, including tumor suppressor genes [12] and tumor antigens. DNMTIs have become therapeutically relevant in hematologic cancers [13,14] and are being explored in solid tumors in combination with chemotherapy [15]. **Here we hypothesize that the use of a novel DNMTI (guadecitabine) will reverse**

silencing of tumor antigens, restore their expression, and potentiate the effects of immune interventions.

Our preliminary data support that treatment with guadecitabine *in vitro* causes significant up-regulation of tumor antigens including *Muc16* (encoding CA-125), *Mage A2, Mage A11*, and *cancer testis antigen (NY-ESO 1)* in OC cell lines (Fig. 1). We speculate that these effects render OC cells more recognizable by the immune system *in vivo*. Furthermore, analyses of tumor material obtained from another ongoing clinical trial using guadecitabine in combination with carboplatin in women with OC[17] showed significant treatment-induced hypomethylation of *Mage A2* and *Mage A11* (as measured by DNA pyrosequencing, Fig. 2A) in tumor biopsies obtained after 2 cycles of guadecitabine compared to baseline biopsies. Re-expression of the tumor antigens *NY-ESO-1* and *MAGE A3* was confirmed by RT-PCR in tumor biopsies obtained after 2 cycles of guadecitabine compared to baseline (Fig. 2), demonstrating that hypomethylating agents induce tumor antigen re-expression in human tumors, by reducing DNA methylation. These data support a strategy combining epigenome editing using guadecitabine along with immunomodulatory approaches, as proposed here. Based on the recent knowledge that the anti-PD1 antibody pembrolizumab (P) has impressive clinical activity in solid tumors[18,19], and is well tolerated and induces durable responses in patients with advanced epithelial ovarian cancer, we propose to use PD-1 blockade (P) in combination with guadecitabine in preclinical and clinical OC models.

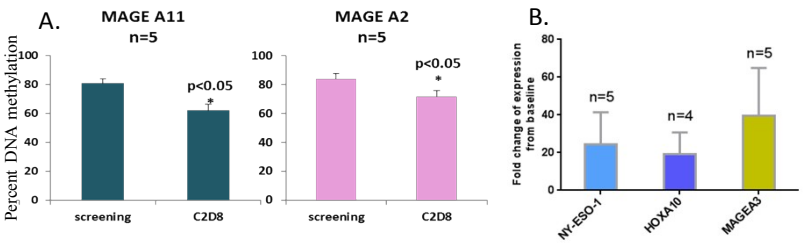

Fig. 2: Methylation and expression of tumor antigens in biopsies obtained from patients enrolled on a clinical trial testing SGI-110 and carboplatin in OC. Tumor biopsies were obtained at baseline and on Cycle 3, Day 8, after 3 cycles of SGI-110. A. DNA methylation measured by pyrosequencing in tumor biopsies. B. Fold change in expression levels measured by RT PCR in tumor biopsies before and after SGI-110 treatment (bars are averages +/- SD).

1.4.1 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) evaluated the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to

IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

To match the required q 3 weeks administration of pembrolizumab, the cycle of guadecitabine has been modified from D1-5 every 4 weeks (as previously administered in Study SGI-110-02) to a regimen using D1-4 every 3 weeks. This modification allows the administration of similar doses of guadecitabine over time (e.g. 480mg/m2 in 12 weeks with the 3 weekly regimen compared to 450mg/m2 in 12 weeks with the 4 weekly regimen). The D1-4 guadecitabine regimen every 3 weeks has been found to be tolerable in a

cholangiocarcinoma study (personal communication from Astex, unpublished).

1.4.2 Safety run-in cohort

As stated earlier, this study proposes to use the combination of pembrolizumab with guadecitabine in subjects with ovarian cancer. The combination of pembrolizumab with guadecitabine has not been previously tested in this subject population. Hence, a safety run-in cohort has been designed for the first six subjects enrolled, in order to ensure the safety of this combination of agents. Refer to section 4.3 for details on how this will be conducted.

1.5 Exploratory Studies

To test the hypothesis that the combination of guadecitabine and pembrolizumab reactivates immune responses by allowing re-expression of tumor antigens silenced epigenetically, we will measure several exploratory endpoints. All biological parameters will be correlated with clinical endpoints (ORR, CBR, and PFS).

- *NY-ESO-1* and *MAGE* antigens' promoter methylation (pyrosequencing) and mRNA expression levels (quantitative RT-PCR) will be measured before and after treatment in DNA (plasma and tumor biopsies) and RNA (tumor biopsies), respectively.
- Cytokine response (IFNγ, IL2, IL6, IL10, TNFα) will be measured in plasma by ELISA.
- Expression of the PD-L1 ligand will be measured by IHC in archival tumors.
- Measure LINE 1 methylation in DNA extracted from PBMCs (D5 vs. D1 of cycles 1 and 2).
- Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC), including in optional samples collected > 6 months on treatment.

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2.0 OBJECTIVES & ENDPOINTS

2.1 Primary Objective & Endpoint

Measure objective response rate (ORR) to guadecitabine and pembrolizumab in subjects with recurrent platinum resistant Ovarian Cancer (OC) using RECIST v1.1.The objective response rate as defined by RECIST v1.1 is the proportion of all subjects with confirmed PR or CR according to RECIST v1.1, from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

2.2 Secondary Objectives & Endpoints

2.2.1 Measure progression free survival (PFS) for the combination of guadecitabine and pembrolizumab.

Progression free survival (PFS) is defined per RECIST v1.1 as the time from the date of registration until the criteria for disease progression is met as defined by RECIST 1.1 or death occurs.

2.2.2 Measure clinical benefit rate (CBR) for the combination of guadecitabine and pembrolizumab.
(Note: CBR is defined as Objective Response Rate (CR + PR) plus Stable Disease for at least 3 months)

2.2.3 Measure toxicity profiles to the combination of guadecitabine and pembrolizumab.

2.2.4 Measure objective response rate (ORR) to the combination of guadecitabine and pembrolizumab using the Immune Related Response Criteria (irRC) *(Note: Response assessments will be made using both irRC and RECIST v1.1, allowing additional comparisons among these criteria for disease response assessment. Measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC. Disease progression will be determined using RECIST 1.1 criteria. (Refer to section 6.0 for details.)*

2.3 Exploratory Objectives & Endpoints

- NY-ESO-1 and MAGE antigens' promoter methylation (pyrosequencing) and mRNA expression levels (quantitative RT-PCR) will be measured before and after treatment in DNA (plasma and/or tumor biopsies) and RNA (tumor biopsies), respectively.
- Cytokine response (IFNγ IL2, IL6, IL10, TNFα) will be measured in plasma by ELISA.
- Measure LINE 1 methylation in DNA extracted from PBMCs (measured on days 1 and 5 of cycles 1 and 2).
- Expression of the PD-L1 ligand will be measured by IHC in archival tumors.
- Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC), including in optional samples collected > 6 months on treatment.

3.0 PATIENT ELIGIBILITY

The target population for this study is patients with platinum resistant recurrent ovarian cancer. This will be a multicenter-trial conducted at Robert H. Lurie Comprehensive Cancer Center of Northwestern University (RHLCCC). Northwestern University will serve as the lead site and coordinating center for this study. Participating sites will include the University of Chicago.

A total of up to 46 subjects will be needed to obtain 35 evaluable subjects for this trial. Approximately 6-8 potentially eligible patients are seen per month, and it is anticipated that at least 1-2 per month will be accrued.

Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

3.1 Inclusion Criteria

3.1.1 Patients must have a histological or cytological evidence/confirmation of epithelial ovarian cancer, primary peritoneal carcinomatosis, or fallopian tube cancer

3.1.2 Patients must have Measurable disease according to RECIST 1.1 ≤28 days prior to registration. (Appendix 3).

3.1.3 Patients must have recurrent platinum-resistant disease, defined as progression < 6 months after completion of platinum-based chemotherapy or as persistent disease that remains after completing the most recent line of platinum-based therapy. The platinum-free interval should be calculated from the last administered dose of platinum therapy.

3.1.4 Prior therapy allowed:

- At least one and no more than 3 platinum based chemotherapy regimens.

- Up to 2 non-platinum, cytotoxic regimens.
- There is no limit on use of prior biological therapies (hormonal or targeted therapy).

NOTE: Prior immunotherapy is not allowed.

3.1.5 Patients must be age ≥ 18 years.

3.1.6 Patients must exhibit an ECOG performance status of 0-1 ≤14 days prior to registration (Appendix 2).

3.1.7 Patients must demonstrate adequate organ function as defined in Table 1 below; all screening labs to be obtained ≤14 days prior to registration.
(Note: Screening labs will be repeated again on C1D1 if they were performed outside of the ≤4 day window.)

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L (without transfusion or growth factor support/EPO dependency)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN.
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR ≤** 5 X ULN for subjects with liver metastases.
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants. ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
[a]Creatinine clearance should be calculated per institutional standard.	

3.1.8 Be willing to allow the use of archival formalin-fixed paraffin-embedded tumor tissue for correlative analyses.
Note: The archived tumor tissue specimens may be from prior surgery or from prior diagnostic biopsy of primary or metastatic tumor specimen. Unavailability of archived tissue will not render subject ineligible for study.

3.1.9 Be willing and able to undergo a core or excisional tumor biopsy according to institutional standards (guided visually or by computed tomography [CT] or ultrasound), paracentesis, or thoracentesis for tumor cells.
Note: This is to be done prior to treatment at C1D1 and post-treatment (Cycle 2 Day 8), if this is clinically and safely feasible to do so. This will allow the use of this freshly obtained tissue for correlative analyses in the study.

3.1.10 Females of child-bearing potential (FOCBP) must agree to use adequate contraception (Appendix 1) prior to registration, for the duration of study participation, and for 120 days following completion of therapy. Should a female patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

NOTE: A FOCBP is *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- *Has not* undergone a hysterectomy or bilateral oophorectomy
- *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.11 FOCBP must have a negative pregnancy test ≤7 days prior to registration on study.

3.1.12 Patients must have the ability to understand and the willingness to sign a written informed consent prior to registration on study.

3.2 Exclusion Criteria

3.2.1 Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy ≤14 days prior to registration or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

NOTE: *Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.*
NOTE: *If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.*

3.2.2 Has had a prior anti-cancer monoclonal antibody (mAb) ≤28 days prior to registration or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

3.2.3 Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device ≤28 days prior to registration.

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

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

3.2.6 Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 28 days prior to registration and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging

brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

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

3.2.8 Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. *Please contact the Principal Investigator for further clarification if needed.*

3.2.9 Hypersensitivity to pembrolizumab or any of its excipients.

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

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

3.2.12 Has a known history of Hepatitis B and/or Hepatitis C infection.

3.2.13 Has known history of (non-infectious) pneumonitis that required steroids, or current pneumonitis.

3.2.14 Has an active infection requiring systemic therapy within 3 days of registration (NOTE: except for uncomplicated Urinary Tract Infection (UTI)).

3.2.15 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

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

3.2.17 Has received a live vaccine ≤30 days of planned start of study therapy. *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*

3.2.18 Female patients who are pregnant or nursing, or expecting to conceive within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment. *Subjects should not breast feed ≤120 days of completing the trial.*

3.2.19 Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling or child) who is investigational site or sponsor staff directly involved with this trial, unless prospective IRB approval (by chair or designee) is given allowing exception to this criterion for a specific subject.

4.0 TREATMENT PLAN

4.1 Overview

All subjects will be treated in cycles lasting 21 days (3 weeks). The drugs being used in this study include pembrolizumab and guadecitabine. Guadecitabine 30mg/m^2 will be administered as a subcutaneous injection on Days 1-4 of every 21 day cycle. Pembrolizumab 200 mg will be administered as a 30 minute IV infusion on Day 5 of every 21 day cycle. ***Patients must begin therapy within (+) 14 days of registration.***

For patients on combination therapy, treatment should be initiated on Day 1 of each cycle. NOTE: For patients receiving the combination of guadecitabine and pembrolizumab, guadecitabine dosing on Day 1 of each cycle should always begin on a Monday in order for the patient to receive pembrolizumab on Friday (Day 5). In the event of holidays or scheduling difficulties for administrative reasons, Day 1 of each cycle may be delayed up to +28 days. All treatments will be administered on an outpatient basis. Treatment will continue until disease progression, unacceptable toxicity, patient withdrawal, or death. NOTE: please see section 4.4.1 for conditions under which patients may be permitted to continue on single agent pembrolizumab only. One dose of guadecitabine may be skipped during a cycle due to scheduling constraints with holidays (e.g. if Memorial Day is on a weekday, the patient may skip that dose of guadecitabine). The decision to skip a dose of guadecitabine vs dose delay will be at the discretion of the treating physician.

Please refer to Section 5.2 (table) for treatment details regarding patients on Pembrolizumab monotherapy.

To exclude prohibitive toxicity, a safety run-in cohort of the initial 6 patients will be done. All subjects in the run-in cohort will be observed for at least 2 weeks after completion of cycle 1 for dose limiting toxicity (DLT) (Refer section 4.3)

4.2 Treatment Administration

Agent[2]	Dose	Route	Schedule[2]	Cycle Length
Pembrolizumab	200 mg	IV infusion	Day 5 of each 3 week cycle (Q3W)	21days (3 weeks)
.Guadecitabine[1,2]	30mg/m^2	SQ injection	Days 1-4 of each cycle (QD for 4 days)	

[1] The starting dose for guadecitabine is 30 mg/m^2; in the event that dose reduction is necessary (see 4.4.1), dose level -1 will be 24 mg/m^2. There are no further dose reductions.
Note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy, after 6 months (8 cycles) at the discretion of the treating physician, if the patient has stable disease or response. If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days. Please refer to Section 5.2 (table) for treatment details regarding patients on Pembrolizumab monotherapy.
For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.

[2] After completion of the safety run in, starting on Day 5 of each cycle, growth factor support filgrastim or peg filgrastim can be administered 24 hours after completion of

guadecitabine administration, at the discretion of the treating physician.

4.2.1 Pembrolizumab

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

Pembrolizumab does not require any pre-medications unless clinically indicated for an infusion reaction. Local institutional practices should be followed in case of an infusion reaction.

Please refer section 8.1 and to the separate Pharmacy Manual for specific instructions regarding the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

4.2.2 Guadecitabine

Guadecitabine 30mg/m^2 will be administered as a subcutaneous injection on Days 1-4 of every 3-week cycle. Patients are required to come to the clinic for administration of the drug. Pre-medications are not required.
Baseline weight will be used throughout. Dose will not change with weight changes.
Note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles), at the discretion of the treating physician, if the patient has stable disease or response. For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.

4.3 Safety Run-in Cohort

Drug interaction and increased toxicity is not anticipated; however, there is no clinical experience with the combination of guadecitabine and pembrolizumab. To exclude prohibitive toxicity, up to 6 subjects will be treated with combined therapy as outlined in Table 2. If a subject goes off study before completing the DLT observation period of safety run-in for reasons other than DLT, then that subject should be replaced. **Accrual will be suspended after the initial 6 subjects have been enrolled. All subjects in the run-in cohort will be observed <u>for at least 2 weeks after completion of cycle 1</u> for dose limiting toxicity (DLT) as defined in Table 3 below.** An event must be at least “possibly related” to either Pembrolizumab or Guadecitabine to be considered as DLT

Table 3: Definition of Dose Limiting Toxicities (DLT)

Toxicity Category	Criteria Defining a DLT
Hematological*	Grade 4 treatment-related neutropenia lasting for ≥ 7 days
	Grade 4 treatment-related febrile neutropenia
	Grade 3 treatment-related thrombocytopenia lasting ≥7 days associated with bleeding or Grade 4 treatment-related thrombocytopenia lasting ≥4 days
Non-hematological	Grade ≥3 treatment-related toxicity despite the use of adequate/maximal medical interventions and/or prophylaxis as dictated by local institutional clinical practices or the judgment of the Investigator.
Any toxicity that is possibly, probably or definitely related to study drug that results in a >14 day delay in cycle 2 Day 1 dosing will also be considered a DLT. *No growth factor is allowed	

If 0 or 1 out of 6 subjects in the safety cohort experiences a DLT, accrual to the rest of the study will commence. If 2 or more of 6 subjects in the safety lead in cohort experience a DLT, the study will be suspended and an amendment to explore lower doses or a longer treatment interval (28 day cycle) will be considered.

4.4 Toxicity Management & Dose Delays/Modifications

Any patient who receives at least one dose of study therapy will be evaluable for toxicity endpoints. Each patient will be assessed for the development of toxicity according to the timeframe referenced in the Schedule of Events table. Toxicity will be assessed according to CTCAE v4.03.

This section provides suggested guidelines for the management of various study drug-related toxicities in subjects receiving guadecitabine and pembrolizumab. *(Note: Drug-related toxicity will be defined as any event, which is considered possibly, probably, or definitely related to the drug. It does not include toxicities clearly not related to the drug such as disease progression, environmental, unrelated trauma, etc.)*

The treating investigator should assess to the best of his/her ability whether an adverse event is possibly related to study treatment, and if so, attribute it to guadecitabine only, pembrolizumab only, or both guadecitabine and pembrolizumab, and treat the subject accordingly by only reducing the study drug that has most likely contributed to the individual toxicity necessitating dose reduction. Preferably, only one study drug should be reduced at any one time.

4.4.1 Dose modifications for Guadecitabine

Guadecitabine-related AEs will be managed with dose reductions and/or skipped doses. Guadecitabine dosing should be held for ≥ grade 3 neutropenia, ≥ grade 3 thrombocytopenia or other ≥ grade 3 guadecitabine-related non-hematologic toxicities until toxicity has resolved to ≤ Grade 1 or baseline levels (NOTE: pembrolizumab dosing may continue for that cycle if the toxicity is documented to be related only to guadecitabine). Treatment with guadecitabine may then be resumed for the next cycle at dose level -1 (which is 24 mg/m^2 – see Section 4.2). Missed doses will not be made up. Exceptions for the dose reductions would include neutropenia and anemia which could be managed by growth factors and transfusion, and in that case dosing may be resumed at the same dose level. After completion of the safety run in, starting on Day 5 of each cycle, growth factor support filgrastim or peg filgrastim can be administered 24 hours after completion of guadecitabine administration, at the discretion of the treating physician.

If the subject still has drug-related clinically significant toxicity that has not resolved to ≤ Grade 1 or baseline by the time of the next cycle, dosing for that cycle may be delayed 1 week (7 days). Dosing may restart at dose level -1 (24 mg/m2) once the toxicity has resolved to ≤ Grade 1 or baseline. If toxicity is still not resolved after a delay of 1 week, guadecitabine will be discontinued. If the patient has already completed 2 cycles of combination therapy at this point, the patient may be a candidate for continuing on single-agent pembrolizumab (see below).

If grade 3-4 toxicity reoccurs after treatment has been restarted at dose level -1 (24mg/m2), and the patient has received at least 2 cycles of guadecitabine priming, then guadecitabine can be discontinued permanently and pembrolizumab can be continued as a single agent on the same dose schedule.

Rationale for continuing single-agent pembrolizumab:
There is presumptive scientific rationale that treatment with a hypomethylating agent will cause re-expression of tumor antigens within the first 2 cycles, and thus epigenetic priming, which enhances the effects of immunotherapy. Therefore, it is reasonable to continue treatment with single agent pembrolizumab after epigenetic priming has been induced. If a patient cannot complete at least 2 cycles of guadecitabine and pembrolizumab combination, then she would be discontinued from the study.
Please note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles), at the discretion of the treating physician, if the patient has stable disease or response. For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.

4.4.2 Dose Modifications for Pembrolizumab

Pembrolizumab-related AEs will be managed with dose withholdings/delays. Dose reductions will not be permitted for pembrolizumab. Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3 below.

Table 3 Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over 1- 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve ≤ 28 days of last dose or if corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within ≤ 28 days.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

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

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

Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, subject vacation, and/or holidays). Subjects should be placed back on study therapy in ≤28 days of the scheduled interruption, unless otherwise discussed with the Principal Investigator and NU DMC. The reason for interruption should be documented in the subject's study record.

Note: If dosing is delayed by more than 28 days from the start of the adverse event which is possibly, probably or definitely related to the drug, then the subject will be withdrawn from treatment. It does not include toxicities clearly not related to the drug such as disease progression, environmental, unrelated trauma, etc.)

4.5 Concomitant Medications/Treatments

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

4.5.1 Allowed Concomitant Medications

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

All concomitant medications received ≤14 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered beyond 30 days after the last dose of trial treatment for management of study-related toxicity should be recorded for SAEs and Events of Clinical Interest (ECIs) (refer section:7.3.1.5).

4.5.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than those used for this study
- Radiation therapy
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: intranasal flu vaccines, measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the PI. Short term steroid preparation prior to tumor imaging is permitted for prophylaxis (e.g., contrast dye allergy).
- Subjects who, in the assessment by the Principal Investigator (PI) or treating physician, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the PI or treating physician deems to be medically necessary.
- Pembrolizumab may be associated with Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). Risk factors for SJS and TEN include certain medications such as anticonvulsants, sulfonamides, aminopenicillins, allopurinol, and NSAIDs. These should be taken after consultation with the treating physician. Non-medication triggers include infection, contrast media, and vaccinations. Malignancy is associated with an increased mortality rate in patients with SJS and TEN
- Certain medications such as anthracyclines, alkylating agents and checkpoint inhibitors as well as radiation may be risk factors for immune-mediated myocarditis. These should be taken after consultation with the treating physician.

4.6 Supportive Care with Pembrolizumab

Subjects should receive appropriate supportive care measures as deemed necessary by the treating physician. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with

corticosteroids, as well as, additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes, such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the treating physician determines the events to be related to pembrolizumab.

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

4.6.1 Pneumonitis:

- For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

4.6.2 Diarrhea/Colitis:

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).• All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

- For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

4.6.3 Type 1 Diabetes Mellitus (T1DM)

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

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

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

4.6.4 Hypophysitis:

- For **Grade 2** events (*defined as **moderate** per general CTCAE criteria*), treat with corticosteroids. When symptoms improve to Grade 1 or less (*defined as **mild** per general CTCAE criteria*), steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- For **Grade 3-4** events (*defined as either **severe or medically significant** but not immediately life-threatening, OR **life-threatening** consequences, per general CTCAE criteria*), treat with an initial dose of

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

4.6.5 Hyperthyroidism or Hypothyroidism:

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

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

4.6.6 Hepatic:

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

4.6.7 Renal Failure or Nephritis:

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

4.6.8 SJS and TEN management

- For signs or symptoms of SJS or TEN, withhold KEYTRUDA® and refer the patient for specialized care for assessment and treatment.
- If SJS or TEN is confirmed, permanently discontinue KEYTRUDA®.

4.6.9 Immune-mediated myocarditis management

- For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies, and administer corticosteroids as appropriate.

4.6.10 Management of Infusion Reactions:

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

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4 Infusion Reaction Treatment Guidelines

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

4.7 Diet/Activity/Other Considerations

4.7.1 Diet

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

4.7.2 Use in Nursing Women

It is unknown whether pembrolizumab and guadecitabine are excreted in human milk. Since many drugs are excreted in human milk, and because of

the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment. Starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment, *subjects should not breast feed.*

4.8 Duration of Therapy

Patients may continue to receive cycles of treatment until any of the following occur:

- Disease progression as defined by RECISTv 1.1
- Delay of more than 28 days due to treatment related toxicity (toxicities possibly, probably or definitely related to the drug) will result in discontinuation of study drugs.
  (Note: It does not include toxicities clearly not related to the drug such as disease progression, environmental, unrelated trauma, etc.)
- Development of an inter-current illness that prevents further administration of treatment.
- Unacceptable adverse event(s).
- Patient decides to withdraw from either study treatment or the study as a whole.
- The treating investigator determines that the patient should be taken off treatment for any reason (i.e. changes in condition, inability to comply with study treatment or procedures).
- Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

4.9 Duration of Follow Up

Patients will have the ***first safety follow-up visit*** 30 days (± 7 days) after the last dose of study therapy or before the initiation of a new anti-cancer treatment, whichever is first.

All AEs that occur prior to the Safety Follow-Up Visit (30 days (± 7 days) after the last dose of study therapy) will be recorded. Subjects with an AE ≥ Grade 2 that occurs ≥ 30 days after discontinuation of study therapy and that is attributed (possibly, probably, or definitely) to the agent(s) will be followed until the AE is ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

SAEs *that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded per the guidelines stated above.*

Subjects who discontinue trial treatment for a reason other than disease progression will move into the ***Long-term follow-Up Phase*** and will be assessed every 9 weeks (± 7 days) by radiologic imaging to monitor disease status until the start of new anti-neoplastic therapy, disease progression, death or end of the study. Information regarding post-study anti- neoplastic treatment will be collected if new treatment is initiated.

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject will move into the ***Survival follow-up phase*** and should be contacted *by telephone or email or during clinic visits, every 3 months for the first 2 years after completing study therapy and then every 6 months thereafter to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.*

4.10 Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must

be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted).
- Patient or legal representative (such as a parent or legal guardian) withdraws consent (no follow-up permitted).
- Patient is unable to comply with protocol requirements.
- Patient demonstrates disease progression. This must be confirmed radiographic disease progression. At the first disease assessment, treatment can be continued, even in the presence of radiographic or clinical progression, if in the opinion of the treating physician, the patient is stable or benefitting clinically. In that circumstance a short term follow up scan should be obtained in 4-6 weeks.
  Patient experiences unacceptable toxicity/adverse events.

  (Note: If dosing is delayed by more than 28 days from the start of the adverse event which is possibly, probably, or definitely related to the drug then the subject will be withdrawn from treatment. It does not include toxicities clearly not related to the drug such as disease progression, environmental, unrelated trauma, etc.)

- Treating physician determines that continuation on the study would not be in the patient's best interest.
- Patient becomes pregnant (confirmed positive serum pregnancy test).
- Patient has intercurrent illness that prevents further administration of treatment.
- Patient develops a second malignancy that requires treatment which would interfere with this study.
- Noncompliance with trial treatment or procedure requirements.
- Principal Investigator's decision to withdraw the subject.
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.
  NOTE: *24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the eligibility requirements. For any patient who has limited treatment options, and is currently re-responding to the combination treatment of pembrolizumab and guadecitabine, an additional year of this combination therapy, beyond cycle #35, may be given, subject to review and approval by the PI and drug sponsors Merck and Astex.*

- Conditions outlined in Section 4.8
- Administrative reasons.
- Patient becomes lost to follow-up (LTF) : This is defined as 3 attempts, made 1 week apart, to contact patient by preferred method of contact.

4.11 Patient Replacement

If a patient is enrolled in the study but comes off study prior to the first dose of treatment, the patient may be replaced.

If a subject in the safety run-in portion of the trial goes off treatment before completing the DLT observation period of safety run-in for reasons other than DLT, then a patient will need to be added to the safety run-in.

5.0 STUDY PROCEDURES AND SCHEDULE OF EVENTS

5.1 COMBINATION TREATMENT GUADECITABINE + PEMBROLIZUMAB

	Screening		On Treatment[15]				Off Treatment	
Time Period	**≤28 days prior to registration**	**≤14 days prior to registration**	**Cycle 1[14]**	**Cycle 2**	**Subsequent cycles**	≥ 6 months of treatment[17]	**30 days post last dose[12]**	**Long term Follow-up**
Required Assessments								
Informed Consent	X							
Medical history[1]	X							
Physical exam[2]		X	D1[2]	D1[2]	D1[2]		X	
CBC[3]		X	D1[3]	D1[3]	D		X	
CMP[3]		X	D1[3]	D1[3]	D		X	
CA 125[3]	X		D1[3]	D1[3]	D		X	
Mg, Phos, LDH		X						
PT/INR & aPTT		X						
Thyroid Function and Uric acid[11]		X			D1[11]			
Pregnancy test	X[8]		D1[8]	D1[8]	D1[8]			
Adverse events[16]		X	D1	D1	D1		X	X
Con Meds[16]		X	D1	D1	D1		X	X
CXR or CT of chest[4]	X				X			X[13]
CT or MRI of abdomen & pelvis[4]	X				X			X[13]
Pembrolizumab[15]			D5	D5	D5			
Guadecitabine[15]			D1-4	D1-4	D1-4			
Archival tumor tissue[5]		X						
Blood for DNA analysis[6]			C1D1	C2D8		X[17]		
Blood for cytokine response[7]			C1D1	C2D1	C3D1	X[17]	X	
Blood for PBMC analysis[9]			X	X		X[17]	X	
Tumor biopsy[10]			X	X		X[17]		
Survival status								X
Subsequent therapy								X

1. Includes diagnosis and staging [pathology report and Tumor Node Metastasis (TNM) staging should be collected].

2. Physical exam includes vital signs [blood pressure, weight, height (screening only)] and ECOG performance status. Physical exam will be conducted on Day 1 (+/-4 days) of each cycle and at the safety follow-up visit, which occurs 30 days post last dose.

3. Labs to be done within ≤4 days of D1. Cycle 1 Day 1 CBC and CMP do not need to be repeated if screening labs

were done ≤4 days prior to starting treatment. CA-125 is to be drawn at screening and Day 1 of each cycle. Exceptions would be if CA-125 was drawn within 14 days from the screening visit or within 14 days of visits where doses have been delayed. In these cases, CA-125 will not need to be re-drawn. CBC = Complete Blood Cell Count with diff. CMP = Na, K, Cl, creatinine, BUN; liver function tests (LFTs) to include AST, ALT, total bilirubin, alkaline phosphatase.

4. Tumor response assessment will consist of evaluation by CXR or CT scans of chest and MRI or CT of abdomen and pelvis. Screening radiologic imaging should be done ≤28 days prior to registration. During treatment, radiologic imaging should be done prior to Cycle 3 and cycle 5 and then prior to every third cycle thereafter (e.g. prior to cycles 8, 11, 14, etc.). *Radiologic imaging can be obtained ≤7 days prior to day 1 of the cycle*. The imaging modality selected for each subject should remain the same throughout the study*. Responses of PD, PR, or CR should be confirmed within 4-6 weeks, at the discretion of the treating physician. (Refer to section 6.0 for more details).*

5. Formalin fixed paraffin-embedded tissue will be requested from an archived tumor specimen from all subjects (required to submit, if available). The archived tumor tissue specimens may be from surgery or from a diagnostic biopsy of primary or metastatic tumor specimen, and may be procured up to 3 months from registration. Unavailability of archived tissue will not render subject ineligible for study. See Section 9 and laboratory manual for additional details.

6. Blood for DNA analysis will be collected on Cycle 1 Day 1[≤7days] and Cycle 2 Day 8 [+2 days]. See Section 9 and laboratory manual for additional details.

7. Blood for cytokine response will be collected at pre-treatment on Cycle 1 Day 1(≤4 days), Cycle 2 Day 1(≤4 days), Cycle 3 Day 1(≤4days), and at the safety follow up visit. See Section 9 and laboratory manual for additional details.

8. Pregnancy test required only for females of child-bearing potential ≤7 days prior to registration. Also, pregnancy test will be done on D1 (≤4 days) of each cycle for females of child bearing potential.

9. Blood for PBMC analysis will be collected at pre-treatment Cycle 1 Day 1(≤4days), Cycle 1 Day 5, pre-treatment on Cycle 2 Day 1(≤4 days), Cycle 2 Day 5, and at the safety follow up visit. See Section 9 and laboratory manual for additional details.

10. Tumor biopsies [guided by CT, ultrasound, or physical exam, according to the discretion of the treating physician], and/or paracentesis, and/or thoracentesis will occur after registration and prior to the start of treatment [(Cycle 1 Day 1 (≤7 days)] and Cycle 2 Day 8 (+2 days) if clinically feasible for the subject. See Section 9 and laboratory manual for additional details.

11. Thyroid Function (TSH with reflex testing only if abnormal findings) and uric acid to be performed at screening and D1 (≤4 days) of every odd cycle beginning with Cycle 3.

12. Safety follow-up visit will occur 30 days (± 7 days) after the last dose of study therapy or before the initiation of a new anti-cancer treatment, whichever is first. Please refer to section 7 for tracking and reporting of AEs/SAEs.

13. Subjects who discontinue trial treatment for a reason other than disease progression should be assessed every9 weeks (± 7 days) by radiologic imaging to monitor disease status until the start of new anti-neoplastic therapy, disease progression, death or end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated. Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject should be contacted *by telephone, email, or a clinic visit every 3 months for the first 2 years and then every 6 months thereafter to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.*

14. Cycle 1 day 1 should be started within (+) 14 days following registration.

15. Patients should begin treatment within 14 days of registration. While on treatment, day 1 of each cycle may be delayed up to +28 days from the target date for scheduling purposes and holidays. *NOTE: Due to the dosing of guadecitabine on days 1-4 followed by pembrolizumab on day 5, it is recommended that day 1 of each guadecitabine cycle should occur on a Monday.*

Note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles), at the discretion of the treating physician, if the patient has stable disease or response. If this is done, please follow the pembrolizumab monotherapy dosing table (section 5.2).

16. ConMeds and AE assessment are to be completed from on D1 of each cycle (+/-4 days). ConMeds will also be recorded up to 30 days after the last dose of trial treatment. Concomitant medications administered beyond 30 days after the last dose of trial treatment for management of study-related toxicity should be recorded for SAEs and Events of Clinical Interest (ECIs).
    AE assessments will also be done 30 days after the last dose of trial treatment OR until start of next anti-cancer treatment (whichever is first).
    SAEs will be recorded for 90 days after the last dose of trial treatment.

17. OPTIONAL tests for patients who have been on treatment for ≥6 months. Samples can be collected at any time from ≥6 months and before the safety follow-up visit.
    Tumor biopsies or paracentesis or thoracentesis may be done. This will be guided by CT, ultrasound, or physical exam, according to the discretion of the treating physician. *All samples will be for research purpose.*
    See Section 9 and laboratory manual for additional details.

5.2 PEMBROLIZUMAB MONOTHERAPY

Pembrolizumab monotherapy is indicated for:

- Patients who discontinue guadecitabine after 2 cycles due to toxicity and who continue on pembrolizumab monotherapy.
- Patients who discontinue guadecitabine after 8 cycles due to SD or PR or CR and who continue on pembrolizumab.

In the above cases, D5 will become D1. D1 assessments will now be performed in respect to pembrolizumab D1 dosing instead of guadecitabine D1 dosing.

	On Treatment	Off Treatment	
Time Period	**D1 of each cycle (-/+ 2 days)**	**30 days post last dose[11]**	**Long term Follow-up**
Required Assessments			
Physical exam[1]	D1	X	
CBC[2]	D1[2]	X	
CMP[2]	D1[2]	X	
CA 125[2]	D1[2]	X	
Thyroid Function and Uric acid[3]	D1		
Pregnancy test[4]	D1		
Adverse events[5]	D1	X	X
Con Meds[5]	D1	X	X
CXR or CT of chest[6]	X[6]		X[12]
CT or MRI of abdomen & pelvis[6]	X[6]		X[12]
Pembrolizumab monotherapy[7]	D1		
Archival tumor tissue[8]	X		
Blood for cytokine response[9]	C3D1	X	
Blood for PBMC analysis[10]	X	X	
Survival status			X[12]
Subsequent therapy			X

[1] Physical exam includes vital signs [blood pressure, weight, height (screening only)] and ECOG performance status. Physical exam will be conducted on Day 1 (+/-4 days) of each cycle and at the safety follow-up visit, which occurs 30 days post last dose.

[2] Labs to be done within ≤4 days of D1. CA-125 is to be drawn at Day 1 of each cycle. Exceptions would be if CA-125 was drawn within 14 days of visits where doses have been delayed. In these cases, CA-125 will not need to be re-drawn. CBC = Complete Blood Cell Count with diff. CMP = Na, K, Cl, creatinine, BUN; liver function tests (LFTs) to include AST, ALT, total bilirubin, alkaline phosphatase.

[3] Thyroid Function (TSH with reflex testing only if abnormal findings) and uric acid to be performed at D1 (≤4 days) of every odd cycle beginning with Cycle 3.

[4] Pregnancy test will be done on D1 (≤4 days) of each cycle for females of child bearing potential.

[5] ConMeds and AE assessment are to be completed from on D1 of each cycle (+/-4 days). ConMeds will also be recorded up to 30 days after the last dose of trial treatment. Concomitant medications administered beyond 30 days after the last dose of trial treatment for management of study-related toxicity should be recorded for SAEs and Events of Clinical Interest (ECIs).
AE assessments will also be done 30 days after the last dose of trial treatment OR until start of next anti-cancer treatment (whichever is first).
SAEs will be recorded for 90 days after the last dose of trial treatment.

[6] Tumor response assessment will consist of evaluation by CXR or CT scans of chest and MRI or CT of abdomen and pelvis. During treatment, radiologic imaging should be done prior to Cycle 3 and Cycle 5 and then prior to every third cycle thereafter (e.g. prior to cycles 8, 11, 14, etc.). *Radiologic imaging can be obtained ≤7 days prior to day 1 of the cycle*. The imaging modality selected for each subject should remain the same throughout the study. *Responses of PD, PR, or CR should be confirmed within 4-6 weeks, at the discretion of the treating physician. (Refer to section 6.0 for more details).*

[7] Pembrolizumab 200 mg will be administered as a 30-minute intravenous (IV) infusion every 3 weeks. Pembrolizumab *monotherapy* dosing will be on Fridays (-/+ 2 days) and D1 assessments will now be performed in respect to pembrolizumab D1 dosing instead of guadecitabine D1 dosing.

[8] Formalin fixed paraffin-embedded tissue will be requested from an archived tumor specimen from all subjects (required to submit, if available). The archived tumor tissue specimens may be from surgery or from a diagnostic biopsy of primary or metastatic tumor specimen, and may be procured up to 3 months from registration. Unavailability of archived tissue will not render subject ineligible for study. See Section 9 and laboratory manual for additional details. *(Please contact Principal Investigator or Lead site study- coordinator for any questions or concerns)*

[9] Blood for cytokine response will be collected at pre-treatment on Cycle 3 Day 1(≤4days), and at the safety follow up visit. See Section 9 and laboratory manual for additional details.

[10]Blood for PBMC analysis will be collected at pre-treatment Cycle 1 Day 1(≤4days), Cycle 1 Day 5, pre- treatment on Cycle 2 Day 1(≤4 days), Cycle 2 Day 5, and at the safety follow up visit. See Section 9 and laboratory manual for additional details.

[11]Safety follow-up visit will occur 30 days (± 7 days) after the last dose of study therapy or before the initiation of a new anti-cancer treatment, whichever is first. Please refer to section 7 for tracking and reporting of AEs/SAEs.

[12]Subjects who discontinue trial treatment for a reason other than disease progression should be assessed every 9 weeks (± 7 days) by radiologic imaging to monitor disease status until the start of new anti- neoplastic therapy, disease progression, death or end of the study. Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject should be contacted *by telephone, email, or a clinic visit every 3 months for the first 2 years and then every 6 months thereafter to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.*

6.0 ENDPOINT ASSESSMENT

6.1 Definitions

Response assessments will be made both using the Immune Related Response Criteria (irRC), and using RECIST v1.1 (See table below), allowing additional comparisons among these criteria for disease response assessment. Measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC. Disease progression will be determined using RECIST 1.1 criteria (The Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Eur J Can, 2009.45:p.228-247). Refer to the RECIST1.1 publication for complete details on these criteria. At the first disease assessment, treatment can be continued, even in the presence of radiographic or clinical progression, if in the opinion of the treating physician, the patient is stable or benefitting clinically. In that circumstance, a short-term follow up scan should be obtained in 4-6 weeks.

Immunotherapeutic agents such as pembrolizumab (MK-3475) may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab (MK-3475).

Therefore, RECIST 1.1 will be used with the following adaptations:
If radiologic imaging shows initial PD, tumor assessment should be repeated ≥4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued / resumed. If repeat imaging confirms progressive disease, subjects will be discontinued from study therapy In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions. In subjects who have initial evidence of radiological PD, it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained a minimum of 4 weeks later. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive pembrolizumab (MK-3475) treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease.

Measurable Disease
Measurable disease per RECIST 1.1 is defined as the presence of at least one measurable lesion. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. For irRC, measurable lesions are defined as as those that can be accurately measured in at least two dimensions as 10x10mm with CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant Lymph Nodes
To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable Lesions

For both RECIST 1.1 and irRC, all other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: *Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.*

6.2 Primary Endpoint

Measure ***Objective Response Rate (ORR)*** to guadecitabine and pembrolizumab in patients with recurrent platinum resistant Ovarian Cancer. Any patient who has completed at ***least 2 cycles*** of treatment will be evaluable for this endpoint.

6.3 Secondary Endpoints

Measure progression free survival (PFS) and clinical benefit rate (CBR) to the combination of guadecitabine and pembrolizumab.

PFS will be defined as the time from first dose of study treatment until disease progression or death from any cause. Any patient who has completed at **least 1 cycle of treatment** will be evaluable for PFS endpoint.

Clinical Benefit Rate(CBR) will be defined as patients with CR + PR or SD (SD for at least 3 months). Any patient who has completed at **least 2 cycles of treatment** will be evaluable for CBR endpoint.

Measure toxicity profiles for the combination of guadecitabine and pembrolizumab. The number, type, grade, severity, and frequency of adverse events will be tabulated; adverse events are assessed at least once per cycle. Any patient who receives **at least one dose** of study therapy will be evaluable for toxicity endpoints.

Measure objective response rate (ORR) to the combination of guadecitabine and pembrolizumab using the Immune Related Response Criteria (irRC)
(Note: Response assessments will be made both using the irRC, and using RECIST v1.1, allowing additional comparisons among these criteria for disease response assessment. Measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC. Disease progression will be determined using RECIST 1.1 criteria).

6.4 Exploratory Objectives:

To test the hypothesis that the combination of guadecitabine and pembrolizumab reactivates immune responses by allowing re-expression of tumor antigens silenced epigenetically, we will measure several endpoints:

- NY-ESO-1 and MAGE antigens' promoter methylation (pyrosequencing) and mRNA expression levels (quantitative RT-PCR) will be measured before and after treatment in *DNA (plasma and/or tumor biopsies)* and *RNA (tumor biopsies),* respectively.
- Cytokine response (IFNγ IL2, IL6, IL10, TNFα) will be measured in plasma by ELISA.
- Measure LINE 1 methylation in DNA extracted from PBMCs (D5 vs. D1 of cycles

1 and 2).
- Expression of the PD-L1 ligand will be measured by IHC in archival tumors.
- Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC).

All biological parameters will be correlated with clinical endpoints (ORR and PFS).

6.5 Immune Related Response Criteria (irRC)

6.5.1 Summary of irRC

This study will evaluate concordance of the Immune Related Response Criteria (irRC) with RECISTv 1.1 and OS. These response criteria were developed to overcome the variable and unusual patterns of response to immunotherapeutic agents, in particular, ipilimumab [34]. The development of the guidelines were prompted by observations, mostly in subjects with metastatic melanoma, of initial disease progression followed by later response, late responses, and mixed responses with an overall decrease in tumor burden.

6.5.2 Antitumor response based on total measurable tumor burden:

For the irRC, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden). At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (5 lesions per organ, up to 10 visceral lesions) is calculated. At each subsequent tumor assessment, the SPD of the

index lesions and of new, measurable lesions (up to 5 new lesions per organ; 10 visceral lesions) are added together to provide the total tumor

burden: Tumor Burden = $SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$

Table 5: Comparison of WHO and irRC criteria:

	WHO	irRC
New, measurable lesions	Always represent PD	Incorporated into tumor burden
New, non-measurable lesions	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart

SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

6.5.3 Time-Point Response Assessment Using irRC:

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions
as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD). Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRC were derived from WHO criteria and, therefore, the thresholds of response remain the same. However, the irRC response categories have been modified from those of WHO criteria as detailed above.

6.5.4 Overall Response Using irRC:

The sum of the products of diameters at tumor assessment using the immune-related response criteria (irRC) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

6.5.5 Definition of Index Lesions Response Using irRC

- irComplete Response (irCR): Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as “CR” by the mWHO criteria.
- irPartial Response (irPR): Decrease, relative to baseline, of 50% or greater in the sum of the products of the 2 largest perpendicular diameters of all *index* and all new measurable lesions (i.e., Percentage Change in Tumor Burden). **Note:** the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥ 25% when compared to SPD at nadir.
- irStable Disease (irSD): Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- irProgressive Disease (irPD): At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

6.5.6 Definition of Non-Index Lesions Response Using irRC

- irComplete Response (irCR): Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as “CR” by the mWHO criteria.
- irPartial Response (irPR) or irStable Disease (irSD): *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.
- irProgressive Disease (irPD): Increases in number or size of non-index

lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount).

6.5.7 Impact of New Lesions on irRC

New lesions in and by themselves do not qualify as progressive disease. However, their contribution to total tumor burden is included in the SPD, which in turn feeds into the irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

6.5.8 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- *Immune-Related Complete Response (irCR):* Complete disappearance of *all* tumor lesions (index and non-index together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.

- *Immune-Related Partial Response (irPR):* The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).

- *Immune-Related Stable Disease (irSD):* irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.

- I*mmune-Related Progressive Disease (irPD)*: It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
  - At least 25% increase in the sum of the products of all index lesions over nadir SPD calculated for the index lesions.
  - At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

6.5.9 Immune-Related Best Overall Response Using irRC (irBOR):

irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

Derivation of irRC overall responses:

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),*%	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR[†]
↓100	Stable	Any	irPR[†]
↓100	Unequivocal progression	Any	irPR[†]
↓≥50	Absent/Stable	Any	irPR[†]
↓≥50	Unequivocal progression	Any	irPR[†]
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25	Any	Any	irPD[†]

*Decreases assessed relative to baseline (scan prior to start of any protocol therapy), including measurable lesions only

[†]Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

7.0 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (http://cancer.northwestern.edu/CRO/data/DataandSafetyMonitoringPlanMay2014.pdf). The level of risk attributed to this study requires high, as outlined in the DSMP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (see Section 5 for time points). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Definitions & Descriptions

7.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention,

whether or not related to the intervention.
Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.
Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

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

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.3. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the site investigator to the study drug.

7.2.2 Severity of AEs

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 90 days after the last administration of study drug, must be reported upon discovery or occurrence.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **Results in *death*.**
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- **Is *life-threatening*.**

  The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- **Requires *in-patient hospitalization* or *prolongation of existing hospitalization* for ≥ 24 hours.**
- **Results in *persistent or significant disability or incapacity*.**
- **Is a *congenital anomaly/birth defect.***
- **Is an *important medical event*.**
  Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.
- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
  - Is a new cancer (that is not a condition of the study);
  - Is associated with an overdose.

7.2.4 Unanticipated Problems Involving Risks to Subject or Others

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is *unanticipated* in terms of nature, severity, or frequency
- Places the research subject or others at a different or *greater risk of*

harm

- Is deemed to be *at least possibly related* to participation in the study.

7.2.5 Suspected Adverse Reaction (SAR)

A SAR is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

7.2.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is a SAR that is serious and unexpected (not consistent with the applicable product information).

7.2.7 Definition of Overdose according to Merck

For purposes of reporting overdose in this trial, Merck considers an overdose of pembrolizumab to be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). *NOTE: NU IRB requires reporting of overdose of any amount as Reportable New Information*. No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

7.3 Adverse Event Reporting

7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF. Routine AEs will be reviewed by the Data Monitoring Committee (DMC) according to the study's phase and risk level, as outlined in the DSMP.

7.3.2 Determining if Expedited Reporting is Required

This includes all events that occur within 30 days of the last dose of protocol treatment. Any ≥ Grade 2 event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly, until the AE is ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

1) Identify the type of adverse event using the NCI CTCAE v 4.03.
2) Grade the adverse event using the NCI CTCAE v 4.03.
3) Determine whether the adverse event is related to the protocol therapy.
   Attribution categories are as follows:
   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.
4) Determine the prior experience of the adverse event.
   Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:
   - the current protocol
   - the drug package insert
   - the current Investigator's Brochure

   All AEs considered related to study drug will be followed until

resolution to ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.

7.3.3 Expedited Reporting to the Northwestern University QAM/DMC

SAEs will be reported from the time of informed consent through 90 days following last dose of study drug, or the initiation of new anti-cancer therapy, whichever comes first regardless of whether or not the event(s) are considered related to the study drug.

All AEs and SAEs will be recorded in the subject's medical record

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Completion of the NU CRO SAE Form, provided as a separate document, is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the DMC at their next meeting.

7.3.4 Expedited Reporting to the Northwestern University IRB

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

7.3.5 Expedited Reporting to the FDA (To be done by QAM)

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

7.3.6 Expedited Reporting to Merck and Astex (To be done by coordinator)

SAEs will be reported to Merck **within one business day** of acknowledgement of the event, using the NU CRO SAE form. Follow-up information will be provided to Merck as reasonably requested. SAE/ECI

Submission Forms and any other relevant safety information are to be forwarded to the **Merck** Global Safety facsimile number: +1-215-993-1220.

SAEs related to guadecitabine will be reported to Astex **within one business day** of acknowledgement of the event, using the NU CRO SAE form. In addition, the Astex SAE Fax Cover Sheet should be used when faxing the form to Astex. Follow-up information will be provided to Astex as reasonably requested. SAE/ECI Submission Forms and any other relevant safety information are to be forwarded to the Astex Drug Safety facsimile number: 1-800-576-6568 or emailed to drugsafety@astx.com

7.3.7 Additional Merck Definitions and Reporting Requirements

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

Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported using the NU CRO SAE form within 24 hours to the PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).
For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the PI and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

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

Events of clinical interest for this trial include:

- an overdose of Merck product, as defined in Section 7.2.1 that is not associated with clinical symptoms or abnormal laboratory results.

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

***NOTE:** These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.*

Reporting of Overdose to Merck

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event,

even if no other seriousness criteria are met. If a dose of Merck's product meeting the protocol definition of overdose (refer section 7.2.1) is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."
All reports of overdose with and without an adverse event must be reported using the NU CRO SAE form within 24 hours to the PI and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

(*Note: This does not apply to Northwestern University, because, as per NU IRB regulations, any amount of overdose will be reported as a reportable New Information [RNI])(Refer Section 11.7.2).*

Reporting of Pregnancy and Lactation to Merck

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

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

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

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

8.0 DRUG INFORMATION

8.1 Pembrolizumab

8.1.1 Other names

Pembrolizumab; MK-3475 [Anti-PD-1 Antibody MK-3475])

8.1.2 Classification - type of agent

Humanized X PD-1_mAb (H409A11) IgG4

8.1.3 Mode of action

Highly selective anti-PD-1 humanized monoclonal antibody which inhibits programmed cell death-1 (PD-1) activity by binding to the PD-1 receptor on T-cells to block PD-1 ligands (PD-L1 and PD-L2) from binding. Blocking the PD-1 pathway inhibits the negative immune regulation caused by PD-1 receptor signaling (Hamid, 2013). Anti-PD-1 antibodies (including pembrolizumab) reverse T-cell suppression and induce antitumor responses.

8.1.4 Storage and stability

Pembrolizumab is provided as a white to off-white lyophilized powder (50 mg/vial) or as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only. Pembrolizumab Powder for Solution for Infusion, 50 mg/vial, is reconstituted with sterile water for injection prior to use. Pembrolizumab is formulated with L-histidine as buffering agent, polysorbate 80 as surfactant, sucrose as stabilizer/tonicity modifier, and hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary). The drug product is stored as a stable lyophilized powder or liquid solution under refrigerated conditions (2°C - 8°C).

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

Store Pembrolizumab vials under refrigeration at 2 to 8 degrees Celsius (C) or 36 to 46 degrees Fahrenheit (F). Do not freeze; do not shake.
Storage following dilution: Store at room temperature for up to 6 hours or refrigerated at 2—8 degrees C (36—46 degrees F) for up to 24 hours. This includes room temperature storage of the diluted infusion solution and the duration of infusion). Do not freeze.

8.1.5 Protocol dose specifics

Pembrolizumab 200 mg will be administered as a 30-minute intravenous (IV) infusion every 21 days (3 weeks).

8.1.6 Preparation

Please refer to the Pharmacy Manual for a comprehensive description of pembrolizumab preparation.

The product after reconstitution with sterile water for injection and the liquid drug product is a clear to opalescent solution, which may contain extraneous and proteinaceous particulates. The reconstituted product and liquid product is intended for IV administration. The reconstituted drug product solution and liquid drug product can be further diluted with normal saline or 5% dextrose in the concentration range of 1 to 10 mg/mL in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be used immediately to prepare the infusion solution in the IV bag, and the infusion solution should be administered immediately. If the diluted pembrolizumab solution is not used immediately, it may be stored for no more than 24 hours at 2°C to 8°C. This 24-hour total hold time from reconstitution may include up to 6 hours at room temperature (at or below 25°C).

8.1.7 Route of administration for this study

Intravenous (IV)

8.1.8 Incompatibilities:

None known

8.1.9 Availability & Supply

Merck will provide investigational supply pembrolizumab directly to Northwestern University Investigational pharmacy, at no charge to subjects participating in this clinical trial.

The contact for drug ordering will be Sloan Stribling (sloan_stribling@merck.com). The Merck Drug Request Form should be

completed and emailed to these contacts.

Pembrolizumab is available in two formulations from Merck as summarized below. NOTE: the primary formulation that will be supplied in this study is the pembrolizumab 100 mg/4mL solution for injection.

Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

8.1.10 Side effects

Please see current Investigator's Brochure for a complete list of adverse events.

Pembrolizumab is generally well tolerated and demonstrates a favorable safety profile in comparison to chemotherapy. Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events are of primary concern. Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis, colitis, thyroid disorders (hypothryoidism/hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitis, uveitis, and nephritis. After a recent review of data, events newly characterized as identified risks also include pancreatitis, myositis, and severe skin reaction; these are included in the reference safety information in the current IB. The majority of immune-mediated adverse events were mild to moderate in severity, were manageable with appropriate care, and rarely required discontinuation of therapy.

In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated; these are also further described in the current IB.

8.1.11 Nursing implications

Pembrolizumab will be administered as an IV infusion ((dilution as stated above) over approximately 30 minutes Every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

When an IV bag is used for the infusion, the IV line will be flushed with a volume of normal saline equal to the priming volume of the infusion set used after the contents of the IV bag are fully administered.

Since the compatibility of w i t h other IV medications and solutions, other than normal saline (0.9% [w/v] sodium chloride for injection) and D_5W, is not known, the Pembrolizumab solution should not be infused through an IV line in which other solutions or medications are being administered. The date, start time, interruption, and completion time of Pembrolizumab administration must be recorded in the source documents.

8.1.12 Return and Retention of Study Drug

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

of the trial.

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

8.2 Guadecitabine

8.2.1 Other names:

SGI-110

8.2.2 Classification - type of agent

Sodium (2R,3S,5R)-5-(4-amino-2-oxo-1,3,5-triazin-1(2H)-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl((2R,3S,5R)-5-(2-amino-6-oxo-1H-purin-9(6H)-yl)-3-hydroxytetrahydrofuran-2-yl)methyl phosphate

Guadecitabine is a dinucleotide incorporating the approved drug, decitabine, and purine nucleoside, deoxyguanosine

8.2.3 Mode of action:

DNA methyltransferase inhibitor targeting
DNA (cytosine-5-)-methyltransferase 1 (DNMT1)

Targets and reverses aberrant DNA hypermethylation. Guadecitabine-mediated demethylation of DNA upregulates tumor-associated antigens, and may sensitize tumor cells to other anticancer agents, including immunotherapeutics, as well as resensitizing resistant cancer cells to chemotherapeutics.

8.2.4 Storage and stability:

Guadecitabine product is supplied in a two-vial configuration:

- SGI-110 (guadecitabine) for Injection (SGI-110), 100 mg or 156 mg, containing lyophilized guadecitabine drug powder for reconstitution; and
- SGI-110 Diluent for Reconstitution (Diluent), 3 mL or 1.2 mL or 1.4 mL.

SGI-110 (guadecitabine) 100 mg and 156 mg vials should be stored under refrigerated conditions of 2 - 8°C (36°- 46°F) in the original packaging in an upright position until use. SGI-110 Diluent, 3 mL should be stored at 2° - 30°C (36°- 86° F); 1.2 mL and 1.4 mL should be stored at 2 - 8°C (36°- 46°F) in upright position until use. Both vials are preservative free and for single use only.

Upon reconstitution, the admixed product is stable for 10 days under refrigerated conditions of 2–8°C (36°-46° F), either in the vial or in syringes. Therefore, if desired, sites may prepare syringes for all treatment days in a dosing cycle in advance (as institutional procedures allow).

Please refer to pharmacy manual Table "Temperature Excursion Guidelines for SGI-110 and Diluent While Stored On Site" for instructions on temperature excursions outside the required storage conditions for SGI-110 for Injection and SGI-110 Diluent for Reconstitution.

Guadecitabine must be stored in a secure, locked facility accessible only to authorized study personnel

Please refer to current pharmacy manual for additional information.

8.2.5 Protocol dose specifics:

Guadecitabine 30mg/m^2 will be administered as a subcutaneous injection on Days 1-4 of every 3 week cycle.
(*Note: Baseline weight will be used throughout. Dose will not change with weight changes.)*
Note: Guadecitabine priming can be discontinued and the patient can continue on pembrolizumab monotherapy after 6 months (8 cycles), at the discretion of the treating physician, if the patient has stable disease or response. If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days).
For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable.

8.2.6 Preparation

Please refer to the Pharmacy Manual of SGI-110 (guadecitabine) for a comprehensive description of guadecitabine preparation.

OSHA Guidelines for handling cytotoxic drugs outlined in the American Journal of Hospital Pharmacy must be followed [23]. As with other potentially toxic anti-cancer agents, care should be exercised in the handling and preparation of guadecitabine. The use of gloves and protective garments is recommended. Preparation should occur in a vertical laminar flow biological hood or per institutional standards using proper aseptic technique. If guadecitabine SGI-110 contacts the skin, it should be immediately be treated with borax buffer solution pH 10 followed by washing immediately and thoroughly with soap and water. If guadecitabine contacts the mucous membranes, flush thoroughly with water.

Drug spilling can be inactivated by 2 N sodium hydroxide solution or Water and Kericide CR Biocide B which consist of a blend of stabilized chlorine dioxide and a quaternary ammonium compound.
Disinfect biological safety cabinet by wiping surfaces with 70% isopropanol or ethanol solution or by established pharmacy standards.

Note: Please follow your institutional SOPs regarding any additional requirements or processes.

Reconstituted drug product is intended for subcutaneous administration at a maximum concentration of 100 mg/mL. The required volume from the diluent vial is drawn into a syringe for reconstitution and emptied into the vial containing guadecitabine for injection.

8.2.7 Route of administration for this study

Subcutaneous. Please refer to pharmacy manual for more details on SGI-110 Administration.

8.2.8 Incompatibilities:

None known

8.2.9 Availability & Supply

Astex will supply guadecitabine directly to Northwestern University at no charge to subjects participating in this clinical trial. Investigational product resupply will need to be requested at least 2 weeks in advance Site-specific drug order forms will be provided prior to study activation and will be used to order drug supply directly from Astex.

8.2.10 Side effects

Please see current Investigator's Brochure for a complete list of adverse events.

8.2.11 Nursing implications

Ice packs can be used for painful injection site as a comfort measure

9.0 CORRELATIVES/SPECIAL STUDIES

To test the hypothesis that the combination of guadecitabine and pembrolizumab reactivates immune responses by allowing re-expression of tumor antigens silenced epigenetically, we will measure several endpoints:

- NY-ESO-1 and MAGE antigens' promoter methylation (pyrosequencing) and mRNA expression levels (quantitative RT-PCR) will be measured before and after treatment in DNA (plasma and/or tumor biopsies) and RNA (tumor biopsies), respectively.
- Cytokine response (IFNγ IL2, IL6, IL10, TNFα) will be measured in plasma by ELISA.
- Measure LINE 1 methylation in DNA extracted from PBMCs (Days 1 and 5 of cycles 1 and 2)
- Expression of the PD1 ligands (L1) will be measured by IHC in archival tumors.
- Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC). All biological parameters will be correlated with clinical endpoints (RR and PFS).

Please see the Laboratory Manual (LM) for specific details on correlative study procedures, handling, preserving, and shipping. The table below summarizes the tests that will be performed for correlative endpoints:

Correlative Samples summary				
Correlative study (sample type)	Archival tissue	Fresh biopsy or paracentesis or thoracentesis.	PBMC	Plasma
Mandatory or Optional	Mandatory	Mandatory except the sample collected at >=6 months which is optional	Mandatory except the sample collected at >=6 months which is optional	Mandatory except the sample collected at >=6 months which is optional

Timing (+/- windows)	Baseline (may be procured up to 3 months from registration)	Pre-treatment C1D1 (≤7 days) but after registration); C2D8 (+2 days) and ONE OPTIONAL sample collection at anytime from ≥6 months and before the safety follow-up visit.	Pre-treatment C1D1(≤4 days); C1D5; Pre-treatment C2D1(-≤4 days); C2D5; Follow up visit; and ONE OPTIONAL sample collection at anytime from ≥6 months and before the safety follow-up visit.	For cytokine response Pre-treatment C1D1 (≤4 days); C2D1 (≤4 days) C3D1 (≤4 days) and at Follow up visit. For DNA analysis C1D1(≤7days); C2 D8(+2 days)-at the time of biopsy /paracentesis/ thoracentesis) and ONE OPTIONAL sample collection for both cytokine response and DNA analysis, at any time from ≥6 months and before the safety follow-up visit.
Volume Needed (blood only)	NA	NA	5-8ml	5-8mL
Tube type needed (blood only)	NA	NA	Sodium citrate	Sodium citrate
Tissue thickness and/or # slides (tissue only)	5-6 microns 6-10 slides	3 core biopsies for research. Tissue should be tested by pathology for tumor confirmation		NA
Processing center		IR	PCF	PCF

Sample handling/ processing instructions	RT, ship as described	The first core will be snap frozen upon collection. The second core will be formalin-fixed, paraffin-embedded (FFPE). The third core will be snap frozen upon collection. Freshly collected paracentesis/ thoracentesis samples will be sent immediately to the Matei lab for processing.	Separate PBMCs and plasma. Freeze PBMCs	Freeze after plasma separation
Shipping/delivery info		Matei Lab Lurie 4-220	Matei Lab Lurie 4-220	Matei Lab Lurie 4-220
Storage needs	Matei Lab Lurie 4-220	Matei Lab Lurie 4-220	Matei Lab Lurie 4-220	Matei Lab Lurie 4-220
Analysis center	Qualtek and Matei Lab	Matei Lab	Matei Lab	Matei Lab
Assay methodology	IHC	DNA, RNA extraction	DNA extraction	DNA extraction Cytokine ELISA

The archived tumor tissue specimens may be from surgery or from a diagnostic biopsy of primary or metastatic tumor specimen, and may be procured up to 3 months from registration study. Unavailability of archived tissue will not render subject ineligible for study. Subjects will be consented to optional storage of any remaining tumor and plasma samples after protocol- specified studies are complete. Stored tumor and plasma samples will be reserved for future cancer-related research. Tumor and plasma samples for future use will be stored at Northwestern University's local laboratory:
Matei Lab
303 E. Superior St, Lurie 4-220
Chicago, IL 60611
Phone: 312-472-4684

10.0 STATISTICAL CONSIDERATIONS

Statistical analysis of this study will be the responsibility of the Biostatistics Core Facility of the Robert H. Lurie Comprehensive Cancer Center.

10.1 Study Design

This is a multi-center non-randomized, open label phase II study. Study endpoints are defined in Section 2.0. A safety run-in cohort has been designed for the first six subjects enrolled, in order to ensure the safety of this experimental combination of

guadecitabine and pembrolizumab.

10.2 Sample Size and Accrual

We will utilize the optimum two-stage design and we anticipate enrolling 35 evaluable patients. This two-stage design to test the null hypothesis that RR<=0.10 versus the alternative that RR>=0.30 (where RR is calculated via RECIST v1.1) has an expected sample size of 22.5 and a probability of early termination of 0.71 when RR=0.10. If the drug combination is actually not effective, there is a 0.047 probability of concluding that it is (the target for this value was 0.050). If the drug is actually effective, there is a 0.098 probability of concluding that it is not (the target for this value was 0.100). Subjects who are not evaluable for response will be replaced so up to 46 subjects may be enrolled.

10.3 Data Analysis Plans

10.3.1 Analysis Plans for Primary Objective

The primary outcome is objective response by RECIST v1.1. The proportion of patients having an objective response by RECIST 1.1 will be estimated with a 95% exact binomial confidence interval for the efficacy population. These confidence intervals will be adjusted for the sequential nature of the two-stage procedure [24].

10.3.2 Analysis Plans for Secondary Objectives

Confidence intervals for the clinical benefit rate will be calculated similarly to RR. Progression-free survival times will be estimated using the Kaplan-Meier method. The objective response rate by irRC will be estimated similarly. Confidence intervals on the median will be constructed. Toxicities will be tabulated.

10.3.3 Analysis Plans for Exploratory Objectives

All biological parameters other than PD-L1 ligand expression will be measured pre and post treatment (either in plasma or tumor) or Day 1 and Day 5 (LINE1 methylation in PBMCs). Pre-treatmentvs post-treatment or Day 1 to Day 5 comparisons will be made by paired t-tests (or Wilcoxon Signed-Rank tests if the data are not normally distributed). PD-L1 ligand expression from archival tissue will be summarized descriptively. Pre-treatment (including PD-L1 ligand expression) and pre-post changes in parameters will be correlated
with response by Fisher-Exact test for RR, CBR, and log rank test for PFS (using the maximal chi-square cut-point for the parameter).

10.4 Interim Analysis/Criteria for Stopping Study

10.4.1 Safety Criteria

The first 6 patients will be part of an initial safety run-in. A safety analysis will be done after all six patients have been observed for at least 2 weeks after completion of cycle 1. All toxicities will be tabulated. The study will move forward if ≤ 1 of 6 in the safety lead in cohort 1 experience a DLT (as defined in Section 4.3 and Table 3). If ≥ 2 of 6 in cohort 1 experience a DLT, the study will be suspended and an amendment to explore alternate dosing schemes will be considered. Toxicity counts and rates and the results of the analysis will be reviewed by the DMC. The patients enrolled in the safety lead in cohort will be included in the efficacy analysis.

10.4.2 Efficacy Criteria

As this is a two-stage design, an interim efficacy analysis is planned after 18 patients are evaluable (see 10.2 for additional details). After testing the drug on 18 patients in the first stage, consideration will be given to trial termination if 2 or fewer patients demonstrate clinical benefit (CR, PR, or SD for at least 3 months). If the trial goes on to the second stage, a total of **35 evaluable**

patients will be studied. If the total number responding is less than or equal to 6, the combination is rejected.

11.0 STUDY MANAGEMENT

11.1 Institutional Review Board (IRB) Approval and Consent

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

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

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

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by Merck and Astex.

11.3 Registration Procedures

11.3.1 Registering a Patient for the safety Run-in portion of the Study

For potential patients for the phase I portion of this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.3.2 Registering a Patient to the Study (after safety run-in)

For potential patients enrolled after the safety run-in portion of this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive a subject identification number:

• Eligibility eCRF (complete in NOTIS)
• Eligibility checklist (signed and dated by the treating physician – upload in NOTIS)
• Signed and dated informed consent document (upload in NOTIS)
• Pathology Report (upload in NOTIS)

The QAM will review the registration, register the patient, assign an identification number, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.4 Data Submission

Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document). Generally, all data for Safety run-in patients during the time period patients are evaluated for Dose Limiting Toxicities (DLTs) must be submitted on a weekly basis. Generally, for all phase II patients, data are due at the end of every cycle.

Please contact croqualityassurance@northwestern.edu for further details.

11.5 Instructions for Participating Sites

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Research Office at Northwestern University:

- Signed and completed Letter of Invitation to participate in the study.
- Signed copy of Northwestern University's Data Monitoring Committee policy pertaining to data submission.
- Draft informed consent form should for review/approval prior to submission to the local IRB
- A copy of the official IRB approval letter for the protocol and informed consent.
- CVs and medical licensure for the local PI and any sub-investigators who will be involved in the study at the site.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation.

Additional activities may be required prior to site activation (i.e. contract execution, study-specific training). Full requirements will be outlined in a memo upon receipt of the signed Letter of Invitation.

11.6 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to NOTIS for additional information). The level of risk attributed to this study requires High Intensity Monitoring as outlined in the DSMP (http://cancer.northwestern.edu/CRO/data/DataandSafetyMonitoringPlanMay2014.pdf). The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the NOTIS for additional data submission instructions.

11.7 Adherence to the Protocol

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

11.6.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

11.6.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.
- Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation may be considered an instance of Reportable New Information (RNI) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.8 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration

of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

11.9 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the DSMC Data Release Policies and Processes. The assigned QAM will prepare a preliminary data set for DSMC approval no later than 3 months after the study reaches its primary completion date, as defined by ClinicalTrials.gov. This is the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated. If the investigator would like data release to be approved by the DSMC prior to when study design specifies, and/or prior to three months after a study's primary completion date, the PI must send a written request for data approval to the QAM which includes justification. Requests must be made a minimum of six to eight weeks in advance of the expected deadline. The request will be presented to the DSMC at their next available meeting. Any DSMC decisions regarding data release will be provided to the PI. If the request is approved, the QAM will present the data set to the DSMC for approval. A final, DSMC-approved dataset, as applicable, will then be released 6-8 weeks after the request was made. The investigators are expected to use only DSMC-approved data and statistical analyses any time they are disseminating trial data. The investigators must send a copy of the draft abstract/poster/manuscript to the study's biostatistician and assigned QAM to confirm that the DSMC-approved data and statistical analyses are used appropriately. Once the biostatistician and QAM gives final approval, the publication may be submitted to external publisher.

REFERENCES

1. Adams, S.F.*, et al.* Intraepithelial T cells and tumor proliferation: impact on the benefit from surgical cytoreduction in advanced serous ovarian cancer. *Cancer* **115**, 2891-2902 (2009).
2. Webb, J.R., Milne, K., Watson, P., Deleeuw, R.J. & Nelson, B.H. Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated with increased survival in high-grade serous ovarian cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **20**, 434-444 (2014).
3. Bukowski, R.M., Ozols, R.F. & Markman, M. The management of recurrent ovarian cancer. *Seminars in oncology* **34**, S1-15 (2007).
4. Vaughan, S.*, et al.* Rethinking ovarian cancer: recommendations for improving outcomes. *Nat Rev Cancer* **11**, 719-725 (2011).
5. Chan, M.W.*, et al.* Hypermethylation of 18S and 28S ribosomal DNAs predicts progression-free survival in patients with ovarian cancer. *Clin Cancer Res* **11**, 7376-7383 (2005).
6. Brahmer, J.R.*, et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *The New England journal of medicine* **366**, 2455-2465 (2012).
7. Hamanishi, J.*, et al.* Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 3360-3365 (2007).
8. Krempski, J.*, et al.* Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. *Journal of immunology* **186**, 6905-6913 (2011).
9. Duraiswamy, J., Freeman, G.J. & Coukos, G. Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer. *Cancer research* **73**, 6900-6912 (2013).
10. Balch, C., Fang, F., Matei, D.E., Huang, T.H. & Nephew, K.P. Minireview: epigenetic changes in ovarian cancer. *Endocrinology* **150**, 4003-4011 (2009).
11. Matei, D.E. & Nephew, K.P. Epigenetic therapies for chemoresensitization of epithelial ovarian cancer. *Gynecol Oncol* **116**, 195-201 (2010).
12. Balch, C.*, et al.* New anti-cancer strategies: epigenetic therapies and biomarkers. *Front Biosci* **10**, 1897-1931 (2005).
13. Kantarjian, H.*, et al.* Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood* **109**, 52-57 (2007).
14. Samlowski, W.E.*, et al.* Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation. *J Clin Oncol* **23**, 3897-3905 (2005).
15. Matei, D.*, et al.* Epigenetic resensitization to platinum in ovarian cancer. *Cancer Research* **72**, 2197-2205 (2012).
16. Yoo, C.B.*, et al.* Delivery of 5-aza-2'-deoxycytidine to cells using oligodeoxynucleotides. *Cancer Res* **67**, 6400-6408 (2007).
17. Gini Fleming, S.G., Yvonne Lin, Angeles Alvarez Secord, ... Mohammad Azab, Daniela Matei. Clinical epigenetic resensitization of platinum-resistant, recurrent ovarian cancer patients with SGI-110, a novel, second-generation, subcutaneously administered hypomethylating agent (HMA). *AACR National Meeting; Proceedings* (2014).
18. Garon, E.B.*, et al.* Pembrolizumab for the Treatment of Non-Small-Cell Lung Cancer. *N Engl J Med* (2015).
19. Robert, C.*, et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* (2015).
20. Foulks, J.M.*, et al.* Epigenetic drug discovery: targeting DNA methyltransferases. *J Biomol Screen* **17**, 2-17 (2012).
21. Singh, V., Sharma, P. & Capalash, N. DNA methyltransferase-1 inhibitors as epigenetic therapy for cancer. *Curr Cancer Drug Targets* **13**, 379-399 (2013).
22. Jean Pierre Issa, G.R., David Rizzieri, Stefan Faderl, Casey O'Connell, Wendy Stock, Raoul Tibes, Elizabeth Griffiths, Karen Yee, Woonbok Chung, Gavin Choy, Aram Oganesian, Pietro Taverna, Mohammad Azab, Hagop Kantarjian. Interim results from a randomized Phase 1-2

first-in-human (FIH) study of PK/PD guided escalating doses of SGI-110, a novel subcutaneous (SQ) second generation hypomethylating agent (HMA) in relapsed/refractory MDS and AML *Porceedings of AACR Meeting* **LB-214** (2012).

23. Yodaiken, R.E. & Bennett, D. OSHA work-practice guidelines for personnel dealing with cytotoxic (antineoplastic) drugs. Occupational Safety and Health Administration. *Am J Hosp Pharm* **43**, 1193-1204 (1986).
24. Atkinson EN & Brown BW. Confidence limits for probability of response in multistage Phase II clinical trials. Biometrics 1985; 41:741-744.)

APPENDICES

Appendix 1: Birth control methods

Barrier Methods	Intrauterine Device Methods	Hormonal Methods
• Male condom plus spermicide • Cap plus spermicide • Diaphragm plus spermicide	• Copper T • Progesterone T • Levonorgestrel-releasing	• Implants • Hormone shot or injection • Combined pill • Minipill • Patch

NOTE: choice contraception should be discussed with primary treating oncologist to discuss the risks and benefits of different modalities of contraception.

APPENDIX 2: ECOG PERFORMANCE STATUS

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

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

APPENDIX 3: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST) 1.1 CRITERIA FOR EVALUATING RESPONSE IN SOLID TUMORS

RECIST version 1.1* will be used in this study for assessment of tumor response.

* As published in the European Journal of Cancer:
E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

Can be accessed at:
http://ctep.cancer.gov/protocolDevelopment/docs/recist_guideline.pdf

APPENDIX 4: EVALUATING ADVERSE EVENTS

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

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

APPENDIX V: HISTORY OF AMENDMENTS (SUMMARY OF CHANGES)

Amendment 1 –August 10, 2016 (*includes FDA mandated changes)*			
Sections(s) Affected	***Prior Version***	***Amendment 1 Changes***	***Rationale***
Section 4.3,4.4,4.8,4.10	Drug-related toxicity mentioned in these sections but was not defined.	Drug-related toxicity defined and language inserted as: 'Drug related toxicity will be defined as any event which is considered possibly, probably, or definitely related to the drug. It does not include toxicities clearly not related to the drug such as disease progression, environmental, unrelated trauma, etc.' This has been defined in section 4.4 and similar language has been inserted in all other appropriate sections.	***Per FDA request during initial review***
Exclusion criteria 3.2.13	Has known history of, or any evidence of active, non-infectious pneumonitis	Has known history of (non-infectious) pneumonitis that required steroids, or current pneumonitis.	***Per action letter for MK-3475 (pembrolizumab, NSC 776864) dated June 24, 2016***
Study parameters (5.0)	Specified TSH, T4, and free T3 for baseline thyroid testing.	Revised to TSH with reflex testing only if abnormal findings noted.	***Clarification***
Section 9.0 Correlative /Special studies	Details regarding ADA antibodies analysis inserted in the table 'Correlative Sample summary'	The entire column containing these details have been removed	***Administrative correction (removal of test that is no longer being done and was mistakenly noted there still).***
Amendment 2 12/16/2016			
Sections(s) Affected	Prior Version	Amendment 1 Changes	***Rationale***
Protocol page	Statistician Alfred Rademaker No affiliate sites listed.	Removed Alfred Rademaker as statistician Added: Masha Kocherginsky as statistician. Contact information included. Added: University of Chicago as affiliate site. Dr. Gini Fleming added as site PI. Contact information inserted.	***Per PI***

Study schema Section 3.0(eligibility) & Section 10.1 Statistical section-study design	Study described as single center	Modified to : Multicenter	***Inclusion of University of Chicago as affiliate site. Potential for more sites to be included.***
Throughout	Grammatical and spelling errors, typos and wrong reference to sections	Corrected grammatical errors, spellings and typos and inserted the correct reference to sections at appropriate places	***Correction of error***
Throughout	Certain sections had "prior to Day1 of study" as the starting time point.	changed to 'prior to registration'	***For convenience and consistency***
Study summary and Section 3.0(eligibility criteria)	RHLCC listed as only site	Modified to state that Lead site: RHLCCC Affiliate site: University of Chicago Language added in section 3.0 to state the same.	***Inclusion of University of Chicago as affiliate site***
Inclusion criteria3.1.1 and study summary	Inclusion criteria: "Histological or cytological evidence/confirmation of recurrent epithelial ovarian cancer, primary peritoneal carcinomatosis, or fallopian tube cancer."	Deleted the word "recurrent" here	***To clarify that the patient does not need histological confirmation of <u>recurrent</u> disease, just of disease***
Inclusion criteria 3.1.3 and study summary	Platinum resistant disease mentioned in the title and study summary , but was not included as an inclusion criteria in section 3.1	Inserted language as inclusion criteria 3.1.3 "Patients must have recurrent platinum-resistant disease, defined as progression < 6 months after completion of platinum-based chemotherapy or as persistent disease that remains after completing the most recent line of platinum-based therapy. The platinum-free interval should be calculated from the last administered dose of platinum therapy." Study summary language also modified to state the same.	***For clarity and consistency. Note: <u>Recurrent disease</u> has been used here and defined.***

Section 2.1, 2.2 and 6.3 Primary and Secondary objectives and Endpoints	Primary objective included irRC assessment as well.	Removed language about irRC from primary objective. Added secondary endpoint 2.2.4 and also in section 6.3 "Measure objective response rate (RR) to the combination of guadecitabine and pembrolizumab using the Immune Related Response Criteria (irRC). *(Note: Response assessments will be made both using the irRC, and using RECIST v1.1, allowing additional comparisons among these criteria for disease response assessment. Measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC. Disease progression will be determined using RECIST 1.1 criteria)"* *Section 2.2.3 added in section 2.2 to include secondary endpoint that was already in section 6.2 but was excluded in error in section 2.2*	***Per PI*** ***irRC is to be used only for secondary objective. Decision to go on to stage II of the study will be based on assessment using RECIST1.1***
Study summary and Section 4.1(treatment administration	Trial treatment may be administered up to 1 week (+7 days) before or after the scheduled Day 1 of each cycle due to administrative reasons	deleted "before or after" and changed to "after." 7 days has been changed to +28 days.	***In order to maintain consistency with Section 4.0 and other windows used in the protocol.***
Section 3.0 Eligibility	Language about referring patients to Dr. Matei at NU. Also, language stating that eligibility waivers are not permitted	Both language removed from the protocol.	***For clarity and to be in alignment with current policies.***

Study summary and section 4.1 and 4.3 (treatment administration)	All subjects in the safety run-in cohort will be observed for at least 2 weeks after administration of cycle 1 for dose limiting toxicity (DLT)	Modification: the word 'administration 'changed to 'completion', to state that subjects will be observed for DLT at least 2 weeks after completion of cycle 1.	***Correction of error.***
Section 3.1.7	Inclusion criteria regarding organ function	**Added note:** *(Note: Screening labs will be repeated again on C1D1 if they were performed outside of the ≤4 day window.)*	***For clarity***
Throughout	'within' word used for windows	Modified to use : '≤' instead of within e.g. ≤28 days;≤14 days;≤30,≤120 days	***For convenience, accuracy and consistency***
Section 4.1 and study Summary	Patients must begin therapy within 7 days of registration. Day 1 may be delayed up to 1 week (7 days).	Patients must begin therapy within(+) 14 days of registration. Day 1 of each cycle may be delayed up to +28 days.	***For convenience and flexibility and consistency***
Section 4.4.2 dose modification for Pembrolizumab	Dose modification for Pembrolizumab (table). All windows were 'within 12 weeks' Subjects should be placed back on study therapy within 3 weeks of schedule interruption	All windows in the table modified to ≤28 days. Subjects should be placed back on study therapy in ≤ 28 days of the scheduled interruption.	***To be consistent with rest of the protocol.***
Section 4.5.1 Allowed Concomitant medications and	"All treatments that the PI considers necessary for a subject's welfare may be administered at the discretion of the PI in keeping with the	Principal Investigator(PI) has been changed to 'treating physician'	***For flexibility and consistency***

Section 4.6 Supportive car with Pembrolizumab	community standards of medical care." "Subjects should receive appropriate supportive care measures as deemed necessary by the PI. The treatment guidelines are intended to be applied when the PI determines the events to be related to pembrolizumab. " "All concomitant medications received ≤ ≤28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded"	The window for Concomitant medication has been changed to ≤14 days in section 4.5.1	
Section 4.5.2 Prohibited Concomitant medications	"Details about prohibition of steroids with restrictions.	Added: "steroid preparation prior to tumor imaging is permitted for prophylaxis (e.g., contrast dye allergy)."	***For safety***
Section 4.9 Duration of follow-up and Section 5.0(Schedule of events)footnote 13	Survival follow-up stated to be 'every 3 months'	Modified to: "every 3 months for the first 2 years after completing study therapy and then every 6 months thereafter."	***For convenience. It is in alignment with standards followed by similar clinical trials.***
Section 4.11 Patient Replacement	"If a patient is enrolled in the study but comes off study before the first dose of guadecitabine, the patient may	Modified to state: "If a patient is enrolled in the study but comes off study prior to the first dose of treatment, the patient may be replaced.	***For clarity***

	be replaced. If a subject in the safety run-in portion of the trial goes off study before completing the DLT observation period of safety run-in for reasons other than DLT, then that subject should be replaced."	If a subject in the safety run-in portion of the trial goes off treatment before completing the DLT observation period of safety run-in for reasons other than DLT, then a patient will need to be added to the safety run-in".	
Section 5.0 Study procedures	Column containing tests to be conducted within -5 days of registration. Footnote 2 only stated assessments included in the physical exam. Footnote3: CA 125 not included as an assessment Footnote 4: During treatment, radiologic imaging should be done every odd numbered cycle starting with Cycle 3 Footnote 6: Details about blood for DNA analysis	-5 day window changed to ≤14 days. Table updated accordingly and with appropriate footnotes. Footnote2: Added language to state that Physical exam will be conducted with a (+/-) 4 day window for D1 of each cycle and at the safety follow-up visit. Footnote 3: Added CA 125 as a standard of care assessment. Footnote 3 updated. Also, C1D1 labs need not be repeated if done within a window of ≤4 days instead of 'within 7 days'. Footnote 4 : added window: Cycle 3(≤7 days prior to day 1 of the cycle) Footnote 6: windows added: Cycle 1 Day 1[≤7days] and Cycle 2 Day 8 [+2 days] (at the time of the biopsy or paracentesis or thoracentesis and with the same window.	***For increased clarity, consistency and convenience.***

	Footnote 7: details about blood for cytokine response.	Footnote 7:windows added: Cycle 1 Day 1(≤4 days), Cycle 2 Day 1(≤4 days), Cycle 3 Day 1(≤4days)	
	Footnote 8: Details about pregnancy test	Footnote 8: Added language that it will be done on D1 (≤4 days) of each cycle in addition to the screening period.	
	Footnote 9: Details about blood for PBMC analysis	Footnote 9:windows added Cycle 1 Day 1(≤4days), Cycle 1 Day 5, pre-treatment on Cycle 2 Day 1(≤4 days)	
	Footnote 10 contained details about CT guided biopsies.	Footnote 10: Added language to state that biopsies can be guided by CT, ultrasound or physical exam and paracentesis or thoracentesis can also be done. Windows added: [(Cycle 1 Day 1 (≤7 days)] and Cycle 2 Day 8 (+2 days)]	
	Footnote 11 :details about PT/INR, aPTT	Footnote 11: Footnote and table updated to indicate that PT/INR & aPTT will not be done at the 'subsequent cycles' time-point. A (≤)4 day window added for TSH testing. Footnote 17 added to state that PT/INR and aPTT is required at screening only, unless otherwise clinically indicated.	
	Footnote 13:folllow-up details. Subjects to be contacted by phone every 3 months.	Footnote 13: Added that subjects may be contacted by email or a clinic visit as well, every 3 months for the first 2 years and then every 6 months thereafter	

	Footnote 14: C1D1 to should be within 7 days of registration. Footnote 15: Treatment details. It states that while on treatment day 1 can be delayed by 1 week.	Footnote14: Cycle 1 day 1 should be started within (+) 14 days following registration. Footnote 15: Modified to state that "While on treatment, day 1 of each cycle may be delayed up to +28 days from target date for scheduling purposes and holidays. Additional modifications: Footnote 16 added to the table. It states: "ConMeds and AE assessment to be completed at the time of the D1 physical exam. See footnote 12 regarding windows for the D1 physical exam". Table updated at appropriate places with the corresponding footnotes. For footnotes 5, 6, 7, 9, 10: added language referring to laboratory manual	
Section 6.1 Endpoint assessment definitions	"The same measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC"	Modified to: "Measurable and non-measurable lesions will be followed by both RECIST v1.1 & irRC" For Measurable Disease, language added: "For irRC, measurable lesions are defined as those that can be accurately measured in at least two dimensions as 10x10mm with CT scan." For Non-measurable Lesions, language added to indicate that for both RECIST 1.1 and irRC the same criteria will be used.	***For clarity and to match thresholds for RECIST1.1 and irRC.***
Section 7.3.5 Expedited	No reference was made as to	Added language: To be done by QAM	***For clarity***

Reporting to the FDA	who would be doing it.		
Section 7.3.6 Expedited Reporting to Merck and Astex	No reference was made as to who would be doing it. Language about SAE reporting stated that : SAEs will be reported to Merck and Astex within one business day of receipt of the NU CRO SAE Submission form.	Added language: To be done by coordinator. Language modified to state that SAEs will be reported to Merck and Astex within one business day of **acknowledgement of the event,** using the NU CRO SAE form.	***For clarity***
Section 8.2.10	list of side-effects for Guadecitabine	Added edema (swelling of tissue) to the 'Less Common' side effects list	***Per updated IB and approval of PI***
Section 9.0 Correlative studies	Statement that left over samples will be stored.	Statement added to specify that left over samples will be stored at Dr. Matei's laboratory at NU. Other language updated in order to be consistent with windows and other updates made to section 5.0	***For improved clarity and specificity***
Section 5.0 footnote 5 and Section 9.0	States that archived tumor tissue specimens may be obtained at any point during conduct of this study	Corrected to "may be procured up to 3 months from registration"	***To be consistent with information in the table in section 9.0 and the lab manual.***
Section 10.3 Statistics –data analysis plans	Analysis for Primary Objective: The objective response rate by irRC will be estimated similarly.	Removed the irRC language from the Primary Objective analysis.	***Correction of error, since irRC is to be used only for secondary objective. Decision to go on to stage II of the study will be based on assessment using RECIST1.1 only***

Section 10.4.2 efficacy criteria in statistics section	interim analysis planned after 18 patients using Clinical Benefit(defined as CR,PR, or SD for at least 3 month).	Changed to state that interim analysis is planned with 18 patients using RR	***Since primary objective is ORR.***
Section 11.5 Instructions for Participating Sites	This section was not in the protocol.	Inserted this section after University of Chicago came on board as affiliate site	***To include information for affiliate sites***
Amendment 3 5/10/2017			
Sections(s) Affected	**Prior Version**	**Amendment 3 Changes**	***Rationale***
Study Schema	Tumor assessments every 2 cycles	Tumor assessments every 2 cycles up to cycle 5; then every 3 cycles	***For safety and convenience***
Section 2.2.4 Secondary objective	Statement of RR assessment with RECIST1.1 and irRC	Added language referring to section 6.0 which has new language regarding RECIST1.1 which will be used here with a few adaptations	***For clarity and accuracy. To accommodate immune response adaptations of RECIST 1.1***
Section 4.2 Treatment administration	Table containing details of administration of pembrolizumab and guadecitabine.	Added footnote: "After completion of the safety run in, starting on Day 5 of each cycle, growth factor support filgrastim or peg filgrastim can be administered 24 hours after completion of guadecitabine administration, at the discretion of the treating physician." Similar language added to section 4.4.1 which pertains to 'Dose modifications for guadecitabine'	***For safety***
Section 4.4.1 Dose modifications for Guadecitabine	In general, guadecitabine dosing should be held guadecitabine-related non-hematologic toxicities until toxicity has resolved to ≤	Modified to : In general, guadecitabine dosing should be held for ≥ Grade 3 neutropenia, thrombocytopenia or other guadecitabine-related non-hematologic toxicities until toxicity has resolved to ≤ Grade 1 or baseline levels	***For clarity and safety***

	Grade 1 or baseline levels		
Section 4.4.2 Table 3 Dose modification guidelines for pembrolizumab related AEs	Previous table which had the window for resolution of toxicity as ≤28 days of last dose.	Updated window to ≤28 days from the start of the event throughout the table and footnotes and notes. Similar update done in section 4.10	***For safety and convenience***
Section 4.5.2 Prohibited Concomitant Modifications	Previous language	Added language regarding Stevens-Johnson syndrome(SJS), Toxic Epidermal Necrolysis(TEN) and immune-mediated myocarditis	***Based on action letter and updated pembrolizumab IB***
Section 4.6.8 and 4.6.9	N/A	Added management details for SJS , TEN and immune-mediated myocarditis	***Based on action letter and subsequently updated pembrolizumab IB***
Section 4.10 Removal of Subjects from Study Treatment and/or Study as a Whole	One of the conditions stated: "Patient demonstrates disease progression. This must be confirmed radiographic disease progression"	Added language to this: "At the first disease assessment, treatment can be continued, even in the presence of radiographic or clinical progression, if in the opinion of the treating physician, the patient is stable or benefitting clinically. In that circumstance a short term follow up scan should be obtained in 4-6 weeks" Full details in section 6.1	***To accommodate immune response adaptations of RECIST 1.1***
Section 5.0 Study procedures table	1. Mg, Phos, LDH and at screening, Day1 of each cycle and at end of study. 2. Uric acid at screening, Day1 of each cycle and at end of study. 3. Footnote4: Tumor assessment every 2 cycles	1. Mg, Phos and LDH to be done only at screening. 2. Uric acid to be done D1 (≤4 days) of every odd cycle beginning with Cycle 3. Footnote 11 updated to reflect this. 3. Footnote 4: During treatment, radiologic imaging should be done prior to cycles 3, 5 and then prior to every third cycle (e.g. cycles 8, 11, 14, etc.) *CTs can be obtained ≤7 days prior to day 1 of the cycle.*	***For safety, clarity and convenience***

	4. Footnote10: Tumor biopsies or paracentesis or thoracentesis 5. Footnote 15 details about treatment 6. Footnote 16: Conmeds and AE assessment was tied to physical exam 7. footnote 17 stating specifics about coagulation parameters	*Reference to section 6.0 made for response assessment. Footnote 4 added to Chest x-ray and CT rows in the table* 4. Footnote 10: Tumor biopsies and/or paracentesis and/or thoracentesis can be done 5. Minor modifications made to Footnote 15 language for clarity 6. Footnote 16 modified to state "ConMeds and AE assessment are to be completed on D1 of each cycle (+/-4 days)." 7. Footnote 17 removed 8. Other minor re-arrangements of footnotes in the table made for increased clarity	
Section 6.1 endpoint assessment definitions	previous definitions	Added language to describe details about adaptations to RECIST1.1 for immunotherapeutic agents like pembrolizumab, wherein if the first scan shows PD, treatment can still continue and a repeat scan will be done in 4-6 weeks for response assessments.	***To accommodate immune response adaptations of RECIST 1.1 which incorporates the delayed anti-tumor effects of immunotherapy***
Section 8.1.10 side effects of pembrolizumab	List of side effects	added 'Very common side effects seen in ≥20% of patients'-itching of skin, diarrhea, cough. Most common side effects changed to 'common side effects (seen in ≥10% of patients. Also added joint pain and back pain to this list. Added 'myasthenic syndrome' to list of rare but serious/life threatening side effects	***Based on action letter and subsequently updated pembrolizumab IB***

Amendment 4 7/24/17			
Section 9.0	Stated "Subjects will be consented to optional storage of any remaining tumor samples after protocol-specified studies are complete"	Added language to clarify that leftover plasma will also be used for future analysis. All other storage details remain the same.	***Per PI, in order to open up possibilities of using both tumor and blood samples for future research***

Amendment 5 10/25/17(re-submitted to SRC with version date 12.6.17)			
Section 4.2, 4.2.2, 4.4.1, 8.2.5	Treatment details	Added language: *"Note: Guadecitabine priming can be discontinued after 6 months (8 cycles) at the discretion of the treating physician, if patient has stable disease or response."*	*For safety. In order to minimize toxicity for patients.* *(approved by sponsor Astex)*
Section 4.4.2 Table 3	Previous pembrolizumab toxicity management and dose modification table for immune related AEs	Replaced with the current **Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab.** **Specifically, Immune-mediated Myocarditis management has been included**	*Based on directive received from Pembrolizumab drug sponsor Merck. (Note: Myocarditis was included as an immune – mediated AE in Amendment 3)*

Study schema and Sections 4.2, 4.2.2, 4.4.1, 8.2.5	Treatment details	Added language: *"Note: Guadecitabine priming can be discontinued after 6 months (8 cycles) at the discretion of the treating physician, if patient has stable disease or response."*	*For safety. In order to minimize toxicity for patients.* *(approved by sponsor Astex)*
Section 5.0 Study procedures table	Footnote 3: C1d1 CBC and CMP had a window of ≤ 4 days prior to start of treatment and Ca-125 at screening had a window of 14 days. Footnote 1 was also inserted in the 'X' accompanying the C1 tumor biopsy	Footnote 3 modified to state that CBC and CMC And CA-125 at every D1 has a window of ≤4 days prior to D1. Ca-125 at screening will still have the 14 days window. Footnotes updated in the table as appropriate. Footnote 3 in Mg, phos and LDH removed since these are only done prior to registration. Footnote 1 removed from this position	***For flexibility and clarity*** ***Correction of error. Pathology not required for this biopsy***
	Footnote 15 details about Guadecitabine administration	Footnote 15: Added language: *"Note: Guadecitabine priming can be discontinued after 6 months (8 cycles) at the discretion of the treating physician, if patient has stable disease or response."*	*For safety. In order to minimize toxicity for patients.* *(approved by sponsor Astex)*

Amendment 6 –April 30, 2018			
Sections(s) Affected	**Prior Version**	**Amendment 6 Changes**	**Rationale**
Title page	Mario Pineda, MD listed as Sub-investigator	Removed Mario Pineda and added Emma Barber, MD as Sub-Investigator Inserted complete details for the Statistician	***Dr. Pineda has left Northwestern University*** ***Administrative***
Throughout (wherever applicable)	Previous statement about Guadecitabine priming followed by Pembrolizumab therapy	Language modified: "Guadecitabine priming can be discontinued *and the patient can continue on pembrolizumab monotherapy* after 6 months (8 cycles), at the discretion of the treating physician, if the patient has stable disease or response." *Language added: "If this is done, the previously scheduled pembrolizumab treatment on Day 5 will become Day 1(+/-2 days)."*	***In order to minimize exposure and streamline scheduling for patients, since the Guadecitabine priming is not necessary after a time point.***
Throughout (wherever applicable)	Previous statement about Guadecitabine priming followed by Pembrolizumab therapy, then monotherapy with pembrolizumab.	*Language inserted:* *"For patients who discontinued guadecitabine for reasons other than toxicity (i.e. disease stability of response > 6 months) and progress on pembrolizumab alone, a re-trial of guadecitabine in combination with pembrolizumab is possible, at the discretion of the treating physician, if the patient is clinically stable."*	***As an addendum to the provision of prembrolizumab monotherapy, in order to give patients another chance before taking them off study.***
Section 1.1 Ovarian cancer background	Previous language	Some language deleted	***Language not appropriate in this context.***
Throughout	Secondary Objective stated as RR and ORR(for Objective Response Rate) in different sections of the protocol.	Corrected to ORR for Objective Response Rate	***For consistency and accuracy***

Section 4.1 Treatment overview	Details regarding Guadecitabine and Pembrolizumab combination therapy.	Inserted language regarding Guadecitabine skipped dose versus dose delay. Language regarding pembrolizumab monotherapy also inserted.	***For clarity and consistency***
Section 2.2.2, 6.3 10.4.2	Definition of Clinical Benefit rate inconsistent between study summary and rest of the protocol.	Language updated to match the statistical portion of the study summary. The correct plan is : "After testing the drug on 18 patients in the first stage, consideration will be given to trial termination if 2 or fewer patients demonstrate clinical benefit (CR, PR, or SD for at least 3 months)."	***Correction of the discrepancy between the sections of the protocol it serves to clarify the initial intent of the two stage study design per the original sponsor-approved statistical plan.***
Section 4.1 Treatment plan overview	Previous language for treatment administration	Added language to clarify scheduling details and skipped doses and dose delays.	***For increased clarity***
Section 4.4.1 Dose modifications for Guadecitabine	Previous language	Added details regarding grades for thrombocytopenia and Guadecitabine-related non-hematologic toxicities. Added other language for clarity.	***For increased clarity***
Section 4.4.2 Table 3 Dose modifications for Pembrolizumab	Dose modification details inserted as per Nov 2017 'Dear Investigator' letter from Merck.	Modification to window for corticosteroid taper and AE resolution to fit the needs of the study.	***To align with specifications stated in the rest of the protocol.***

Section 5.0 Study Procedures table	All study procedures listed in one table	Study procedures now illustrated in two separate tables: 5.1 Study procedures for Combination treatment (Guadecitabine and pembrolizumab) 5.2 Study procedures for Pembrolizumab Monotherapy for ➢ Patients who discontinue guadecitabine after 2 cycles due to toxicity and who continue on pembrolizumab monotherapy ➢ Patients who discontinue guadecitabine after 8 cycles due to SD or PR or CR and who continue on pembrolizumab Footnotes have been customized as appropriate for each table.	***To align with the treatment modifications made to the study and for increased clarity regarding each scenario.***
Section 5.1 and 5.2	C1D1 CA-125 does not need to be repeated if screening CA125 was conducted within 14 days prior to starting treatment.	Language modified to state: "CA-125 is to be drawn at screening and Day 1 of each cycle. Exceptions would be if CA-125 was drawn within 14 days from the screening visit or within 14 days on visits where doses have been delayed. In these cases, CA-125 will not need to be re-drawn." (Language appropriately modified for section 5.2).	***For safety, clarity and convenience***
Section 5.1 footnote 16 and Section 5.2 footnote 5	AE and ConMeds assessment were stated to be done on Day1 (+/-4 days) of each cycle.	Added language to state that ConMeds will also be recorded 30 days after the last dose of trial treatment. Concomitant medications administered beyond 30 days after the last dose of trial treatment for management of study-related toxicity should be recorded for SAEs and Events of Clinical Interest (ECIs). AE assessments will also be done 30 days after the last dose of trial treatment OR until start of next anti-cancer treatment (whichever is first). *SAEs will be recorded for 90 days after the last dose of trial treatment."* **Corresponding updates to the table have been made.**	***For clarity and for consistency with other sections of the protocol.***

Section 4.9 Duration of follow-up Section 5.1 Footnote 13 Section 5.2 footnote 12	Subjects who discontinue trial treatment for a reason other than disease progression will move into the ***Long-term follow-Up Phase*** and will be assessed every 6 weeks (± 7 days) by radiologic imaging to monitor disease status until the start of new anti-neoplastic therapy, disease progression, death or end of the study.	The 6 week time point has been moved to 9 weeks (+/-7 days). Deleted language: "After 1 year, subjects will continue to should be assessed every 9 weeks (± 7 days) by radiologic imaging to monitor disease status until the start of new anti-neoplastic therapy, disease progression, death or end of the study."	***In order to reduce the exposure of patients to frequent imaging.***
Section 6.1 Endpoint assessment definitions	Previous language	Some language deleted as it is not in alignment with the current protocol specifications, and may cause confusion.	***For accuracy and clarity.***

Amendment 7 –August 30, 2018

Sections(s) Affected	***Prior Version***	***Amendment 1 Changes***	***Rationale***
Section 1.5 and 2.3	One of the exploratory objectives states:"Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC)."	Added additional time point to this objective: "Tumor infiltrating lymphocytes (TILs) will be quantified in tumor biopsies before and after treatment (IHC), **including in optional samples collected > 6 months on treatment."**	***PI has determined this to be a critical scientific point, and analysis of samples at this point could advance our knowledge of how epigenetic and immunotherapy works.***

Section 5.1 Study procedures and schedule of events (Combination treatment guadecitabine+pembrolizumab)	Previous details	added column **"≥ 6 months of treatment"** with corresponding **footnote 17.** This indicates inclusion of OPTIONAL research tests once anytime from ≥6 months and before the safety follow-up visit. Tests will include blood for DNA analysis, cytokine response and PBMC analysis ; also tumor biopsies or paracentesis or thoracentesis may be done. This will be guided by CT, ultrasound, or physical exam, according to the discretion of the treating physician.	***PI has determined this to be a critical scientific point, and analysis of samples at this point could advance our knowledge of how epigenetic and immunotherapy works.***
Section 9.0 Correlative/ special studies	Previous details	Added corresponding language for the above mentioned OPTIONAL research tests once anytime from ≥6 months and before the safety follow-up visit.	***To be in alignment with updates made to rest of the protocol regarding additional optional research testing.***
Section 10.3.3	Previous details	Language updated to include additional time point for sample collection.	***To be in alignment with updates made to rest of the protocol regarding additional optional research testing.***

Amendment 8 –November 9, 2018

Sections(s) Affected	***Prior Version***	***Amendment 8 Changes***	***Rationale***
Section 4.10 Removal of subjects from study treatment and/or study as a whole	A provision for re-trial with combination therapy was available if patient progressed with pembrolixzumab monotherapy.	In order to accommodate patients who are benefiting from this re-trial, an additional flexibility has been added: *"For any patient who has limited treatment options, and is currently re-responding to the combination treatment of pembrolizumab and guadecitabine, an additional year of this combination therapy, beyond cycle #35, may be given, subject to review and approval by the PI and drug sponsors Merck and Astex."*	***For increased flexibility and benefit for patients***

Section 5.2 Pembrolizumab monotherapy	The pembrolizumab monotherapy was to begin on a Friday, like the combination therapy schedule	This requirement has been removed. Pembrolizumab monotherapy can begin any day that is feasible for the treating physician and clinic.	***For convenience***
Section 7.3.6 Expedited Reporting to Merck and Astex	Only a fax number was listed for Astex for safety reporting	In addition to the fax number, an email ID has been included: emailed to drugsafety@astx.com	***For convenience***
Section 8.1.4, 8.1.6, 8.1.10 Pembrolizumab drug information	Previous information	Updated with the most current information based on Investigator's brochure and pharmacy manual.	***To be in alignment with current information from Merck.***
Section 8.2.6, 8.2.10 Guadecitabine drug information	Previous information	Updated with the most current information based on Investigator's brochure.	***To be in alignment with current information from Astex.***

Amendment 9 –January 23rd , 2019			
Sections(s) Affected	***Prior Version***	***Amendment 9 Changes***	***Rationale***
Study summary and Section10 Statistical considerations. (Sample size and statistics)	Maximum accrual was stated to be 38 in order to obtain 35 evaluable patients. *(with an anticipated 1-3 patients that may be inevaluable).*	Maximum accrual has been increased to 46 in order to obtain 35 evaluable patients. *[This has been done after DSMC approved that 8 more patients can be added to reach 35 evaluable. This request to DSMC was based on the fact there were 5 patients who were enrolled and didn't start study treatment. There were another 7 patients who registered and only had one cycle of treatment, which meant that they were not evaluable for the primary end point. This is a total of 12 patients, when it was initially anticipated that only 1-3 patients would not be evaluable for response. Thus, it became essential to update the total enrollment to 46 in the hope that we will obtain 35 evaluable, with a drop out of 13-14 patients].*	***Based on the initial DSMC approval that this study can add up to 8 more patients to obtain a total of 35 evaluable patients.***
Section 8.1 Drug Section: Pembrolizumab	Previous specifications regarding drug preparation.	Updated with most current specifications for drug preparation	***Based on current IB and pharmacy manual.***
Section 8.2 Drug section Guadecitabine	Previous specifications regarding drug preparation. and storage	Updated with most current specifications. *[Astex sent notification that the current stock of 3ml diluent will be depleted by July 2019 and they will start supplying 1.2ml diluent around this timeframe. So, all documents need to be updated to reflect this.]*	***Based on updated pharmacy manual from Astex.***

Section 11.9 Publication policy	Previous template language	Updated with most current template language for publication policy	***Administrative.***

Protocol Amendment 10 – Version date: October 15, 2020			
Section	**Prior Version**	**Changes in Amendment 10**	**Rationale**
Cover page and throughout	Protocol amendment: 9 Protocol date: January 23, 2019	Protocol amendment: 10 Protocol date: October 15, 2020	Administrative update
Section 4.9 (Duration of Follow Up)	All AEs that occur prior to the Safety Follow-Up Visit (30 days (± 7 days) after the last dose of study therapy) will be recorded. Subjects with an AE ≥ Grade 2 will be followed until the AE is ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.	Addition of bolded text: All AEs that occur prior to the Safety Follow-Up Visit (30 days (± 7 days) after the last dose of study therapy) will be recorded. Subjects with an AE ≥ Grade 2 **that occurs ≥ 30 days after discontinuation of study therapy and that is attributed (possibly, probably, or definitely) to the agent(s)** will be followed until the AE is ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier.	Corrects discrepancy. Section 4.9 and Section 7.3.2 contained contradictory language and have been revised to align with one another. The revision clarifies the requirements for AE tracking beyond 30 days during the follow-up period.
Section 7.3 (Adverse Event Reporting)	Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.	Addition of bolded text: Any **≥ Grade 2** event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly**, until the AE is ≤ Grade 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier**.	

Protocol Amendment 11 – Version date: November 18, 2020			
Section	**Prior Version**	**Changes in Amendment 11**	**Rationale**
Cover page and throughout	Protocol amendment: 10 Protocol date: October 15, 2020	Protocol amendment: 11 Protocol date: November 18, 2020	Administrative update
Section 8.2 (Guadecitabine)	SGI-110 (guadecitabine) lyophilized drug powder is supplied as a 100mg vial. SGI-110 Diluent is supplied as 1.2mL or 3mL vials. Upon reconstitution, the admixed product is good for 8 days under refrigerated conditions.	SGI-110 (guadecitabine) lyophilized drug powder is supplied as either a 100mg or 156mg vial. SGI-110 Diluent is supplied as 1.2mL, 1.4mL, or 3mL vials. Upon reconstitution, the admixed product is stable for 10 days under refrigerated conditions.	Updates information to align with guadecitabine (SGI-110) investigator brochure version 10.0. The description of the guadecitabine drug product is updated to include the 156 mg vial. The description of the guadecitabine diluent product is updated to include the 1.4 mL vial. The stability of the reconstituted guadecitabine solution when stored refrigerated is extended from 8 days to 10 days.