Abbreviated Title: DAC-THU/Pembro: NSCLC, EsC, PM

NIH Protocol #: 17C0140

Version Date: 08/01/2022

NCT Number: NCT03233724

Title: Phase I/II Evaluation of Oral Decitabine/Tetrahydrouridine as Epigenetic Priming for Pembrolizumab Immune Checkpoint Blockade in Inoperable Locally Advanced or Metastatic Non-Small Cell Lung Cancers, Esophageal Carcinomas, or Pleural Mesotheliomas

NCI Principal Investigator: David S. Schrump, M.D., M.B.A.
Thoracic Surgery Branch (TSB), NCI, NIH
10 Center Drive, Room 4-3942
Bethesda, MD 20892-1502
Phone: 240-760-6239
Email: schrumpd@mail.nih.gov

Investigational Agents:

Drug Names:	Decitabine (DAC)	Tetrahydrouridine (THU)	Pembrolizumab
Sponsor:	Center for Cancer Research, NCI		
IND Number:	126827		
Manufacturer:	IriSys, LLC and DCTD, NCI	KP Pharmaceutical Technology Inc.	Generic
Supplier:	CC Pharmacy	CC Pharmacy	CC Pharmacy

Commercial Agents: None

PRÉCIS

Background:

- Non-small cell lung cancers (NSCLC), esophageal carcinomas (EsC) and malignant pleural mesotheliomas (MPM) account for approximately 185,000 deaths annually in the United States, with over two thirds of patients presenting with advanced, incurable disease. 1st-line platinum-based chemotherapy for advanced NSCLC, EsC, or MPM produces transient responses at best, with most patients succumbing to disease within 12-16 months following diagnosis.
- Recent randomized clinical trials have demonstrated response rates approximating 20% in unselected patients with advanced NSCLC or EsC, and nearly 45% in patients with tumors exhibiting high level expression of programmed death ligand 1 (PD-L1) following administration of pembrolizumab, a humanized monoclonal anti-PD-1 antibody.
- Approximately 17% of unselected MPM patients have exhibited objective responses following administration of pembrolizumab or other PD-1 inhibitors.
- Preclinical studies have demonstrated that epigenetic drugs such as DNA demethylating agents and histone deacetylase (HDAC) inhibitors can "prime" cancer cells and tumor microenvironments thereby enhancing efficacy of immune checkpoint inhibitors.
- Although a potent DNA demethylating agent, Decitabine has poor bioavailability and inconsistent distribution in solid tumors due to rapid inactivation by cytidine deaminase (CDA) which is present in high levels in many organs.
- Recent studies in rodents and nonhuman primates, as well as a Phase 1 clinical trial (NCT#01685515) in patients with sickle cell disease have demonstrated that oral tetrahydrouridine (THU), an inhibitor of CDA, significantly enhances bioavailability/solid-tissue-distribution of low dose oral DAC, thereby enhancing systemic DNA demethylation with acceptable toxicities.
- Preliminary results of a recent clinical trial suggest that oral DAC-THU can increase the frequency and magnitude of responses to immune checkpoint inhibitors in lung cancer patients with low or absent intra-tumoral PD-L1 expression.
- These data support further evaluation of oral DAC-THU in combination with immune checkpoint inhibitors for therapy of thoracic malignancies.

Objectives:

Phase I

- To define pharmacokinetics, toxicities and maximum tolerated dose of oral DAC-THU in combination with pembrolizumab in patients with inoperable, or unresectable, locally advanced or metastatic NSCLC, EsC, or MPM.

Phase II

- To determine clinical response by RECIST criteria to oral DAC-THU in combination with pembrolizumab in patients with inoperable, or unresectable, locally advanced or metastatic NSCLC, EsC, or MPM.

Eligibility:

Inclusion Criteria

- Male or female, 18 years or older with histologically or cytologically-proven, inoperable, or unresectable locally advanced, or metastatic NSCLC, EsC, or MPM.
- Measurable disease.
- Patients with high PD-L1 expression (≥ 50%) and low PD-L1 expression (0-49%) in cancer cells by immunohistochemistry are eligible.
- NSCLC patients with no prior systemic treatment, or those with prior first line treatment including an immune checkpoint inhibitor are eligible for study.
- MPM patients who have received, refused, or are ineligible for first line chemotherapy are eligible.
- Patients with EsC including Seiwert-Stein Type I and Type II gastro-esophageal junction (GEJ) carcinomas who have received or refused standard of care first line therapy and/or targeted therapy are eligible.
- Patients who received DNA demethylating agents or PD-1/PD-L1 inhibitors for another malignancy may be eligible for study if there were no dose-limiting immune related events, and there has been either no clinical evidence of disease or minimal residual disease that has been stable for at least three years.
- Willingness to undergo tumor biopsies if safely accessible per PI discretion before and after treatment.
- ECOG performance status 0 – 2.
- No evidence of unstable or decompensated myocardial disease; adequate pulmonary reserve.
- Adequate renal, hepatic and hematopoietic function.

Exclusion Criteria

- Patients with any targetable mutation for which there is approved first or second line therapy.
- Serious cardiovascular conditions.
- Active Hepatitis A, Hepatitis B or Hepatitis C.
- Human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness.
- Other active infection requiring systemic therapy.
- Pregnant or breastfeeding women.
- Patients who are receiving systemic corticosteroids.
- Patients receiving another investigational agent.
- Another malignancy.

Design:

- The Phase I component will be a standard 3+3 design combining high and low PD-L1 expressers with incremental dose escalation of oral DAC-THU to define MTD.
- Simon 2-stage design for Phase II studies will be used to determine clinical response at the MTD.

- Patients will receive oral DAC-THU on T-W for two weeks out of every 3 for 9 weeks.
- Pembrolizumab will be administered on Wednesday, Thursday or Friday at a fixed intravenous dose of 200 mg every 3 weeks.
- One cycle is three weeks; one course is 9 weeks. Treatment evaluation using RECIST 1.1 every 10 +/- 1 weeks.
- Those patients exhibiting disease progression or unacceptable toxicities will be removed from study. Patients exhibiting stable disease or disease regression will be offered an additional course of therapy followed by treatment evaluation. Treatment will continue in this manner until off-study criteria have been met.
- Once the MTD for DAC/THU has been identified, the MTD dose level will be expanded by 4 patients to confirm its safety. Then, including these 10 patients at the MTD, a total of 10 NSCLC patients with high (50% or greater) intratumoral PD-L1 expression and 10 NSCLC patients with low (0-49%) intratumoral PL-L1 expression will be accrued to the first stage of each of two separate Phase II cohorts using individual Simon optimal designs. If 5 or more patients of the 10 first stage NSCLC patients in the high PD-L1 cohort respond to treatment, the cohort will be expanded to 23 patients. If 11 of 23 of these patients respond to treatment, the trial will be deemed positive for NSCLC with high PD-L1 expression. If 2 or more of the 10 first stage NSCLC patients in the low PD-L1 expression cohort respond to treatment, the cohort will be expanded to 29 patients. If 6 or more of these 29 patients experience a response, the trial will be deemed positive for NSCLC with low PD-L1 expression. Up to 10 EsC patients, including those considered to be part of the Phase I component after the MTD has been identified, will be enrolled into a separate cohort to examine responses to DAC-THU/pembrolizumab at the MTD. If 2 or more of these 10 EsC patients respond to treatment, these findings may warrant an amendment or a separate Phase II trial to determine response rates to DAC-THU/pembrolizumab in EsC patients. Similarly, if 2 or more of 10 MPM patients respond to treatment, these findings may warrant an amendment or a separate Phase II trial to determine response rates to DAC-THU/pembrolizumab in MPM.
- Biopsies of index lesions will be obtained at baseline and at treatment evaluation following the first course of therapy for analysis of pharmacodynamic endpoints.
- Patients will be followed for toxicity for at least 30 days after treatment has been discontinued, start of new anti-cancer treatment or until death, whichever occurs first.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

- To define pharmacokinetics, toxicities and maximum tolerated dose of oral DAC-THU in combination with pembrolizumab in patients with inoperable, or unresectable, locally advanced or metastatic NSCLC, EsC, or MPM. (Phase I Component)
- To determine clinical response by RECIST criteria to oral DAC-THU in combination with pembrolizumab in patients with inoperable, or unresectable, locally advanced or metastatic NSCLC, EsC, or MPM. (Phase II Component)

1.1.2 Secondary Objectives

- To examine if oral DAC-THU modulates DNA methylation, as well as gene, micro-RNA, and endogenous retroviral expression profiles of NSCLC, EsC, and MPM cells and alters the tumor micro-environment.
- To examine if oral DAC-THU modulates plasma tumor DNA methylation, circulating tumor cells and peripheral immune subsets.

1.2 BACKGROUND AND RATIONALE

1.2.1 Background and Significance:

Lung cancer is the leading cause of cancer-related mortality worldwide and presently accounts for ~1.8 million annual deaths globally, of which nearly 160,000 occur in the United States (1, 2). The vast majority of these malignancies are directly attributable to cigarette smoking. Survival is stage dependent, and ranges from > 80% for patients with early stage tumors to less than 15% for patients with locally advanced or disseminated disease (3). The majority present with inoperable disease; as such median overall survival for lung cancer patients is 12-16 months. Approximately 80% of pulmonary carcinomas are non-small cell lung cancers (NSCLC) comprised primarily of

adeno-, squamous cell and large cell undifferentiated carcinomas with distinct histologic and molecular features. The remaining 20% are highly lethal small cell lung cancers (SCLC) (4) which exhibit varying degrees of neuroendocrine differentiation, and are typically widely metastatic at presentation with a high propensity for recurrence despite initial, often dramatic responses to chemotherapy (5).

Esophageal cancer (EsC) is the sixth leading cause of cancer-related mortality worldwide, accounting for ~509,000 deaths annually of which nearly 16,000 occur in the US (1, 6). Esophageal squamous cell cancers (ESCC) predominate in high incidence areas in Asia, South America, and the Middle East; these neoplasms typically arise in the upper and mid-esophagus, and are attributable to smoking, ethanol and cultural dietary practices. Esophageal adenocarcinomas (EAC) predominate in Western Europe and the US; these malignancies typically arise in the distal esophagus and gastro-esophageal junction and are attributable to obesity, gastro-esophageal reflux, Barretts esophagus, and to a lesser extent, cigarette smoking. Most patients (~70%) present with locally advanced or metastatic disease. Survival is stage dependent, ranging from ~40% for localized disease, to 20% for regional disease, and 5% for disseminated disease. With the exception of Her2 overexpression in a small percentage of cases, esophageal cancers typically have no actionable mutations (7, 8).

Malignant pleural mesotheliomas (MPM) are rare, lethal neoplasms attributable primarily to environmental or occupational exposure to asbestos and related fibers, influenced in some cases by predisposing germline mutations (9-12). An estimated 40,000 individuals succumb to mesotheliomas worldwide each year despite asbestos being banned in many industrialized countries (13, 14). Most patients present with advanced, inoperable disease; median survival of these individuals is approximately 6 months with supportive care alone, and 12 months with systemic chemotherapy (15, 16). Whereas patients with limited disease burden may benefit from cytoreductive surgery- typically in the context of multimodality therapy, the role and extent of surgery in patients with MPM remains controversial (16-18). MPM typically have no actionable mutations.

Immune checkpoint inhibitors are revolutionizing treatment of diverse malignancies including NSCLC, gastroesophageal carcinomas (2), and MPM. Humanized monoclonal antibodies such as pembrolizumab, which inhibit engagement of programmed death ligand -1 (PD-L1) on cancer cells and immune regulatory cells with programmed death-1 (PD-1) receptors on activated T cells (19), break tolerance and mediate objective responses in approximately 20% in patients with advanced NSCLC and gastroesophageal carcinomas when administered as second line therapy (20, 21). Response rates appear to be higher in smokers/former smokers due to increased mutational burden and neoantigen load (22-25), as well as patients whose tumors expressed high levels of PD-L1(20, 25). The favorable toxicity profiles, impressive, durable response rates and survival benefit of pembrolizumab have led to FDA approval of this immune checkpoint inhibitor for second and third line therapy of NSCLC and gastroesophageal carcinomas, respectively, irrespective of tumor PD-L1 expression status, as well as first line therapy for non-squamous NSCLC with 50% or greater expression of PD-L1 and no EGFR or ALK mutations (http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm526430.htm). Although presently not approved for the treatment of MPM, pembrolizumab is listed in NCCN guidelines for second-line treatment of these neoplasms, with far fewer toxicities compared to chemotherapy.

Epigenetic Strategies for Cancer Therapy: Alterations in chromatin structure perturb gene expression during malignant transformation (26). DNA demethylation results in de-repression of

endogenous retroviruses, loss of imprinting, and activation of cancer-testis genes, which normally exhibit stage-specific expression during germ cell development in testes or placenta, and either no expression or highly restricted expression in normal somatic tissues. In the context of genome wide DNA demethylation, numerous tumor suppressor genes are silenced by site specific DNA hypermethylation and/or polycomb repressive complexes. These alterations in chromatin structure, which recapitulate epigenomic states in normal germ cells provide the rationale for epigenetic therapy for cancer (27-29).

Aberrant activation of cancer-testis (CT) genes (also referred to as cancer-germline genes) in somatic cells during malignant transformation results in expression of highly restricted tumor antigens that induce serologic as well as cell-mediated immune responses in cancer patients; as such, cancer-testis antigens (CTAs) have emerged as attractive targets for cancer immunotherapy (30). To date, over 200 CTAs have been identified, approximately half of which are encoded on the X chromosome (31) (**Figure 1**). CT-X chromosome (CT-X) genes are normally expressed in spermatogonia, and typically comprise extended families associated with inverted DNA repeats. The non-X CT genes tend to be expressed during later stages of germ cell differentiation (i.e. spermatocytes), and do not appear to be associated with extended families or inverted repetitive DNA sequences (32, 33). Relative to autosomal CT genes, CT-X genes are more frequently activated in cancer cells, and particular gene families appear to be coordinately de-repressed in a tumor-specific manner.

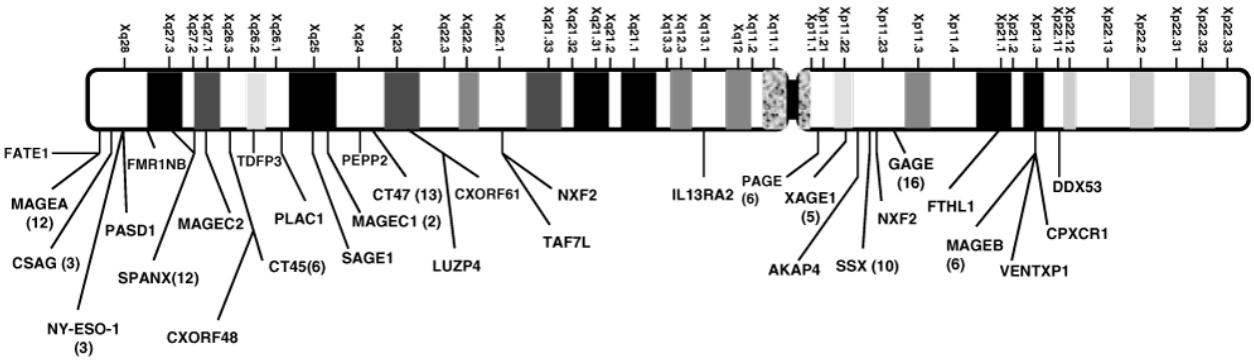


Figure 1: Location of cancer testes (CT) genes on X chromosome, with corresponding number of family members in parenthesis. Copied from ref (34).

In general, the frequency and magnitude of CT-X gene de-repression in human cancers coincides with advanced stage of disease. Typically, tumors with CT-X mRNA copy numbers > 10% of testes exhibit corresponding proteins detectable by immunohistochemistry techniques. In contrast, tumors with CT-X mRNA levels < 1% of testes do not have detectable antigen expression. With the exception of synovial sarcomas or Hodgkin Lymphomas that tend to exhibit homogeneous expression of NY-ESO-1 and CT-45, respectively, the majority of human cancers exhibit extremely focal CTA expression. These observations, together with recent reports demonstrating high level CT-X gene expression in cancer stem cells (35-37), raise the possibility that CT-X antigens are preferentially expressed in pluripotent tumor cells. As such, pharmacologic interventions that enhance expression and recognition of CT-X antigens may be novel strategies to eradicate cancer stem cells contributing to systemic metastases.

Oral Decitabine and Tetrahydrouridine: Decitabine (5-AZA-2′-deoxycytidine, DAC) was originally synthesized in 1964 by Pliml and Storm. This analogue of deoxycytidine has been extensively evaluated as an experimental anti-neoplastic and differentiation agent (38). Unlike cytidine analogues such as cytarabine, the sugar moiety of decitabine is unmodified, enabling incorporation of the analogue into DNA without termination of chain elongation or cytotoxicity at low doses. Through mechanisms that have not been fully delineated, the incorporated DAC induces rapid ubiquitination and proteosomal degradation of the "maintenance" DNA methyltransferase (DNMT) 1, but not "de-novo" DNMTs 3a or 3b (39, 40). In normal cells, DNMT1 functions primarily to restore DNA methylation following DNA replication; in cancer cells, DNMT1 is frequently over-expressed and contributes to de-novo DNA methylation and aberrant silencing of tumor suppressor genes; DNMT1 is indispensable for viability of cancer stem cells (41, 42). Depletion of DNMT1 in cancer cells by DAC results in passive DNA demethylation, and de-novo induction of a variety of genes including those encoding cancer-testis antigens, as well as epigenetically silenced tumor suppressors (43). At high concentrations DAC exhibits anti-metabolite, DNA damaging, cytotoxic effects.

In published studies we have demonstrated induction of NY-ESO-1 and MAGE-A3 as well as p16 in NSCLC, EsC, and MPM cells but not normal respiratory epithelial cells or fibroblasts following 72 hour exposure to DAC (44, 45); up-regulation of NY-ESO-1 and MAGE-A3 persisted for approximately one month following DAC treatment, and facilitated recognition and lysis of tumor cells by cytotoxic T-lymphocytes (CTL) or PBL expressing T cell receptors for these CT as shown in **Figure 2** and **Figure 3**) (45-48). We have also demonstrated eradication of pulmonary metastases in immunocompetent mice following systemic treatment with DAC and subsequent adoptive transfer of CTL specific for the murine CTA, P1A (**Figure 4**) (49).

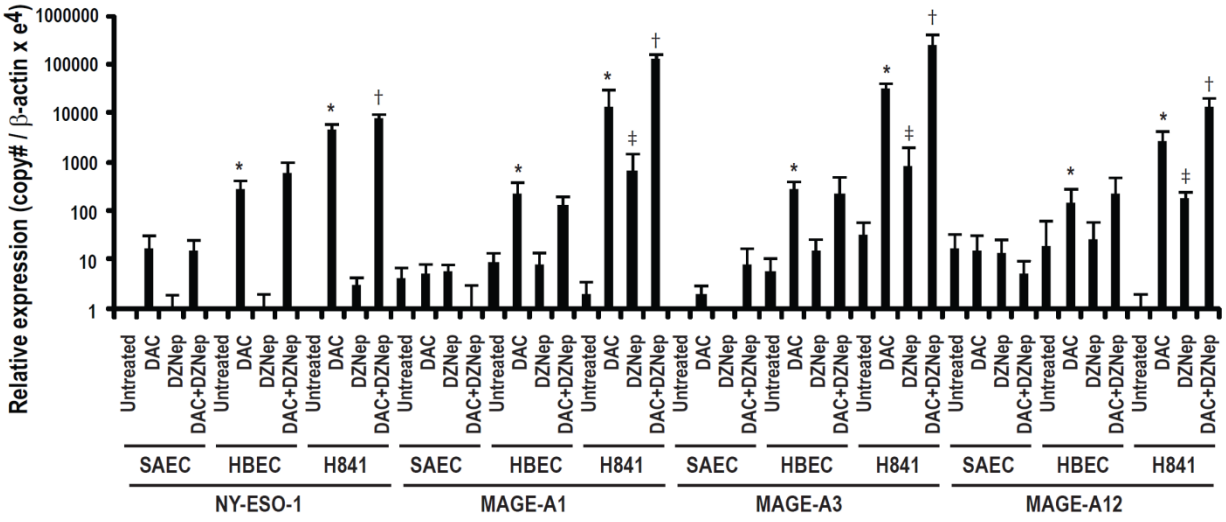


Figure 2: qRT-PCR analysis of CT-X gene activation in H841 lung cancer cells, immortalized human bronchial epithelial cells (HBEC) and normal small airway epithelial cells (SAEC) following exposure to DAC, 3-deazaneplanocin (DZNep) or DAC +DZNep. DAC exposure markedly up-regulates CT-X gene expression, which can be further increased with DZNep in lung cancer cells and to a lesser extent in HBEC. Up-regulation of CT-X genes in SAEC is minimal following drug treatment.

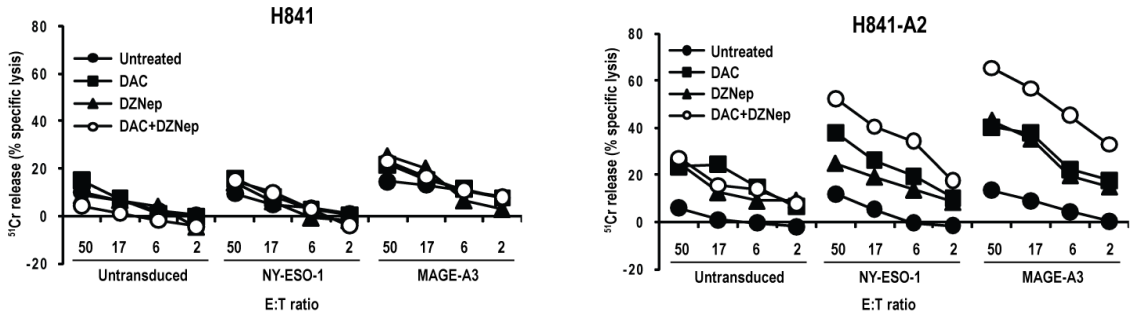


Figure 3: Chromium release assays demonstrating that up-regulation of NY-ESO-1 and MAGE-A3 expression by DAC, DZNep and DAC+DZNep enhances lysis of lung cancer cells by PBMC expressing recombinant T-cell receptors (TCRs) recognizing these CTAs.

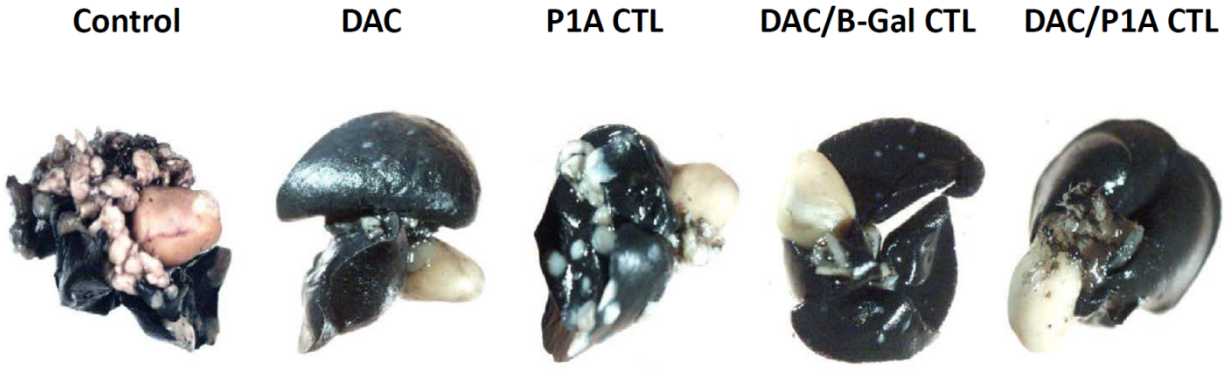


Figure 4: Systemic administration of DAC followed by adoptive transfer of P1A CTL eradicates pulmonary metastases in immunocompetent mice.

A Phase I dose escalation study was undertaken to examine the feasibility of using gene induction strategies to simultaneously augment immunogenicity and apoptosis in thoracic malignancies (50). Because DAC is an S-phase agent with a plasma t ½ alpha and t ½ beta of 7 and 35 minutes, respectively, following intravenous infusion (51), the study utilized a continuous 72-hour infusion to optimize the duration of DAC exposure in cells which are cycling at different rates within solid tumors, in an attempt to recapitulate treatment conditions in our preclinical experiments. The primary endpoints included identification of the MTD of DAC administered under these conditions, clinical response at the MTD, and analysis of NY-ESO-1, MAGE-A3 and p16 expression in tumor tissues before and after DAC treatment. The MTD of DAC administered by 72h continuous infusion was 60 mg/m^2 for patients with three or more prior treatment regimens, and 75 mg/m^2 for individuals with two or less prior treatments. Myelosuppression was the most common dose limiting toxicity. No objective responses were observed in 25 evaluable patients; however, stabilization of disease was observed in eight individuals. Two patients with advanced lung cancers refractory to prior therapy remained on study for approximately 12 months until being removed either because of inability to image residual disease in a previously radiated hilum, or slow disease progression. Pharmacokinetic analysis revealed average steady-state DAC plasma concentrations approximating 10 to 20 ng/ml (~50 nM), which corresponded with threshold concentrations for target gene induction in our preclinical tissue culture experiments.

Immunohistochemistry analysis revealed induction of NY-ESO-1, MAGE-A3, and p16 in 5 of 13, 6 of 20, and 4 of 22 patients, respectively, whose tumor biopsies were sufficient for analysis. Virtually all of the non–small cell lung cancers that exhibited induction of NY-ESO-1, MAGE-A3, and/or p16 had intense focal protein expression. In contrast, NY-ESO-1 or MAGE-A3 protein expression was markedly uniform in one small-cell lung cancer specimen following DAC exposure (representative results of NY-ESO-1, MAGE-A3, and p16 immunohistochemical analyses are depicted in **Figure 5**).

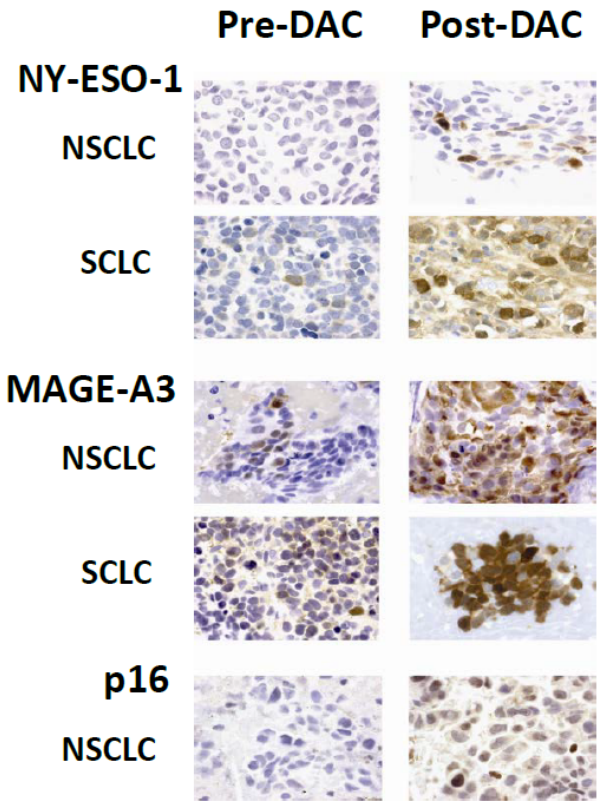


Figure 5: Representative immunohistochemical analyses demonstrating up-regulation of NY-ESO-1, MAGE-A3 and p16 lung cancer tissues following DAC treatment.

Four patients exhibited simultaneous up-regulation of NY-ESO-1 and MAGE-3; two patients exhibited p16 with either NY-ESO-1 or MAGE-A3 induction, and one lung cancer patient exhibited simultaneous induction of NY-ESO-1, MAGE-A3, and p16 in tumor tissues following DAC infusion. Post-treatment antibodies to NY-ESO-1 were observed in three patients who exhibited NY-ESO-1 induction in biopsy specimens following DAC infusion. The lack of purified recombinant MAGE-A3 protein precluded analysis of immune recognition of this CTA. Of particular interest was a lung cancer patient with recurrent endobronchial tumor that exhibited prolonged stabilization of disease following DAC treatment. This patient underwent numerous bronchoscopic biopsies, which were of sufficient quality to enable reliable qRT-PCR analysis of target gene expression. NY-ESO-1 as well as MAGE-A3 mRNA copy numbers increased dramatically, whereas p16 expression remained relatively stable over the course of DAC treatment (**Figure 6**).

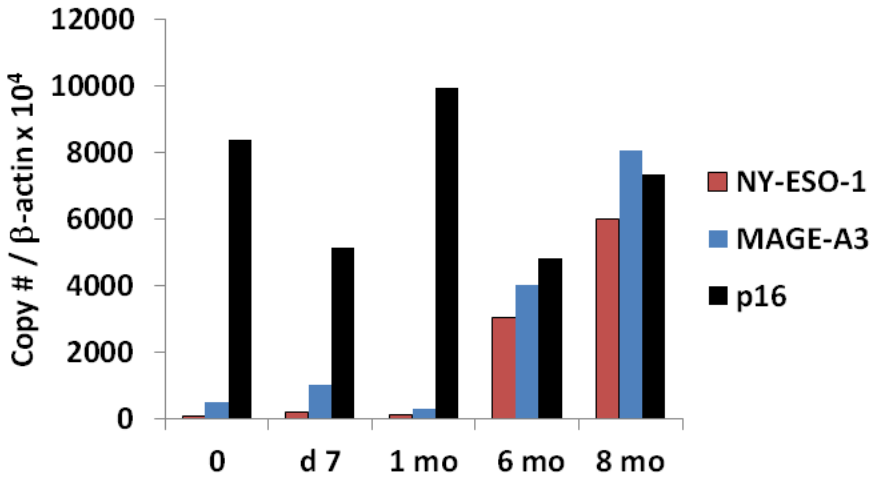


Figure 6: qRT-PCR analysis depicting progressive increases in NY-ESO-1 and MAGE-A3 expression in an endobronchial lung tumor in a patient receiving monthly IV DAC infusions.

Immunohistochemistry and serum ELISA (performed in a blinded manner), revealed NY-ESO-1 expression as well as NY-ESO-1 antibodies at the time of the fifth biopsy and thereafter. These data are consistent with a cumulative molecular treatment effect in this individual and suggest that chronic exposures to DNA demethylating agents are necessary to activate gene expression in solid tumors.

As this trial was nearing completion, we attempted to perform more comprehensive evaluation of gene expression in lung cancer cells mediated by DAC exposure. In particular, we sought to ascertain the effects of DAC treatment relative to gene expression profiles detected in laser-captured tumor cells and adjacent histologically normal bronchial epithelia from resected lung cancer specimens. Consistent with our observations regarding DAC treatment of lung cancer cells in vitro (data not shown), considerable interpatient heterogeneity was observed in baseline as well as post-treatment gene expression profiles of laser captured tumor cells. Data derived from eight resected specimens were sufficient for exploratory statistical analysis, allowing detection of induction/repression of gene expression with threshold of 1.2-fold and using $P < 0.05$ (two-tailed t test) to define potentially important modulation of gene expression. The limited number of arrays from DAC-treated patients precluded rigorous statistical analysis of gene modulation in these individuals. Seventy-five genes were induced, whereas 324 genes were repressed > 2-fold following DAC treatment. Interestingly, those genes that were induced or repressed > 2- fold by DAC seemed to be down-regulated or overexpressed, respectively, in resected primary lung cancers relative to adjacent, histologically normal bronchial epithelial cells (**Figure 7**). Sixty of the 75 genes induced, and 287 of 324 genes repressed, by DAC were mapped to the PANTHER database. Subsequent Gene Ontology analysis identified several potentially enriched functional groups, including signal transduction and protein modification. Although limited and exploratory in nature, these data suggested that prolonged DAC exposure can shift global gene expression profiles in lung cancer cells from a malignant signature to one more reflective of normal respiratory epithelial cells (50).

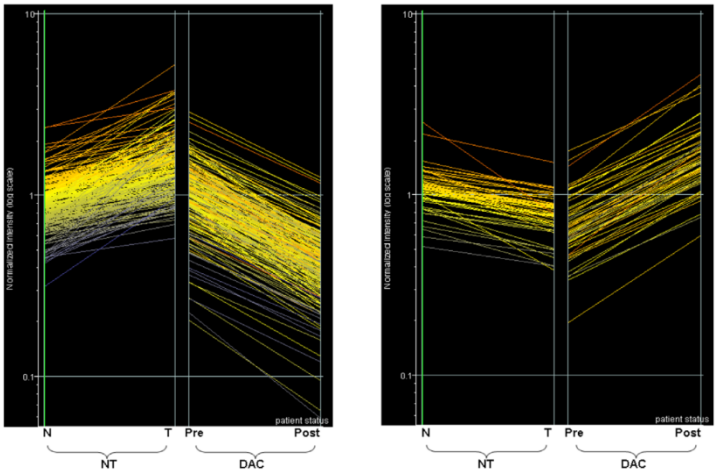


Figure 7: Affymetrix array analysis of gene expression in laser captured lung cancer cells from patients receiving IV DAC, relative to gene expression in laser captured lung cancer cells and adjacent normal respiratory epithelia from patients undergoing resection of their tumors. Genes that were up-regulated in lung cancers relative to normal respiratory epithelia were repressed in lung cancers by DAC treatment. Conversely, genes that were down-regulated in lung cancers relative to normal respiratory epithelia were induced in lung cancer cells by DAC treatment. These data suggest that DAC therapy can "normalize" gene expression profiles in lung cancer cells.

Inhibition of gene-silencing chromatin modifying enzymes such as DNMT1 induces terminal differentiation of myeloid and other cancer cells *in vitro* (reviewed in ref (52)). These differentiation-mediated cell cycle exits, which are similar to those that occur during normal tissue differentiation, do not require p53, and are readily induced in p53/p16-null cancer cells (53). The same non-cytotoxic conditions also increase differentiation of normal progenitors, but in contrast, increase self-renewal of normal hematopoietic stem cells (52, 54). In cancer cells, DAC treatment facilitates differentiation-driving transcription factor-mediated up-regulation of maturation factors which antagonize MYC-function (e.g., CEBPE, p27/CDKN1B) and terminate proliferation independent of p53. In contrast, normal stem cells express master stem cell transcription factors (e.g., HLF, HOXB4), and activate stem cell genes and stem cell fate in response to DAC treatments (52). These observations suggest that dosing of DAC to avoid systemic toxicities may be a novel strategy to induce growth arrest, differentiation or apoptosis of pluripotent cancer cells (52, 54).

When administered by infusion or subcutaneous injection, DAC has pharmacologic limitations that impede translation to chronic epigenetic therapy for solid tumors. For example, DNMT-1 depletion requires DAC levels to overlap with cellular S-phase entry, yet the plasma half-life of DAC is < 20 minutes, which severely curtails the probability of such overlap. This problem is not solved by increasing DAC dose, since off-target effects from higher C_{max} cause cytotoxicity (i.e. myelosuppression) that paradoxically decreases efficacy by restricting the frequency of drug administration. Similarly, continuous infusion is likely not the answer, since toxic increases of DAC can occur in some tissues while inadequate exposure remains in others. This unbalanced distribution may be attributable to widely diverging tissue expression of cytidine deaminase (CDA), the enzyme that rapidly deaminates DAC and the related DNMT1-depleting cytidine analogue 5-azacytidine (5Aza) (55). Moreover, CDA expression is particularly high in the intestines and liver, suggesting that CDA could be a barrier to oral bioavailability of cytidine analogues (**Figure 8**).

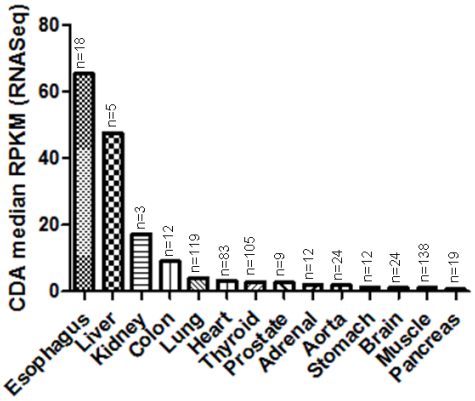


Figure 8: Cytidine deaminase (CDA) expression in normal tissues (RNA-sequencing).

One potential strategy to overcome these limitations is to combine oral DAC with a CDA inhibitor (oral tetrahydrouridine, THU), which has been used extensively with no appreciable toxicities in cancer patients. THU is a competitive inhibitor of CDA with K_i values of 3-5 x 10^{-8}M (56, 57). The very high affinity of THU for CDA may be due to its resemblance to a tetrahedral intermediate of cytidine formed by addition of water across the 3, 4 double bond (57). CDA binds THU approximately 4-orders of magnitude more tightly than uridine, the substrate for the reverse reaction catalyzed by CDA (57). Slow onset of inhibition suggests that structural reorganization precedes the formation of a stable enzyme-inhibitor complex (57). Preclinical studies in mice and nonhuman primates (described in references (58, 59) and extensively summarized in the Investigators Brochure) have established that oral THU administered prior to oral DAC increases bioavailability of DAC approximately 10-fold, prolongs plasma $t_{½}$ from < 20 minutes to > 3 hours, and produces significant in vivo pharmacodynamic effects at a DAC oral dosage of 2.5-5 mg/m^2 (59). Preclinical studies in non-human primates and results of a recent proof of concept Phase I clinical trial in sickle cell disease (SCD) are summarized below.

1.2.2 Pharmacokinetics of DAC Alone Following Intravenous (IV), Subcutaneous (SC) and Oral Administration in Non-Human Primates

The pharmacokinetics of DAC were assessed in female baboons following IV (n=2), SC (n=2), and oral (n=2) administration. The average initial concentration (C_0) following IV DAC 10 mg/m^2 (0.5 mg/kg) in baboons PA7482 and PA7472 was > 3 µM (690 ng/mL), with mean alpha and beta half-lives 2.13 and 180.5 minutes respectively (**Figure 9**). SC DAC 10 mg/m^2 in PA7258 and PA7254 produced peak drug levels < 0.5 µM (114 ng/mL) and a wider concentration-time profile than IV DAC at the same dose (**Figure 9**). Oral DAC 200 mg/m^2 (10 mg/kg) in PA7470 and PA7484 produced peak levels of ~0.2 µM (46 ng/mL) and ~ 0.01 µM (2.3 ng/mL) respectively, and a wider concentration-time profile than SC administration (**Figure 9**).

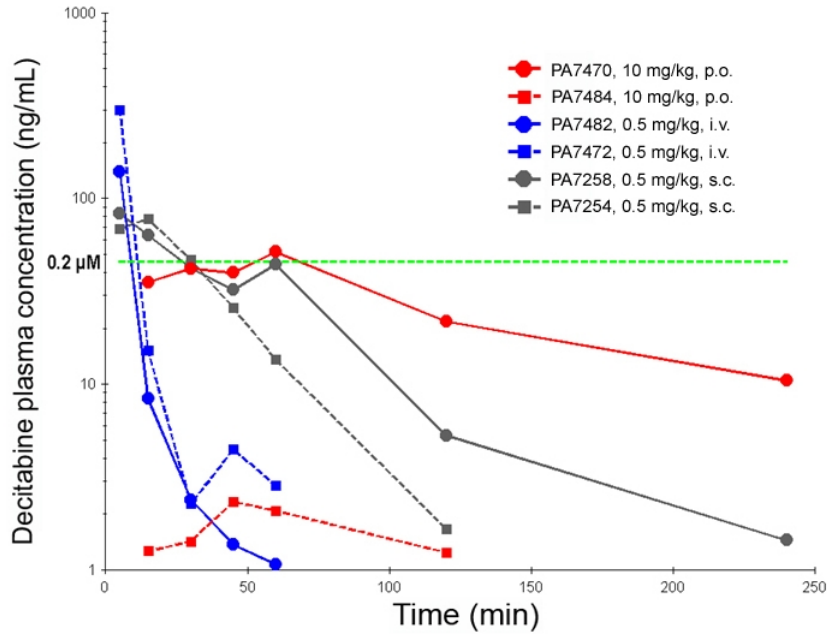


Figure 9: Plasma concentration-time curves of decitabine following intravenous (i.v.), subcutaneous (s.c) and oral gavage (p.o) administration to baboons. i.v. (0.5mg/kg), s.c. (0.5mg/kg) and p.o. (10mg/kg). Blood was collected at 7 time-points and plasma concentrations were determined using an LC/MSMS method. The horizontal green line indicates a concentration of 0.2 µM.

1.2.3 Identification of Dose and Timing of Oral THU Required to Increase Oral Bioavailability of DAC in Non-Human Primates

In two female baboons, PA7470 and PA7484, selected for high and low bioavailability of oral DAC alone respectively, THU 400 mg/m^2 (20 mg/kg), 60 minutes before DAC 100 mg/m^2 (5 mg/kg), produced higher DAC concentrations than THU 40 mg/m^2 (2 mg/kg) (**Table 1**) (59). In the same baboons after a wash-out period, THU 400 mg/m^2 60 minutes before DAC produced higher DAC concentrations than THU 400 mg/m^2 administered simultaneously or 30 minutes prior to DAC (**Table 1**) (59).

1.2.4 Effects of Oral THU on DAC Oral Bioavailability and Inter-Individual Variability in Non-Human Primates

Eight female baboons were treated with oral DAC 200 mg/m^2 (10 mg/kg) (59). The median AUC_{last} with oral DAC alone was 463 min*ng/ml, with a range between 190 to 6279 min*ng/mL (~33-fold variation) (**Table 1**). After a wash-out period of > 2 weeks, the same animals were treated with oral THU 400 mg/m^2 60 minutes prior to DAC 100 mg/m^2 (5 mg/kg). The median AUC_{last} with THU-DAC (decitabine at half the dose) was 2284 min*ng/ml, with a range between 534 to 7515 min*ng/mL (~14-fold variation) (**Table 1**). The increase in AUC_{last} in the THU group was statistically significant (p=0.02, Wilcoxon test) (**Table 1**), even though AUC_{last} was calculated over 25% more time in the DAC alone group (240 minutes versus 180 minutes for THU-DAC). Furthermore, in many animals in the THU-DAC group, the concentration-time trends suggested DAC levels may have continued to increase beyond the last measured time-point at 180 minutes.

Hence, AUC_{last} values for THU-DAC are underestimates. The coefficient of variation (as a measure of inter-individual variability) of AUC_{last} was 141 in the DAC alone group and 97 in the THU-DAC group. The increase in AUC_{last} and accompanied by decrease in coefficient of variation with THU-DAC was because the largest increases in DAC AUC_{last} occurred in animals which had the poorest response to decitabine alone (**Table 1**): this converged the pharmacokinetic profiles in low oral bioavailability animals towards the animals with higher intrinsic oral bioavailability (**Table 1**). The average decitabine C_{max} was 0.05 µM (10.85 ng/mL) for DAC alone and 0.12 µM (26.98 ng/mL) for THU-DAC (decitabine at half the dose).

Table 1: The largest increases in AUC_{last} produced by co-administration of oral THU occurred in baboons which had the lowest AUC_{last} with DAC alone

		AUC_{last} (min*ng/mL)[#]		
Baboon Number	**Weight (kg)**	**Decitabine 10 mg/kg alone**	**Decitabine 5 mg/kg alone**	**THU 20 mg/kg 60 mins before Decitabine 5 mg/kg**
PA7482	12.3	49.98	Not done	Not quantifiable
PA7484	11.5	190.35	252.73	5621.05
PA7256	19.8	299.02	Not done	760.27
PA7472	10.7	327.25	Not done	444.825
PA7254	14.4	463	Not done	2587
PA7255	19.9	807.8	Not done	533.47
PA7258	12.6	2863.6	Not done	2284.48
PA7470	9.6	6278.78	1219.98	7515.32
Mean±SD		1604 ± 2262		2821 ± 2749
Median±IQR		463 ± 2565		2284 ± 5088
Median±IQR per mg of decitabine*		160 ± 226		457 ± 1017
Fold-variation		~30-fold		~14-fold
Coefficient of Variation		141		97

[#]AUC_{last} calculated over 240 minutes for decitabine alone, 180 minutes for THU-decitabine. *p=0.02. Wilcoxon test. SD=standard deviation. IQR = inter-quartile range

1.2.5 Effects of Repeat Administration of Oral THU-DAC on Blood Counts, DNA Methylation and Fetal Hemoglobin Levels in Non-Human Primates

To evaluate pharmacodynamic effects with repeat dose administration, oral THU-DAC 2X/week for 8 weeks was administered to baboons PA7484 and PA7472 (59). DAC doses were 10 and 5 mg/m^2 respectively, administered by oral gavage 60 minutes after oral THU 400mg/m^2, dosages that were expected to produce C_{max} within the range (0.005-0.5 µM) that was demonstrated to deplete DNMT1 in normal hematopoietic precursors without causing significant DNA damage or cytotoxicity (**see Investigators Brochure)**. In PA7484, platelet counts increased during the first 6

weeks of drug administration, with a decrease during the last 2 weeks (but not below the lower limit of normal) (**Figure 10A**). In the same animal, neutrophil counts decreased during the first 3 weeks of drug administration (but not below the lower limit of normal), and increased during the last 2 weeks (**Figure 10B**). In PA7472, platelet counts increased during drug administration (**Figure 10A**), while neutrophil counts decreased but not below the lower limit of normal (**Figure 10B**). These alterations in blood counts are characteristic of the shifts in hematopoietic differentiation produced by DNMT1 depletion by DAC (60, 61). In PA7472, a bone marrow aspirate 96 hours after THU-DAC administration provided sufficient cells for analysis of DNA damage/repair by γH2AX staining. There was a small increase in γH2AX compared to negative control that was not suggestive of major DNA damage, although early DNA damage induction would have been missed (**Figure 10C**). Oral THU-DAC was also administered 3X/week with 50% of the above DAC doses, again with concurrent increases in platelet and decreases in neutrophil counts (**Figure 11**).

One potential application of long-term DNMT1-depleting therapy is to increase fetal hemoglobin (HbF) in red blood cells as a means to treat hemoglobinopathies such as sickle cell disease (as envisaged in the proposed clinical trial). HbF levels increased progressively during treatment in both PA7484 and PA7472, although at late time-points in PA7484, there was a partial reverse of this trend (**Figure 10D**). In repeat dose experiments with 50% of the above DAC doses administered 3X/week, progressive HbF increases were again noted (**Figure 11**). One intended biological effect of therapy is to hypomethylate promoter CpG that regulate expression of target genes (e.g., γ-globin gene [*HBG*] promoter CpG). *HBG* promoter CpG that are hypomethylated at the developmental stages associated with HbF expression (and which therefore potentially play a role in regulating *HBG* expression) were identified by comparing methylation of β-globin gene cluster CpG in DNA isolated from baboon fetal liver, fetal bone marrow, and adult bone marrow erythroid precursors (**Figure 10E**) (methylation levels of embryonic [*HBE*] and β-globin gene [*HBB*] CpG were similar in fetal and adult erythroid precursors; in both fetal and adult erythroid precursors, CpG in the locus control region, an enhancer for the β-globin genes, were hypomethylated compared to the methylation level in white blood cells). The developmentally responsive *HBG* promoter CpG, but not other CpG such as *HBE* and *HBB* gene promoter CpG, were hypomethylated by up to 20% by the 2X/week oral THU-DAC administration to PA7484 and PA7472, and by up to 50% by 10 days of daily administration of SC decitabine 5 mg/m^2 in PA7002 (**Figure 10E**). The SC treatment with DAC alone was used as a positive control and increased HbF levels up to 81.3% (data not shown).

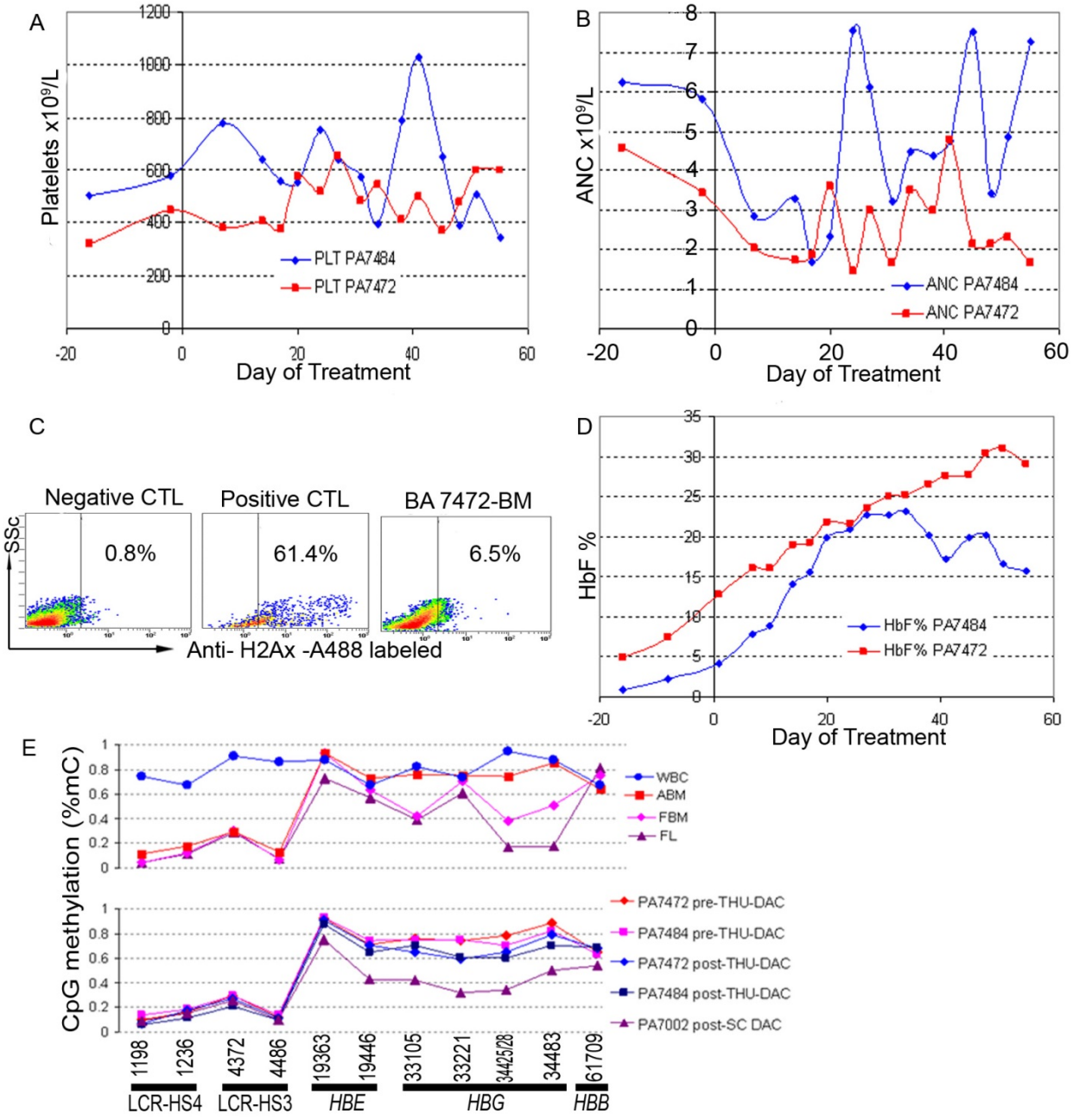


Figure 10: Pharmacodynamic effects of repeat dose oral THU-DAC in non-human primates. DAC 10 mg/m² (PA7484) or 5 mg/m² (PA7472) 60 minutes after THU 400 mg/m² was administered 2X/week for 8 weeks. First dose Day 1. Last day of treatment Day 51 in PA7484, Day 50 in PA7472. A) Platelet counts during treatment. B) Absolute neutrophils counts during treatment. C) Phospho-H2AX (γH2AX) labeling of bone marrow cells 96 hours after THU-DAC administration in week 8 to PA7472. Positive control Hela cells treated with camptothecin 10 µM. Negative control vehicle treated Hela cells. D) HbF expression during treatment. E) Specific hypomethylation of CpG sites in the γ-globin gene (*HBG*) promoter in erythroid precursors. *Top panel*: Methylation levels of CpG in the beta globin cluster in erythroid precursors isolated from baboon fetal liver (FL), fetal bone marrow (FBM), adult bone marrow (ABM) measured by LCMSMS to identify CpG hypomethylated at the developmental stages associated with HbF expression, and to compare methylation of these CpG in erythroid versus non-erythroid cells (white blood cells, WBC). *Bottom panel*: Methylation levels of these CpG before and after 8 weeks of 2X/week oral THU-DAC administration in PA7484 and PA7472, and before and after 10 days of daily treatment with SC DAC 5 mg/m² in PA7002.

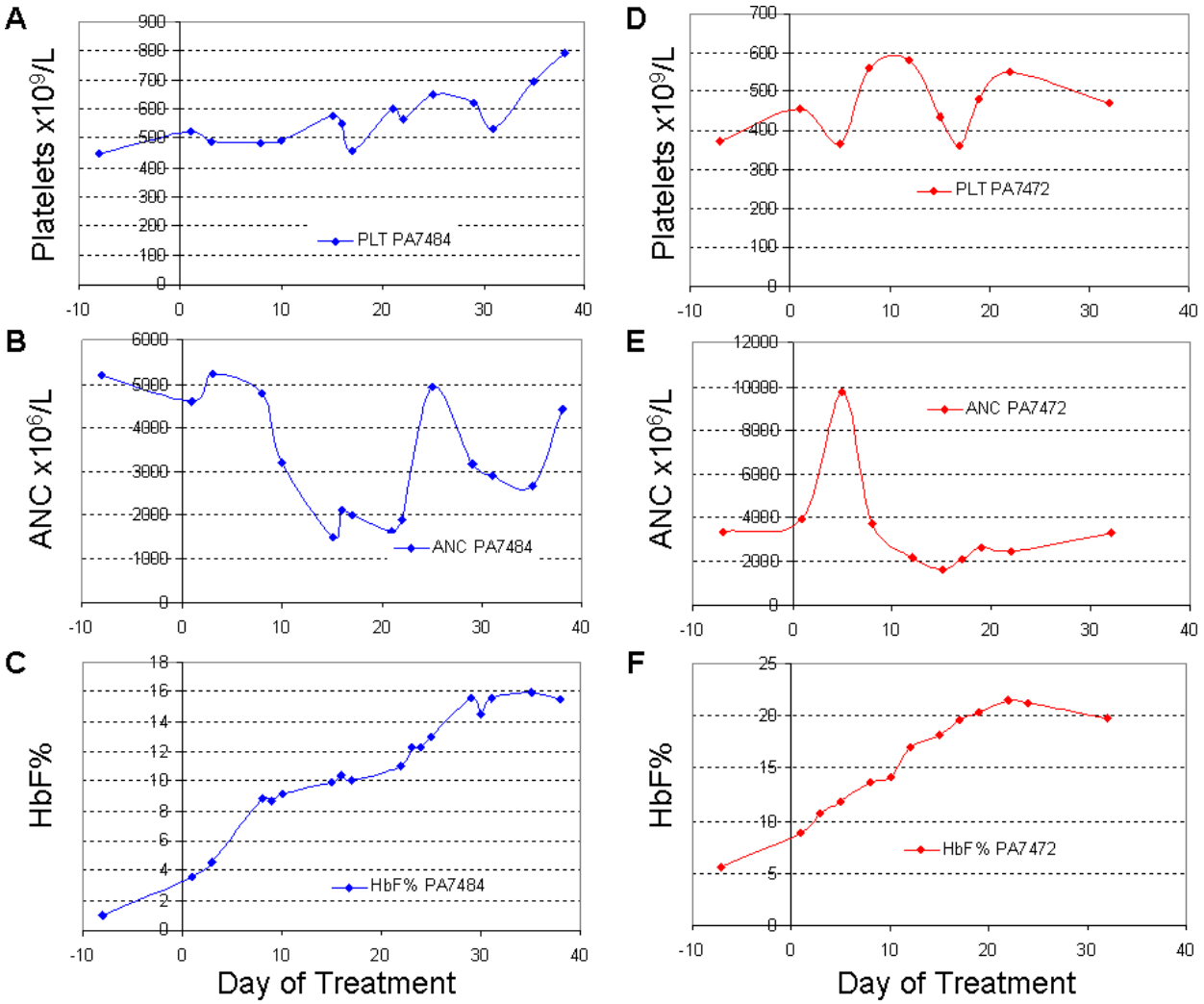


Figure 11: Pharmacodynamic effects of repeat dose oral THU-DAC in non-human primates. DAC 5 mg/m² (PA7484) or 2.5 mg/m² (PA7472) 60 minutes after THU 400 mg/m² was administered 3X/week for 5 weeks (PA7484) or 3X/week for 3 weeks (PA7472). First dose Day 1. Last day of treatment Day 31 in PA7484, Day 19 in PA7472. A) Platelet counts in PA7484. B) Absolute neutrophil counts (ANC) in PA7484. C) HbF% in PA7484. D) Platelet counts in PA7472. E) ANC in PA7472. F) HbF% in PA7472.

1.2.6 Clinical Pharmacokinetics: THU Alone

As previously discussed, THU is a competitive inhibitor of CDA, the enzyme that is largely responsible for the metabolism of cytidine analogues (62). THU has no therapeutic utility as a separate entity, instead, it is frequently used in clinical studies to inhibit CDA and block the metabolism of cytidine analogues which are co-administered as the active therapeutic agent. The clinical pharmacokinetics of THU have been extensively characterized (63).

In these studies, with the exception of time-to-peak THU levels, the pharmacokinetics of intravenous, SQ and oral THU at a dose of 200 mg/m^2 were essentially identical (63). By each route of administration, the initial and secondary half-lives were ~8 and 80 minutes, respectively with a plasma half-life or approximately 7 hours. THU elimination was primarily in the urine and with intact drug but no metabolites identified in urine or plasma.

These observations were extended through the use of [^{14}C]-THU (64). In this study, 50 mg/kg [^{14}C]-THU was rapidly cleared from the blood with a plasma half-life of about 1 hour following intravenous administration. Approximately 10% of the same dose was absorbed from the GI tract following oral administration. The main pathway of excretion was through the kidneys with approximately 50% of the administered drug excreted within 24h and 100% elimination within 48h.THU at 10, 25, and 50 mg/kg given 15 minutes before [3H]-cytosine arabinoside (ara-C) at a dose of 0.003 mg/kg produced an approximately two-fold increase in ara-C blood levels at all times measured from 5 minutes to 4 hours, with only slight increases in the half-life of ara-C (64). A dose-related effect of THU upon the deamination of ara-C was obvious only during the time from 15 minutes to 1 hour after the injection of 3H-ara-C. The inhibitory effect of THU upon CDA was also reflected in a considerably increased ratio of ara-C/uracil arabinoside in the urine.

1.2.7 Clinical Pharmacokinetics: DAC Alone

The clinical pharmacokinetics of three doses of DAC administered as 6-hour infusions are shown in **Figure 12** (65). In this study, steady state concentrations are reached within 1-2h of the onset of the infusion, depending upon the dose administered, and are maintained throughout the infusion cycle. Upon cessation of the infusion, decitabine is rapidly removed from circulation ($t_{½}\alpha$ = 7 minutes) with a slower β-elimination phase ($t_{½}\beta$ = 35 minutes).

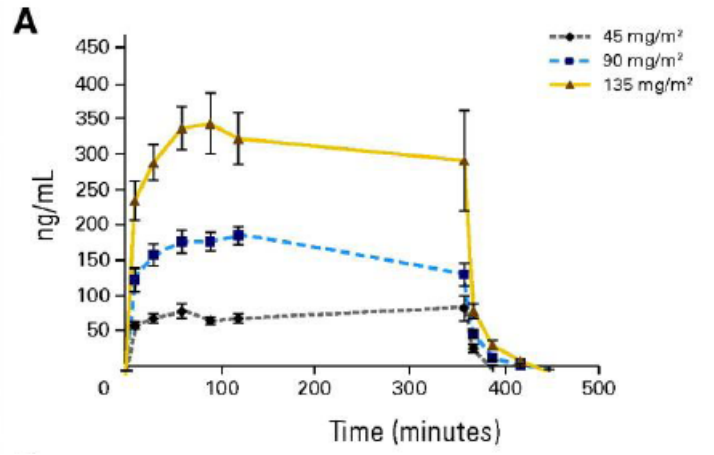


Figure 12: Mean peak plasma levels of DAC were 83, 185.4, and 342.8 ng/mL and a t1/2 of 7 minutes and t1/2 β of 35 minutes (data extracted from (2))

1.2.8 Clinical Safety: THU Alone

Administration of THU to humans by the IV, SQ (SQ), and oral (PO) routes, in combination with various cytidine analogues, in studies spanning over 30 years, suggest it has a benign toxicity profile (63, 64, 66-70) (summarized in **Table 2**). Reports of adverse events following treatment are infrequent. In a study of THU alone administered by IV, SQ and PO routes at a single dose of 200 mg/m^2 to 5 patients with malignant melanoma, there was no mention of toxicities (63). Similarly, THU administered at a daily dose of approximately 900-1800 mg/m^2 for 5 consecutive days together with cytosine arabinoside did not produce toxicities other than those expected with cytosine arabinoside alone (70). A similar experience is described when THU was administered at 700 mg/m^2 daily for 4 consecutive days together with cytosine arabinoside (68). Currently, THU is being used in three NIH-sponsored clinical trials: at 350-500 mg/m^2 administered daily for 3-5 consecutive days in combination with 5-fluoro-2'-deoxycytidine (e.g. NCT00077051), or daily for 7 or more days in combination with other oncotherapeutics (e.g. NCT00378807, NCT00521183).

Table 2: Clinical trials with tetrahydrouridine (published data).

THU dose	THU Route	Combination drug	Number of patients	Toxicity attributed to THU?	Reference
350 mg/m^2 Q12 hours x 4 days	IV	IV Ara-C 100-200 mg/m^2 Q12 hours x 4 days	32	None reported	(68)
350 mg/m^2 daily x 5 days	IV	IV 5-fluoro-2'-deoxycytidine 5-80 mg/m^2 daily x 5 days	11	None reported	(71)
2,100 – 2,800 mg/m^2 total dose	IV	IV Ara-C 1,200 - 1,600 mg/m^2 and carboplatin 900 mg/m^2 total dose	8	Myelosuppression, unacceptable hepatotoxicity, diarrhea. No attribution to direct THU effect	(72)
2 - 4 mg/kg (approx 75-150 mg/m^2) for 3 consecutive days every week	oral	Oral 5-azacytidine 0.2mg/kg for 3 consecutive days every week	2	None reported	(69)
25 – 50 mg/kg (approx 900 – 1800 mg/m^2) daily x 5 days	IV	IV Ara-C 0.1 – 0.2 mg/kg (approx 4 – 8 mg/m^2) daily x 5 days		None reported. Toxicities were as expected from Ara-C alone	(70)
200 mg/m^2 x 1	IV, SQ and oral	none	5	None reported	(63)
350 mg/m^2 daily x 5 days every 21 days OR for 5 days in each of 2 consecutive weeks every 28 days	IV	IV 5-fluoro-2'-deoxycytidine 2.5-180 mg/m^2/day x 5 days every 21 days OR for 5 days in each of 2 consecutive weeks every 28 days	58	None reported	(73)

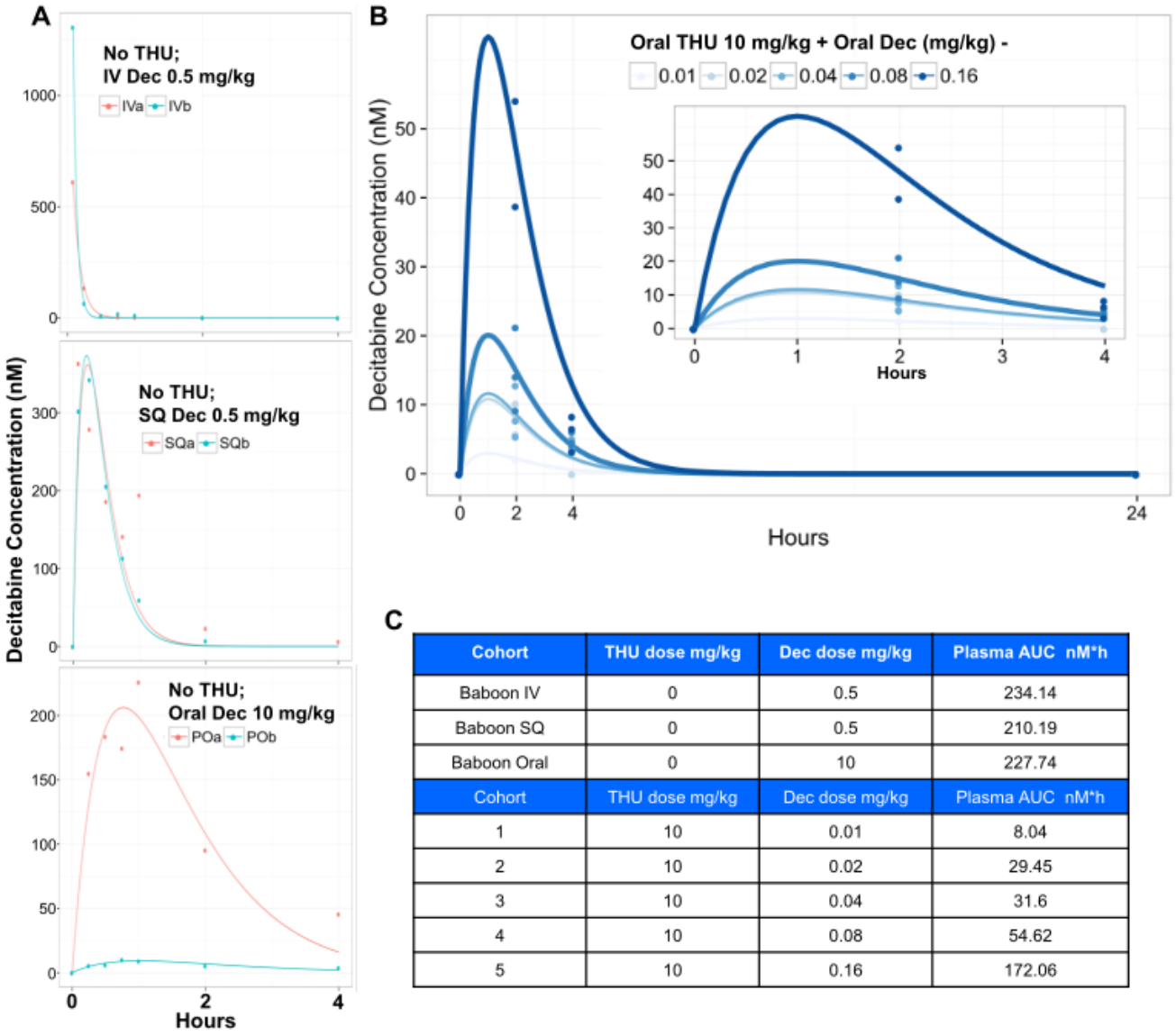
1.2.9 Clinical Safety and Efficacy: Oral THU and DAC in Combination, Repeat Dose Phase 1/2 Study in SCD

A recent Phase I clinical trial was performed to evaluate the toxicities and potential efficacy of oral DAC in combination with oral THU in patients with SCD (Saunthararajah, et al, submitted for publication). The objectives of the study were to (i) extend DAC half-life/T_{max} to hours instead of minutes, to increase S-phase dependent DNMT1-depletion (ii) without high DAC C_{max} that causes off-target effects and cytotoxicity (intended C_{max} > 5nM and < 200nM), (iii) decrease within and between patient variability in DAC distribution (that is, to produce stable distribution of DAC into tissues that otherwise provide sanctuary to myeloid cancer cells from DAC treatment effects), (iv) increase HbF to clinically meaningful levels in patients with SCD.

The timings of administration between oral THU and oral DAC, and the dosages of THU and DAC in this clinical trial, were selected based on the above pharmacologic/pharmacodynamics objectives and studies in mice, and non-human primates (58, 59).

Pharmacokinetic results: For the purposes of non-cytotoxic DNMT1-depletion, a wide concentration-time profile with low peak concentration or C_{max} but extended exposure time or extended T_{max} is desired (**Figure 13**) (59). To evaluate effects of different routes of administration on the DAC concentration-time profile, plasma DAC levels were compared after intravenous (IV), subcutaneous (SQ) and oral administration to non-human primates (baboons). IV DAC 0.5 mg/kg produced peak plasma drug concentrations (C_{max}) > 600 nM and plasma $t_{½}$ < 5 minutes (**Figure 13**). SQ DAC at the same dose produced C_{max} ~300 nM and $t_{½}$ ~40 minutes. Oral DAC at 20-fold greater dose (10 mg/kg) produced widely differing C_{max} of ~220 and 10 nM in two baboons, with $t_{½}$ > 100 minutes (**Figure 13A**). Thus, DAC oral absorption is very limited and very variable, although it does produce the wide concentration-time profile desired for non-cytotoxic DNMT1-depletion.

Pre-clinical studies in mice and baboons suggested that combining oral THU with oral DAC could address the very poor oral bioavailability and very wide inter-individual variability, creating the rationale for this first-in-man clinical trial of oral THU-DAC (**Figure 13**) (59). Samples for PK analyses were obtained at 0, 2, 4 and 24 hours in 12 of the 15 subjects who received study drug (dictated by venous access). DAC levels were measurable even in subjects receiving the lowest DAC dose of 0.01 mg/kg. A dose-dependent increase in plasma DAC was observed. The highest dose of oral Dec, 0.16 mg/kg, produced DAC plasma concentrations at 2 hours between 39-54 nM. The dose level below this, 0.08 mg/kg, produced concentrations at 2 hours of between 9-21 nM. These concentrations of DAC were in the range predicted to have non-cytotoxic epigenetic therapeutic effects (59), and accordingly, these dose levels were the only ones also associated with clinical pharmacodynamic effects, as described below. The model fit suggested C_{max} was at approximately 1 hour, prior to the 2 hour time-point measured in this study. At all dose levels, by 4 hours, DAC plasma levels had decreased to < 50% of the level measured at 2 hours.



C

Cohort	THU dose mg/kg	Dec dose mg/kg	Plasma AUC nM*h
Baboon IV	0	0.5	234.14
Baboon SQ	0	0.5	210.19
Baboon Oral	0	10	227.74
Cohort	THU dose mg/kg	Dec dose mg/kg	Plasma AUC nM*h
1	10	0.01	8.04
2	10	0.02	29.45
3	10	0.04	31.6
4	10	0.08	54.62
5	10	0.16	172.06

Figure 13: Pharmacokinetics (PK) of DAC and oral THU-DAC. A) DAC (Dec in this figure) PK after intravenous (IV), subcutaneous (SQ) and oral (PO) administration to baboons. B) DAC PK in human subjects from this clinical trial. Samples for PK analysis were obtained in 12 of the 15 subjects who received study drug. Stated times are hours after administration of oral Dec, data-points are measured values while curves were fitted by a global fit model using the R package *PKLMfit.R*. The inset shows a close up for hours 0-4, to facilitate comparison to the 4 hour time-frame of the PK studies in baboons. Dec was quantified by a validated LC-MS/MS method. C) Dec AUC estimations in the baboons and in the different cohorts of the human clinical trial *(model-dependent AUC estimates PKLMfit.R)*. The objectives with combination with THU were a wider concentration-time profile (that is low Cmax but extended Tmax or t½) and Dec distribution through CDA rich organs such as the intestines and liver.

Adverse events: Non-cytotoxic modification of hematopoietic differentiation by DAC is expected to simultaneously increase platelet counts and decrease absolute neutrophil counts (shown *in vitro* and in clinical studies with DAC alone (60, 61, 74, 75)). This expected pattern in blood counts was seen in subjects receiving oral DAC 0.08 and 0.16 mg/kg (dose levels 4 and 5) with the steepest

blood count changes observed at the highest dose (**Figure 14**). Platelet and neutrophil counts on therapy did not reach the *a priori* defined triggers for dose modification, that is, neither a platelet count of > 1200 x 10^9/L, nor an absolute neutrophil count < 1.5 x 10^9/L, were reached (the highest platelet count on therapy was 1122 x 10^9/L in a subject with a pre-treatment count of 733 x 10^9/L, and the lowest absolute neutrophil count on therapy was 1.6 x 10^9/L in a subject with a pre-treatment count of 1.8 x 10^9/L) (**Figure 14**). Of particular relevance to this protocol, absolute lymphocyte counts remained stable during and subsequent to the eight-week THU-DAC therapy (**Figure 15**). The pattern of adverse events was similar between subjects receiving placebo or oral THU-DAC in all dose cohorts (**Table 3A**). There were no significant adverse events, including no significant gastro-intestinal adverse events, attributed to study drug (**Table 3B**). The most frequent adverse event in placebo and study drug treated subjects was pain from vaso-occlusive sickle cell crisis (**Table 3A**). There were no Grade 4 adverse events in placebo or study drug treated subjects (**Table 3**). No subjects discontinued placebo or study drug because of adverse events.

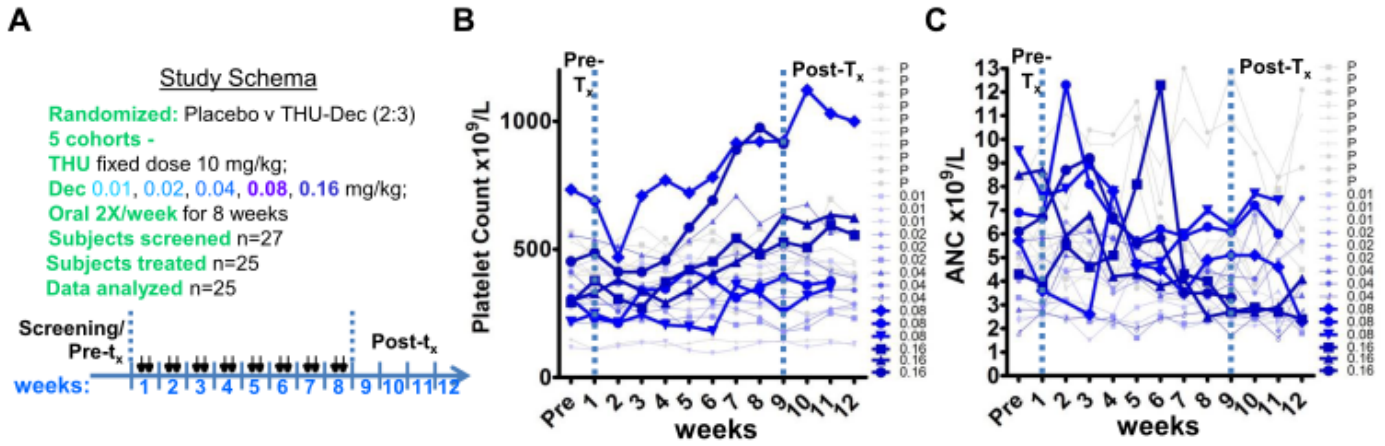


Figure 14: Platelet counts increased and absolute neutrophil counts simultaneously decreased during repeat dose administration, the blood count profile expected from non-cytotoxic DNMT1 depletion. A) Study schema showing repeat dose administration 2X/week for 8 weeks. B) Platelet counts. C) Absolute neutrophil counts (ANC).

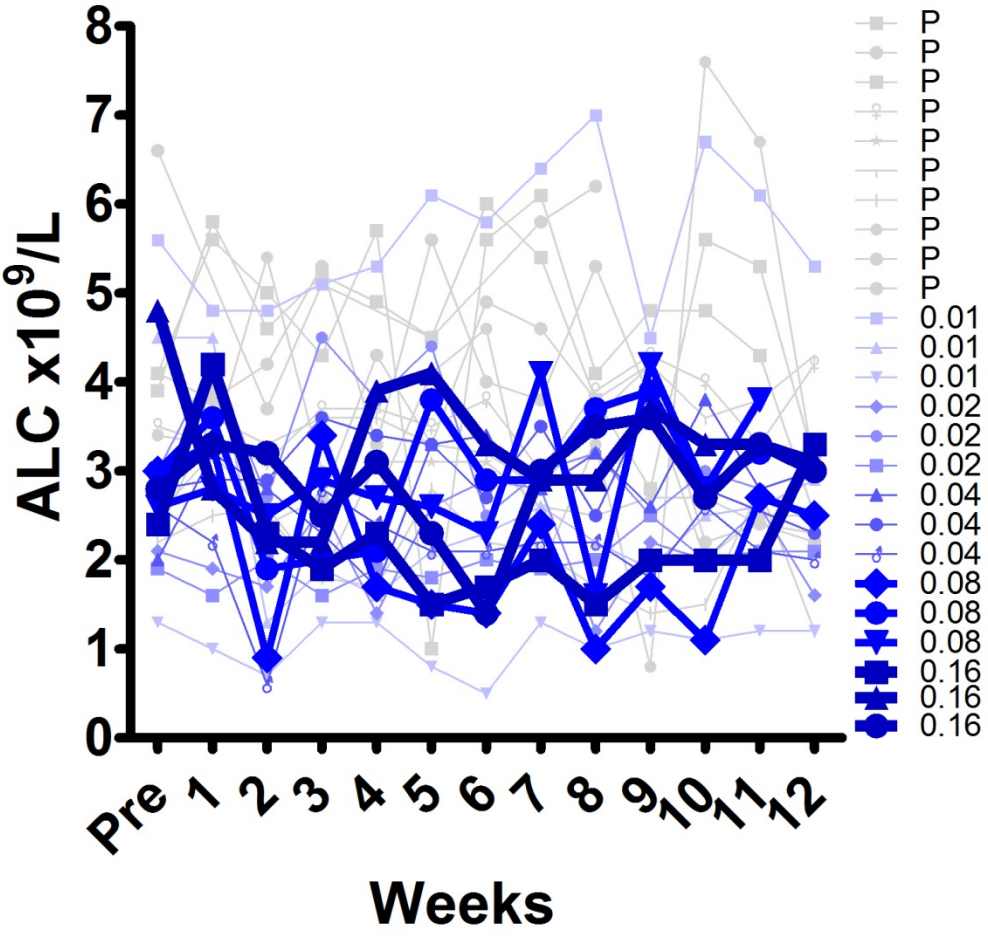


Figure 15: Absolute lymphocyte counts in SCD patients treated with increasing doses of THU-DAC.

Efficacy/Pharmacodynamic Effects: Hemoglobin F levels were measured by HPLC through the Clinical Pathology laboratory and also by flow cytometric quantification of HbF in individual red cells. Significant increases in HbF were observed by both these measurements at dose levels 4 and 5 (DAC 0.08 and 0.16 mg/kg respectively), with the greatest increases occurring at dose level 5 (**Figure 16**). These HbF increases were associated with trends for increasing total hemoglobin, decreasing LDH and total bilirubin (as biomarkers of hemolysis) and decreasing D-dimer levels (as a biomarker of coagulation pathway activation and vaso-occlusion) (**Figure 17**). Increases in HbF were not sustained once study drug was stopped in Week 8 (**Figure 16**).

Hematologic side-effects and toxicity: There were no hematologic toxicities warranting treatment hold (please see above).

Non-hematologic toxicity: No significant non-hematologic toxicities were observed (please see above).

In summary, results from the aforementioned Phase 1 clinical trial of oral THU-DC demonstrate that the non-human primate data essentially mapped to subjects with high risk SCD: combining a

mini-dosage of oral DAC (0.16 mg/kg; ~5 mg/m^2) with THU 10 mg/kg produced major improvement in DAC tissue distribution and exposure, creating a wide DAC concentration-time profile that is desired for DNMT1-depletion without cytotoxicity in vivo. This DAC oral dosage compared very favorably to parenteral dosages FDA approved for administration to humans (20-45 mg/m^2/day for 3-5 days every 4-6 weeks). The THU-DAC was very well-tolerated without significant gastro-intestinal or other drug-related adverse events with repeat administration 2X/week for 8 weeks. Changes in hematologic parameters were those expected from non-cytotoxic DNMT1-depletion by DAC, with simultaneous increases in platelet counts and hemoglobin and decreases in absolute neutrophil counts. The platelet and absolute neutrophil count changes did not cross thresholds that would have triggered treatment holds (> 1,200 x 10^9/L for platelets, < 1.5 x 10^9/L for neutrophils). Increases in HbF that would be considered clinically significant if generated by standard of care HU therapy were observed at DAC dosage of 0.08 and 0.16 mg/kg. The HbF increases were associated with favorable trends in total hemoglobin and in peripheral blood markers of hemolysis and coagulopathy/vaso-occlusion – serum LDH, total bilirubin and D-dimer.

A

Number of Events	PLACEBO (n=10)			COHORT 1 (n=3)			COHORT 2 (n=3)			COHORT 3 (n=3)			COHORT 4 (n=3)			COHORT 5 (n=3		
SYMPTOM	Grade			Grade			Grade			Grade			Grade			Grade		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Pruritus	1			1						1						3		
Upper respiratory infection	1	2		2						1			1			3		
Diarrhea	1			1	1								1			1		
Vomiting		2		1						1	1		1			1		
Urinary tract infection	1				1									3		1		
Pain	8	13	4	3	1	1	1	1	3	1	3	3	1		2	2	2	1
Acute Chest Syndrome			1															
Pneumonia		1												1				
Headache	3	1		1			1			1	1		1	1				
Acute kidney injury	1																	
Hematuria				1	1													
Nausea	1	1		1			1			2	1					1		
Constipation					1					1	1		1					
Rash maculo-papular	4			1			1			1								
Skin hyperpigmentation							1											
Hypomagnesemia							1			1								
Hypokalemia										2								
Leukocytosis		1											1			1		
Anemia											1			1				
Leukocytosis		1										2						
Hypomagnesemia										1						1		
Chest pain - cardiac		1																
Hyperkalemia													2					
Hypertension				1						1				1				
Dysmenorrhea, Oligomenorrhea														1				
Transaminitis		2											1					
Light-headed				1							1							
Abdominal pain or cramps		2		1						1	4		1					
Calve cramps				1														
Vaginal spotting	1			1														
Allergic rhinitis	1																	
Pinpoint pupils	1																	
Enlarged tonsils							1		1									
Tachycardia	1						2			2								
Drowsiness							1											
Pulmonary embolus									1									
Otitis media							1											
Decreased range of motion								1										
Diaphoresis							1											
Vaginal yeast infection										1								
Lip ulcer										1								
Lower extremity swelling											1					1		
Skin ulceration										1								
Leukopenia										1								
Wheezing										1								
Iron deficiency													1					
Tounge swelling	3																	
Cough																1		

B

	COHORT 1				COHORT 2				COHORT 3				COHORT 4				COHORT 5			
	Grade				Grade				Grade				Grade				Grade			
ADVERSE EVENT	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Pruritus	Probable x1																			
Diarrhea	Unlikely x1	Possible x1																		
Vomiting	Possible x1								Unlikely x1											
Urinary tract infection		Unlikely x1																		
Nausea	Unlikely x1									Unlikely x1										
Rash maculo-papular	Unlikely x1																			
Abdominal pain or cramps	Probable x1																			
Calve cramps	Probable x1																			
Leukopenia									Possible x1											

Table 3: Adverse events in subjects treated with placebo and with oral THU-DAC at the various dose levels (Cohorts 1-5). A) All adverse events as per CTCAE v 4.0. There were no Grade 4 adverse events. B) Adverse events with attribution other than unrelated to study drug per the judgement of the treating clinical team.

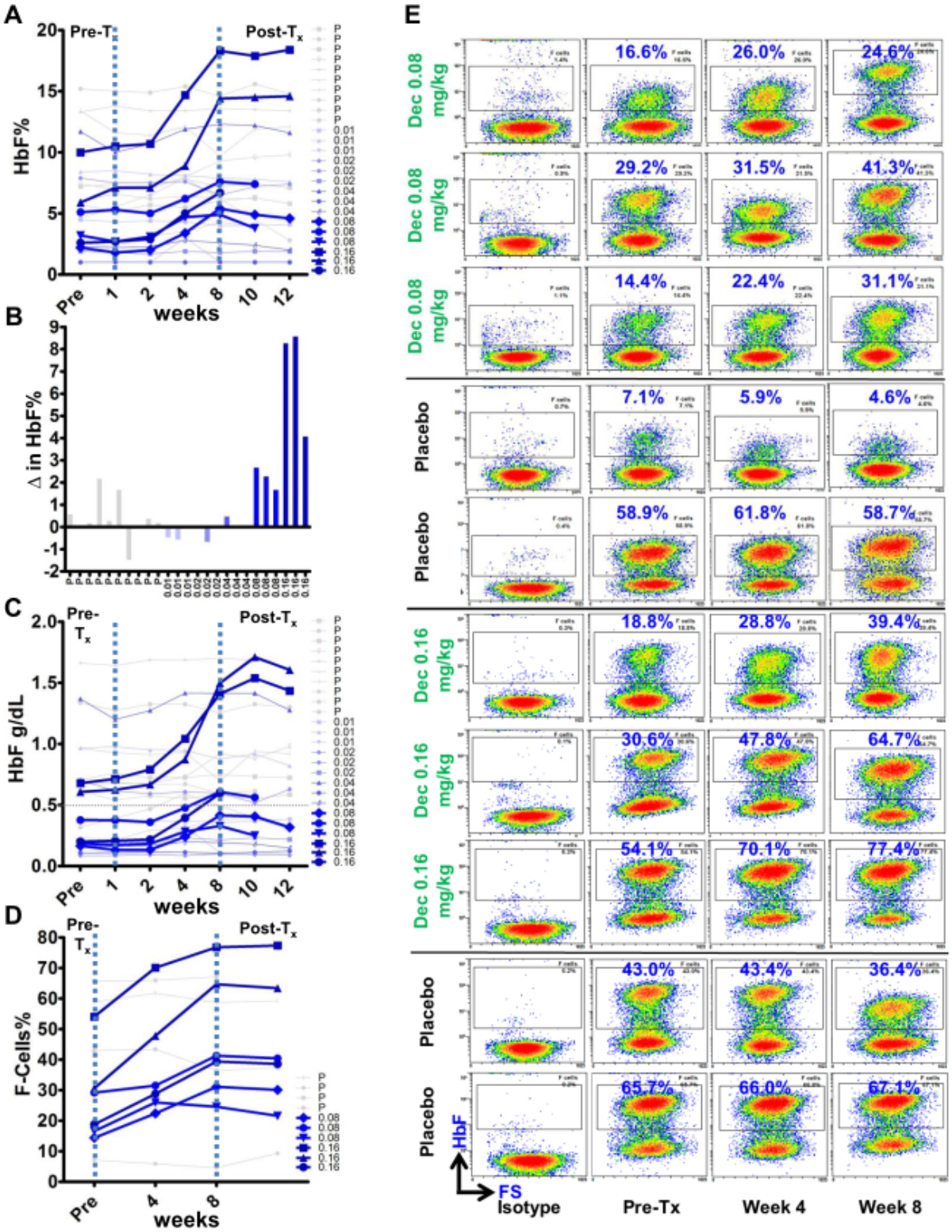


Figure 16: Efficacy. A) Increase in HbF% measured by HPLC was observed in subjects receiving THU-DAC 0.08 and 0.16 mg/kg (fixed dose THU 10 mg/kg 60 mins prior to DAC). B) Change in HbF% from pre-treatment to Week 8 or 10 was most prominent at THU-Dec 0.08 and 0.16 mg/kg. C) Absolute HbF levels.

Horizontal grey line shows 0.5 g/dL threshold associated with better survival. D) Proportion of RBCs expressing high levels of HbF (F-cells) in subjects receiving THU-DAC 0.08 or 0.16 mg/kg. E) Raw F-cell flow cytometry data in Cohort 4 and 5 subjects.

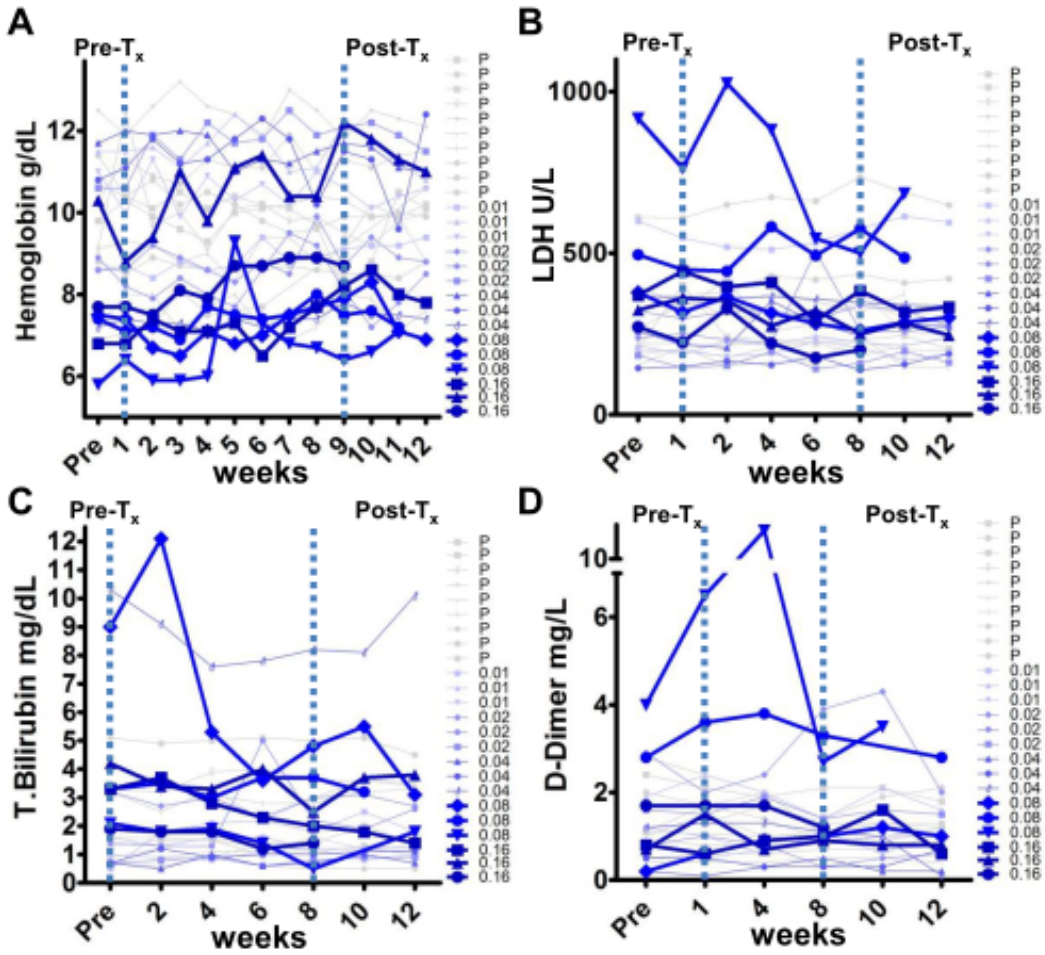


Figure 17: Efficacy: hematologic and chemistry parameters impacted by changes in HbF expression in subjects receiving DAC 0.08 and 0.16 mg/kg. A) Total hemoglobin. B) LDH (biomarker of hemolysis). C) Total bilirubin (biomarker of hemolysis). D) D-dimer (biomarker of coagulation pathway activation and vaso-occlusion).

Because FOXP3 is regulated by epigenetic mechanisms (76), it is possible that DAC-THU therapy will mediate expansion and enhanced immunosuppressive function of Tregs in cancer patients. In-vitro treatment of human Tregs with DAC induced hypomethylation of the Treg specific DNA demethylated region (TDMR), which is required for stable expression of FOXP3, and increased expression of Treg specific genes; however, the cells did not exhibit full suppressive function (77). On the other hand, 5-Aza which is converted to DAC before incorporation into DNA increases Tregs and significantly reduces airway inflammation in mice (78). Furthermore, adoptive transfer of Tregs treated in vitro with DAC mediates resolution of lung inflammation in a murine ARDS model (79). Collectively these data suggest that DNMT depletion may enhance Treg function in humans; as such strategies to inhibit Treg activity may facilitate more effective antitumor effector responses in cancer patients receiving DAC-THU therapy.

Rationale to Combine DAC-THU with Immune Checkpoint Inhibitors for Treatment of Thoracic Malignancies:

Although substantial preclinical studies including those cited above as well as a recent report by Kim et al (80) support evaluation of epigenetic regimens in combination with immune checkpoint inhibitors, observations that 3 of 5 lung cancer patients previously treated with sequential azacytidine/etinostat without success exhibited objective responses to anti-PD-1 or anti-PD-L1 therapy (81-83) provided the clinical impetus to develop combinatorial epigenetic immunotherapies for cancer. Numerous trials have been initiated to evaluate azacytidine with or without etinostat or other HDAC inhibitors and either pembrolizumab or nivolumab in cancer patients who have failed first-line cytotoxic or TKI therapies (84).

Several recent studies have further evaluated potential mechanisms by which DNA demethylating agents augment antitumor immunity **Figure 18**. Wrangle et al (85) examined gene expression profiles in several lung cancer lines following treatment with low dose 5-azacytidine (AZA) which is converted to DAC upon incorporation into DNA, and observed that AZA up-regulated cancer-testis antigen expression as well as numerous pathways mediating innate and adaptive immunity. In addition, AZA up-regulated PD-L1, which could dampen cellular immune responses to cancer cells. The effects of AZA on immune pathways in lung cancer cells were very similar to those observed in HCT116 colon cancer cells following knock-out of DNMT1 and DNMT3b.

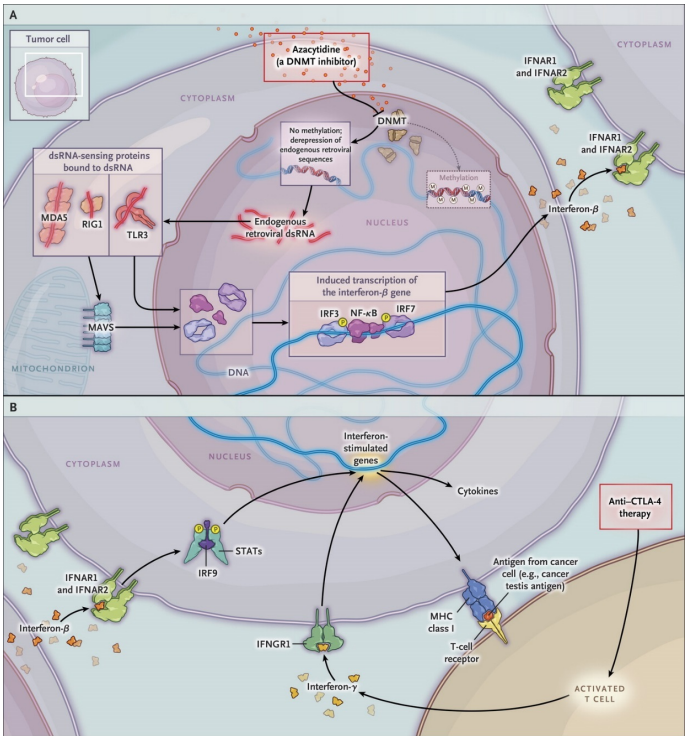
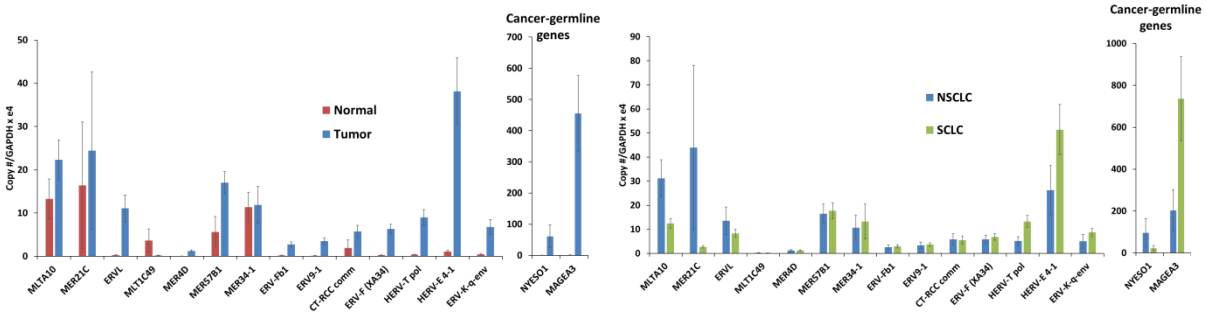


Figure 18: DNA demethylating agents augment antitumor immunity

Li et al (86) examined gene expression profiles in a large panel of breast, colorectal and ovarian cancer lines following treatment with low dose AZA, and observed up-regulation of cancer-testis antigens as well as significant enrichment for immunomodulatory pathways including antigen

processing and presentation, cytokines/chemokines, and interferon signaling. Based on expression profiling, an AZA-IMune (AIM) gene set was defined, which appeared to be up-regulated in primary tumor tissues from cancer patients receiving AZA treatment. More recently, Chiappinelli et al (87) observed that low dose AZA de-represses endogenous retroviruses (ERV) which trigger the double stranded RNA (dsRNA) sensing machinery to induce a type I interferon response similar to a viral response. Analysis of melanoma, breast, ovarian, colon and lung cancers in TCGA revealed that cancers with high levels of viral defense genes were more likely to have better outcomes. Furthermore, high-level intratumoral expression of viral defense genes was associated with improved outcomes in melanoma patients receiving the immune checkpoint inhibitor, anti-CTLA-4. Roulois et al (88) observed that low dose AZA targets colorectal cancer initiating cells (CICs) by induction of "viral mimicry" (**Figure 8**) secondary to up-regulation of ERV, activation of the MDA5/MAVS RNA recognition pathway and up-regulation of IRF7. Knock-down ofMDA5/MAVS, or IRF7 significantly attenuated the inhibitory effects of AZA on proliferation and clonogenic potential of CICs.

A series of experiments have been performed in the Thoracic Epigenetics Laboratory, TSB to examine endogenous retroviral (ERV) expression in lung cancer cells, and to evaluate the feasibility of using DNA hypomethylating agents to augment ERV expression as a potential adjunct to immune check point inhibitor therapy for lung cancer. We also sought to identify potential PD endpoints that could be incorporated into our epigenetic-immunotherapy protocols. Briefly, SYBR Green quantitative RT-PCR techniques were used to examine expression of nine ERV family sequences (MLTA10, MLT1B, MER21C, ERVL, MLT1C627, MER4D, MER57B1, MTE2B4) and cancer-germline genes in normal human bronchial epithelial (NHBE) cells, normal small airway epithelial cells (SAEC), 13 NSCLC lines, 20 SCLC lines, and 5 NSCLC lines and NHBE treated with 5-aza-2'-deoxycytidine (DAC; 0.1 uM x 72 hours). ERV expression patterns in lung cancer lines and normal respiratory epithelial cells were primer specific with no consistency in SCLC or NSCLC lines (**Figure 19**). Lung cancer lines generally showed higher ERV expression than normal cells. MLT1B, ERVL, and MLT1C627 were up-regulated more than two-fold in 82%, 73%, and 70% of lung cancer lines, respectively, compared to normal respiratory epithelial cells. Activation of ERV in lung cancer lines did not appear to coincide with de-repression of cancer-testis genes. DAC mediated relatively modest (two-fold average) up-regulation of ERV, with MLTA10 (2.7 fold), MER21C (3.6 fold) and ERVL (2.6 fold) showing highest induction. Collectively these data demonstrate that cultured lung cancer cells exhibit heterogeneous endogenous ERV expression profiles, as well as variable patterns of ERV induction following exposure to DNA demethylating agents.



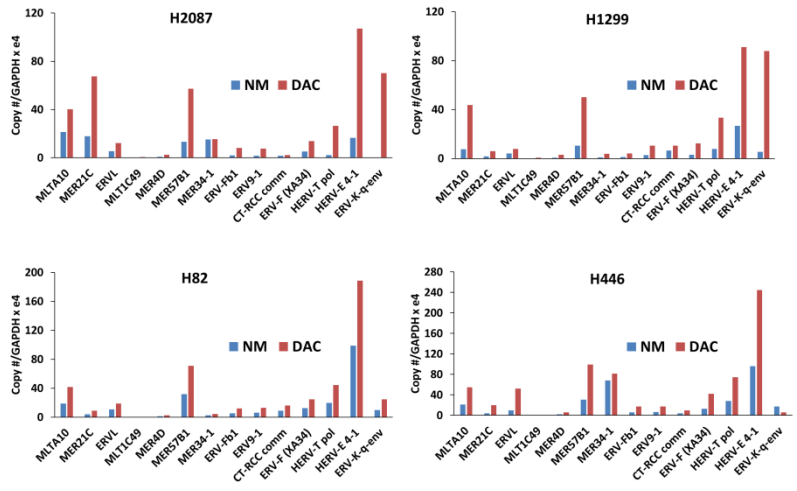


Figure 19: Top panel: qRT-PCR analysis of endogenous ERV expression in a panel of NSCLC specimens and matched normal lung tissues; middle panel: ERV expression levels in NSCLC vs SCLC lines; lower panel: effects of DAC on ERV expression in cultured NSCLC cells.

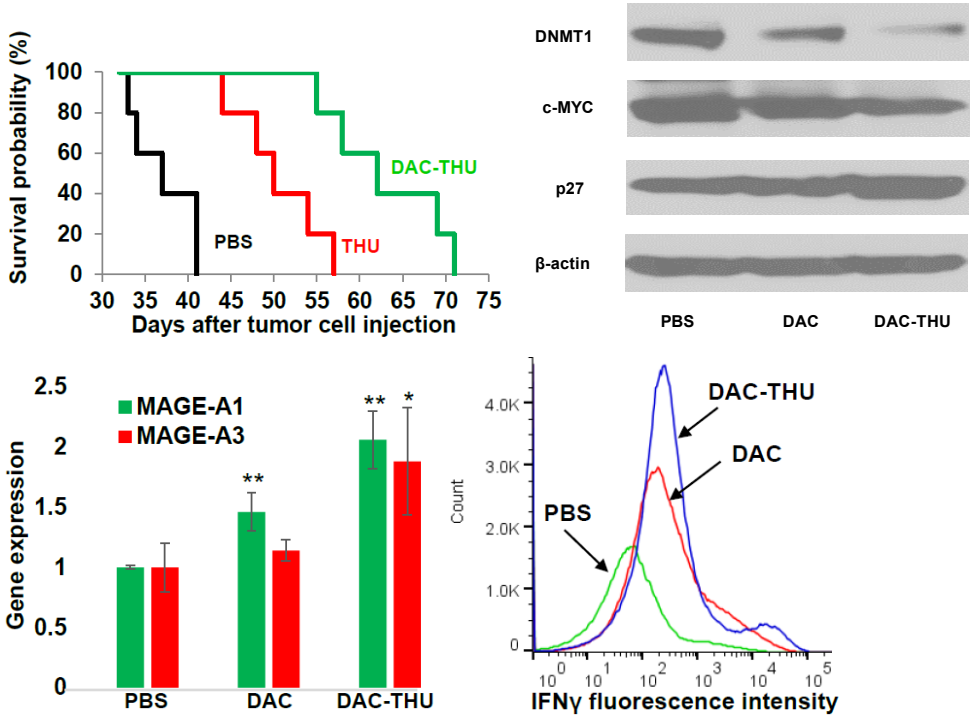


Figure 20: Top left: survival of mice with Lewis lung cancer metastases treated with PBS, DAC, or DAC-THU. Top right: immunoblot demonstrating depletion of DNMT1 and MYC in lung cancer tissues following DAC-THU exposure. Lower left: qRT-PCR demonstrating effects of DAC-THU on cancer-testis gene expression in-vivo lung cancers. Lower right: THU enhances DAC-mediated induction of IFNγ in lung cancer tissues.

Recently a series of preclinical experiments have been performed by Drs. Vamsi Velchetti and Yogen Saunthararajah at the Cleveland Clinic to examine potential efficacy of DAC-THU and anti-PD-1 therapy for lung cancer. Briefly, immunocompetent C57/BL6 mice were inoculated via tail vein with Lewis lung cancer cells. Eighteen days later, mice were imaged to confirm lung colonization and then randomized to receive PBS SC, DAC 0.2mg/kg SC, or DAC (0.1mg/kg) SC/ THU (10mg/kg IP) q M-W-F. On Day 32, mice were bled for flow cytometry experiments and subsequently euthanized when ill-appearing. DAC-THU therapy significantly prolonged survival relative to DAC alone or PBS (**Figure 20**). Combination treatment markedly decreased DNMT1 and to a lesser extent, MYC protein levels while increasing p27 expression, consistent with the aforementioned p53- independent cell cycle exit effect of DNMT1 depletion. DAC-THU therapy also significantly up-regulated intratumoral MAGE-A1 and MAGE-A3 cancer-testis gene expression, as well as intratumoral interferon-γ levels. Furthermore, relative to PBS controls, mice treated with either DAC or DAC-THU exhibited significantly higher peripheral CD4 lymphocytes (approaching those in untreated, nontumor bearing mice), as well as significantly decreased MDSC in peripheral blood (**Figure 21**).

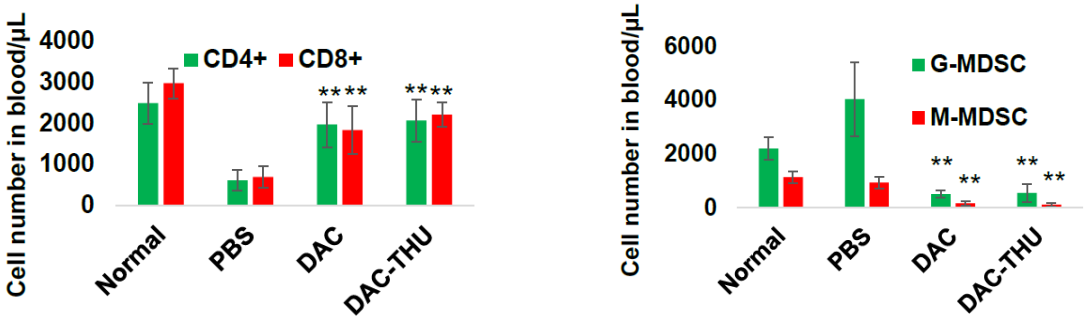


Figure 21: Effects of systemic DAC and DAC-THU on peripheral T cell subsets and MDSC in mice bearing Lewis lung cancer metastases.

In additional experiments, C57/BL6 mice with Lewis lung cancer metastases were randomized to receive PBS SC, DAC (0.1 mg/kg) SC/THU (10 mg/kg IP) q M-W-F, a murine anti-PD-1 antibody (100 ug) q 5 days, or a combined DAC-THU/ anti-PD-1 regimen. Both DAC-THU and anti-PD-1 alone prolonged survival; a combinatorial effect was observed, with 40% of mice receiving DAC-THU-anti-PD-1 therapy appeared to be cured of their disease (**Figure 22**; left panel). Relative to tumor bearing mice treated with PBS alone, mice receiving epigenetic treatment, immunotherapy, or combination DAC-THU and anti-PD-1 therapy exhibited significantly higher peripheral CD4 and CD8 lymphocyte counts (**Figure 22**; right panel). Collectively these findings, together with recent observations that DNA demethylating agents and other epigenetic drugs enhance the efficacy of immune checkpoint inhibitors by blocking immunosuppressive activities of Tregs and MDSC in the tumor microenvironment provide strong rationale for evaluation of DAC-THU and pembrolizumab for cancer therapy.

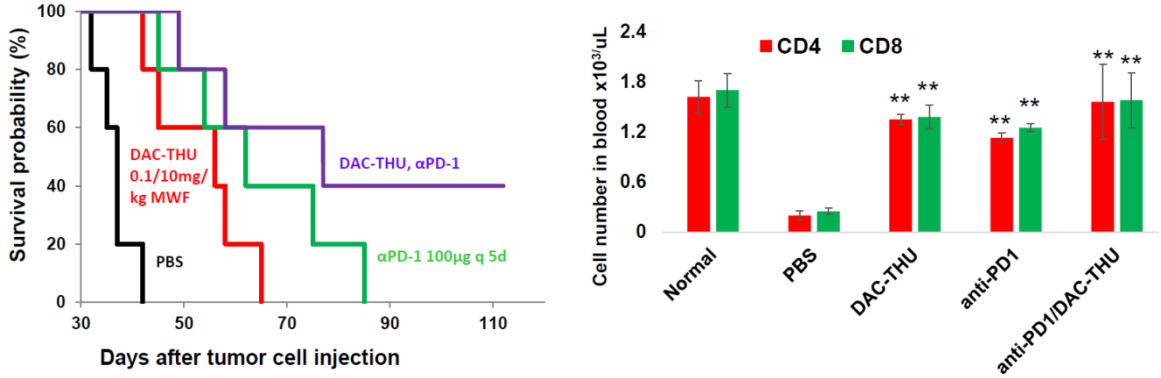


Figure 22: Left: survival of mice with Lewis lung cancer metastases following treatment with PBS, DAC-THU, anti-PD-1, or combined DAC-THU/anti-PD-1 therapy. Right: effects of therapy on peripheral T cell subsets in mice with metastatic Lewis lung cancers.

1.2.10 Pembrolizumab

The importance of immune surveillance in controlling outgrowth of neoplastic transformation was initially postulated by Ehrlich (89) nearly a century ago, and subsequently revised by Burnet (90). Recent accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies (91). In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The programmed death (PD)-1 receptor-ligand interaction is a major pathway utilized by tumors to establish equilibrium and ultimately escape immune surveillance (92). PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 that is normally expressed on the surface of activated T-cells, and functions to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The mechanism by which PD-1 attenuates 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 is expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and natural killer 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 normally 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 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 malignancies including lung cancers express abundant levels of this T-cell inhibitor; PD-L1 expression is also elevated in tumor associated macrophages and fibroblasts. As a result, the PD-1/PD-L1 pathway has emerged as an attractive target for therapeutic intervention in a wide variety of human malignancies (19, 84).

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype which directly inhibits interaction of PD-1 with its ligands, PD-L1 and PD-L2 (20). In 2015, pembrolizumab was approved in the US for second-line therapy of NSCLC (93), and in 2016, this immune checkpoint inhibitor was approved as first-line therapy for NSCLC with high level (≥ 50%) PD-L1 expression. In 2017, the FDA approved pembrolizumab for recurrent locally advanced, or metastatic gastroesophageal cancer with PD-L1 expression (CPS) greater than or equal to 1. In a Phase I trial (Keynote 001), 495 patients with advanced NSCLC were assigned to either training or validation groups, and received pembrolizumab at 2 mg/kg or 10 mg/kg every three weeks, or 10 mg/kg every two weeks (94). Fatigue, decreased appetite and pruritus were the most common side effects (single digit numbers); severe pneumonitis was observed in 1.8% of patients, including one death. The ORR, median PFS and median OS for treatment naïve patients were 24.8%, 6.0 months and 16.2 months, respectively, vs 18%, 3.0 months, and 9.3 months, respectively, for previously treated patients. Subsequent analysis demonstrated ORR and median PFS of 43.9% and 6.1 months vs 50.0% and 12.5 months, respectively, for treatment naïve and previously treated patients whose tumors had a proportion score (PS) of at least 50% cancer cells showing positive membrane staining for PD-L1 (94).

In a Phase III trial (Keynote 024), Reck et al (95) randomly assigned 305 patients with untreated, advanced NSCLC with no sensitizing EGFR or ALK mutations and PD-L1 expression on 50% or more tumors cells to receive either pembrolizumab (200 mg Q3W) or platinum-based chemotherapy. Cross-over from the chemotherapy to pembrolizumab was allowed. The objective response rate was 44.8% in the pembrolizumab arm compared to 27.8% in the chemotherapy arm. Median progression free survival was 10.3 months (95% CI: 6.7 months to not reached) compared to 6.0 months (95% CI: 4.2 to 6.2 months) in the chemotherapy arm. Median duration of response was longer (not reached; range 1.9 to 14.5 months) in the pembrolizumab arm compared to 6.3 months (range: 2.1 to 12.6 months). Grade 3, 4, or 5 treatment related adverse events were significantly lower in the pembrolizumab arm compared to the chemotherapy arm (26.6% vs 53.3%).

Table 4: Systemic Toxicities of Pembrolizumab vs Chemotherapy in Treatment Naïve NSCLC Patients.

Adverse Event	Pembrolizumab Group (N=154)		Chemotherapy Group (N=150)	
	Any Grade	Grade 3, 4, or 5	Any Grade	Grade 3, 4, or 5
	number of patients (percent)			
Treatment-related†				
Any	113 (73.4)	41 (26.6)	135 (90.0)	80 (53.3)
Serious	33 (21.4)	29 (18.8)	31 (20.7)	29 (19.3)
Led to discontinuation	11 (7.1)	8 (5.2)	16 (10.7)	9 (6.0)
Led to death	1 (0.6)	1 (0.6)	3 (2.0)	3 (2.0)
Occurred in ≥10% of patients in either group‡				
Nausea	15 (9.7)	0	65 (43.3)	3 (2.0)
Anemia	8 (5.2)	3 (1.9)	66 (44.0)	29 (19.3)
Fatigue	16 (10.4)	2 (1.3)	43 (28.7)	5 (3.3)
Decreased appetite	14 (9.1)	0	39 (26.0)	4 (2.7)
Diarrhea	22 (14.3)	6 (3.9)	20 (13.3)	2 (1.3)
Neutropenia	1 (0.6)	0	34 (22.7)	20 (13.3)
Vomiting	4 (2.6)	1 (0.6)	30 (20.0)	1 (0.7)
Pyrexia	16 (10.4)	0	8 (5.3)	0
Constipation	6 (3.9)	0	17 (11.3)	0
Stomatitis	4 (2.6)	0	18 (12.0)	2 (1.3)
Decreased neutrophil count	0	0	20 (13.3)	6 (4.0)
Increased blood creatinine level	3 (1.9)	0	15 (10.0)	1 (0.7)
Decreased platelet count	0	0	18 (12.0)	9 (6.0)
Thrombocytopenia	0	0	17 (11.3)	8 (5.3)
Decreased white-cell count	1 (0.6)	0	16 (10.7)	3 (2.0)
Dysgeusia	1 (0.6)	0	15 (10.0)	0
Immune-mediated§				
Any	45 (29.2)	15 (9.7)	7 (4.7)	1 (0.7)
Hypothyroidism	14 (9.1)	0	2 (1.3)	0
Hyperthyroidism	12 (7.8)	0	2 (1.3)	0
Pneumonitis	9 (5.8)	4 (2.6)	1 (0.7)	1 (0.7)
Infusion reaction	7 (4.5)	0	2 (1.3)	0
Severe skin reaction	6 (3.9)	6 (3.9)	0	0
Thyroiditis	4 (2.6)	0	0	0
Colitis	3 (1.9)	2 (1.3)	0	0
Myositis	3 (1.9)	0	0	0
Hypophysitis	1 (0.6)	1 (0.6)	0	0
Nephritis	1 (0.6)	1 (0.6)	0	0
Pancreatitis	1 (0.6)	1 (0.6)	0	0
Type 1 diabetes mellitus	1 (0.6)	1 (0.6)	0	0

In a Phase 2, open-label, single-arm, multicohort study (KEYNOTE-059), 259 patients with recurrent gastric or gastroesophageal cancer received pembrolizumab, 200 mg intravenously every 3 weeks until disease progression, investigator or patient decision to withdraw, or unacceptable toxic effects (96). The primary end points were objective response rate and safety. Secondary end points included response duration. Expression of PD-L1 was assessed by immunohistochemistry. Tumors were considered positive if the combined positive score (CPS) of PD-L1 positive cells (tumor are well as lymphoid cells) per total number of cells was ≥ 1%. The objective response rate was 11.6% (95% CI, 8.0%-16.1%; 30 of 259 patients), with complete response in 2.3% (95% CI, 0.9%-5.0%; 6 of 259 patients). Median (range) response duration was 8.4 (1.6+ to 17.3+) months

(+ indicates that patients had no progressive disease at their last assessment). Objective response rate and median (range) response duration were 15.5% (95% CI, 10.1%-22.4%; 23 of 148 patients) and 16.3 (1.6+ to 17.3+) months and 6.4% (95% CI, 2.6%-12.8%; 7 of 109 patients) and 6.9 (2.4 to 7.0+) months in patients with PD-L1-positive and PD-L1-negative tumors, respectively. Forty-six patients (17.8%) experienced 1 or more Grade 3 to 5 treatment-related adverse events. Two patients (0.8%) discontinued because of treatment-related adverse events, and 2 deaths were considered related to treatment.

A subsequent analysis compared outcomes for three patient cohorts in this trial (97). Cohort 1 patients received pembrolizumab alone after ≥ 2 prior lines of therapy. Cohort 2 patients received pembrolizumab + cisplatin (80 mg/m^2 Day 1) + 5-fluorouracil (800 mg/m^2 Days 1-5 Q3W) or capecitabine (in Japan only, 1000 mg/m^2 twice daily) as first-line. Cohort 3 patients received pembrolizumab alone as first-line. Patients were enrolled in Cohorts 1 and 2 regardless of tumor PD-L1 expression; only patients with PD-L1-positive tumors (combined positive score of ≥ 1%) were enrolled in Cohort 3. In all cohorts, pembrolizumab was given at 200 mg Q3W for up to 2 years. Median (range) follow-up was 6 (1-25), 14 (2-24), and 18 (2-21) months for Cohorts 1 (259 patients), 2 (25 patients) and 3 (31 patients), respectively. Confirmed ORR (95% CI) were 16% (11-23) in PD-L1-positive, and 6% (3-13) in PD-L1-negative tumors in Cohort 1, 73% (45-92) in PD-L1-positive, and 38% (9-76) in PD-L1-negative tumors in Cohort 2, and 26% (12-45) in Cohort 3. Median PFS (95% CI) were 2 (2-2), 7 (6-11), and 3 (2-6) months in Cohorts 1, 2, and 3, respectively. Median OS (95% CI) were 6 (4-7) and 14 (9-not estimable) months for Cohorts 1 and 2 respectively, and not reached (9-21) in Cohort 3. In Cohorts 1, 2 and 3, Grade 3-5 treatment-related adverse events (TRAE) incidence occurred in 46 (18%), 19 (76%), and 7 (23%) patients in Cohorts 1, 2, and 3, respectively.

In a recent randomized Phase III trial, 395 patients with previously treated advanced gastric or gastro-esophageal junction cancers with PD-L1 CPS ≥ 1% were randomized to receive pembrolizumab 200 mg q 3 weeks for up to 2 years or standard dose paclitaxel (98). Median overall survival was 9.1 months (95% CI 6.2–10.7) with pembrolizumab and 8.3 months (7.6–9.0) with paclitaxel (hazard ratio [HR] 0.82, 95% CI 0.66–1.03; one-sided p=0.0421). Median progression-free survival was 1.5 months (95% CI 1.4–2.0) with pembrolizumab and 4.1 months (3.1–4.2) with paclitaxel (HR 1.27, 95% CI 1.03–1.57). Grade 3–5 treatment related adverse events occurred in 42 (14%) of the 294 patients treated with pembrolizumab and 96 (35%) of the 276 patients treated with paclitaxel.

Whereas pembrolizumab did not significantly improve overall survival compared with paclitaxel as second-line therapy for advanced gastric or gastro-oesophageal junction cancer with PD-L1 CPS of 1 or higher, it is noteworthy that the survival curves for the treatment cohorts crossed at 8 months in favor of the pembrolizumab arm suggesting a delayed and sustained benefit from the immunotherapy (**Figure 23**). The authors stated that this violation of the proportional hazards assumption suggests that alternative statistical methods such as a weighted log-rank test should be used to evaluate potential efficacy of immunotherapy in clinical trials. Post-hoc analysis using a weighted log-rank test that placed more weight on the events that occurred around the middle of the follow-up duration (i.e., around the time at which the overall survival curves crossed) and less weight on events that occurred early or late resulted in a one-sided p-value of 0.0009. The survival curves and results of the weighted log-rank test, as well as the relative toxicity profiles of pembrolizumab and paclitaxel clearly suggest that pembrolizumab is preferable to chemotherapy for second-line treatment of gastric or gastroesophageal junction carcinomas.

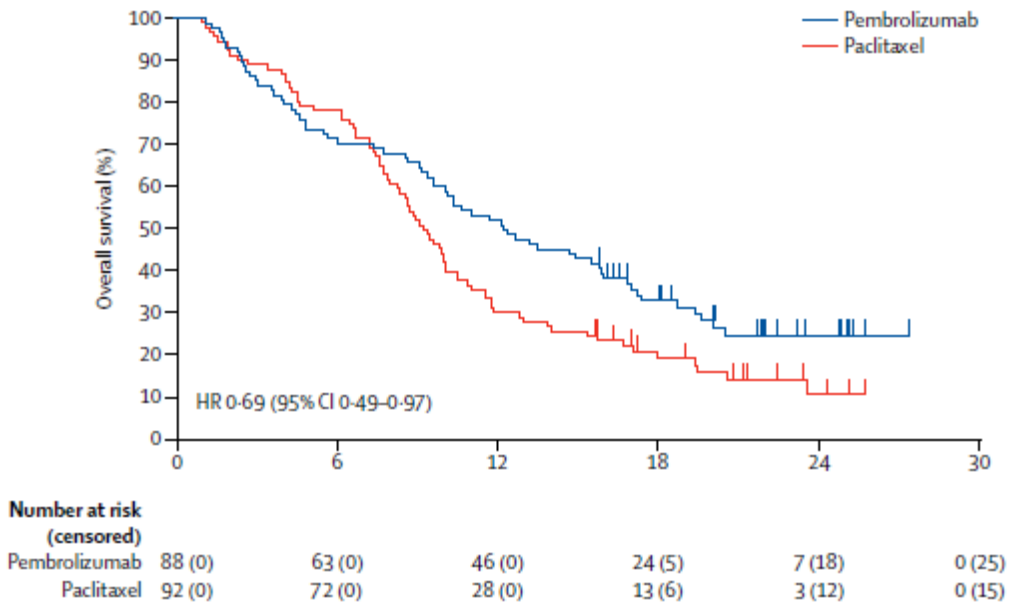


Figure 23: Overall survival of patients receiving pembrolizumab or paclitaxel in Keynote-061.

In a multi-institutional phase 1b trial, 25 previously treated MPM patients received pembrolizumab 10 mg/kg q2 weeks (99). Five (20%) patients experienced Grade 3 treatment-related adverse events. Three (12%) patients required dose interruption because of immune-related adverse events. No treatment-related deaths or discontinuations were observed. Five patients (20%) had a partial response; objective response: 20% (95% CI 6·8-40·7); 13 patients (52%) had stable disease. The responses were durable (median response duration 12·0 months [95% CI 3·7 to not reached]). These preliminary findings demonstrated safety and potential efficacy of pembrolizumab in MPM patients.

In a subsequent study, Metaxas et al (100) reported results of 93 MPM patients who received pembrolizumab off-label in Switzerland and Australia. 48 patients (52%) received pembrolizumab as second-line treatment. 68 patients (73%) had epithelioid MPM, and 67 (72%) had an Eastern Cooperative Oncology Group performance status of 0 or 1. 66 of the 93 patients (71%) had PD-L1 expression analysis: 68% were negative, 18% were intermediate, and 14% had high intra-tumoral PD-L1 expression. The overall response rate (ORR) was 18%, with a median progression-free survival (mPFS) of 3.1 months (**Figure 24**); median overall survival was 7.2 months. ORR was 37%, mPFS was 3.7 months, and the median overall survival was 10.2 months in patients with ECOG PS of 0 or 1 and only one previous systemic treatment (n = 35), Patients with nonepitheloid histological subtype showed an improved ORR (24% versus 16% [p = 0.54) and mPFS (5.6 versus 2.8 months [p = 0.02]). Compared with intermediate and negative PD-L1 expression, high PD-L1 expression was associated with an improved ORR (44% versus 42% versus 11% [p = 0.01]) and mPFS (6.2 versus 3.9 versus 2.7 months [p = 0.04]). Toxicities in MPM patients were similar to those observed in patients with other malignancies.

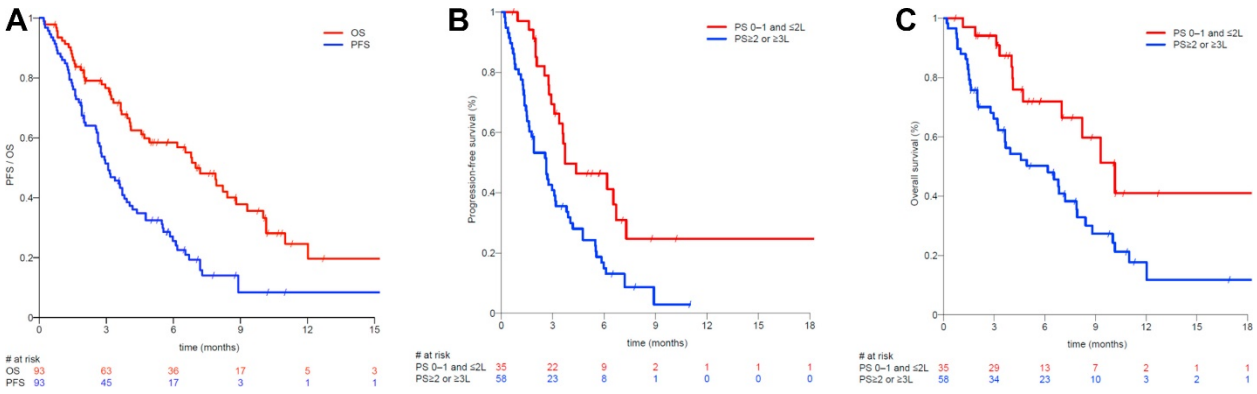


Figure 24: Progression-free survival (PFS) and overall survival (OS) in the entire cohort. Kaplan-Meier curves of survival in the entire cohort (A), PFS according to line of treatment and performance status (PS) (B), and OS according to line of treatment and PS (C) (see the text).

In an abstract presented at ESMO 2019, Popat and colleagues (101) reported preliminary results of the Phase III European Thoracic Oncology Platform (ETOP 9-15) PROMISE-meso trial. 144 patients with relapsed MPM following platinum-based chemotherapy were randomized 1:1 to receive 200 mg of pembrolizumab q 3 weeks (n = 73) or physician's choice of gemcitabine or vinorelbine at standard doses (n = 71). Treatment was administered until disease progression by RECIST1.1 criteria up to 2 years, and patients were assessed every 9 weeks for 6 months, followed by every 12 weeks thereafter. Upon disease progression, those in the chemotherapy arm were permitted to cross over to the PD-1 inhibitor arm. The primary endpoint of the trial was PFS; secondary endpoints were ORR, time to treatment failure (TTF), overall survival (OS), investigator-assessed PFS, and adverse events (AEs). Outcomes by PD-L1 status served as a correlative endpoint.

Baseline characteristics were similar between arms. The median age was 70 years, most patients had epithelioid histology (88.9%), and the majority had an ECOG performance status of 1 (75.1%). Prior therapies included carboplatin/pemetrexed, cisplatin/pemetrexed, platinum or without pemetrexed, and cisplatin/pemetrexed plus carboplatin/pemetrexed.

The ORR was 22% with pembrolizumab compared with 6% in those who received chemotherapy (*P* = .004). The median DOR was 4.6 months for pembrolizumab and 11.2 months with chemotherapy (95% CI, 2.2-10.3; 95% CI, 6.2-15.3). Median PFS was 2.5 months for pembrolizumab compared with 3.4 months for chemotherapy (HR, 1.06; 95% CI, 0.73-1.53; *P* = .76). The 6-month PFS rates were 25.0% and 27.4% with pembrolizumab and chemotherapy, respectively. At a median follow-up of 11.8 months, the median OS was 10.7 months in the pembrolizumab arm versus 11.7 months in the chemotherapy arm (HR, 1.04; 95% CI, 0.66-1.67; *P* = .85). Even when censoring for crossover, there was no OS benefit observed with pembrolizumab (HR, 1.44; 95% CI, 0.77-2.67; *P* = .25), nor for inverse probability weighting (HR, 1.07; 95% CI, 0.67-1.71; *P* = .79). PFS was similar in both PD-L1–positive and –negative subsets. Toxicities of pembrolizumab therapy were similar to those previously reported.

Collectively, the above results indicate that pembrolizumab has activity in MPM patients. Although pembrolizumab is not approved for MPM, it is listed in the current NCCN guidelines for palliative therapy of this disease. In light of the profound immunosuppressive

microenvironment associated with MPM (102), there is clear rationale to utilize epigenetic priming regimens as a strategy to enhance the efficacy of immune checkpoint inhibitors for MPM therapy.

1.2.11 Preliminary Experience with Oral DAC-THU and Immune Checkpoint Inhibitors in NSCLC

1.2.11.1 DAC-THU and Nivolumab

A Phase II trial of oral DAC-THU and Nivolumab as second line therapy for NSCLC (NCT02664181) was recently conducted at the Cleveland Clinic and the NCI (D. Schrump, PI). Patients were randomized 2:1 to receive oral DAC/THU on two consecutive days each week with Nivolumab every two weeks, or Nivolumab alone. Two of five (40%) evaluable patients on the experimental arm had objective responses, one of which was a CR (**Figure 25**). Interestingly, both of these patients were never smokers (1 ALK positive (PDL1 unknown) patient and 1 HER2 insertion site mutation (PDL1- 1-49%). 1 other patient who had PD-L1 0% had SD after 2 cycles. Typically, patients who are never smokers exhibit low mutational burden, and low or no detectable PD-L1 expression; response rates following immune checkpoint inhibitor therapy in patients with these lung cancers range from 0-5% (103, 104). These preliminary findings suggest that oral DAC-THU modulates the tumor and/or microenvironment to enhance efficacy of immune checkpoint blockade in NSCLC patients. In light of current controversies regarding the accuracy of determining PD-L1 expression in NSCLC based on single biopsies (105), and inconclusive data pertaining to the validity of PD-L1 expression as a biomarker of response to immune checkpoint inhibitors (106, 107), these findings support the use of this oral immune-modulatory regimen in conjunction with immune checkpoint inhibitors for the treatment of thoracic malignancies.

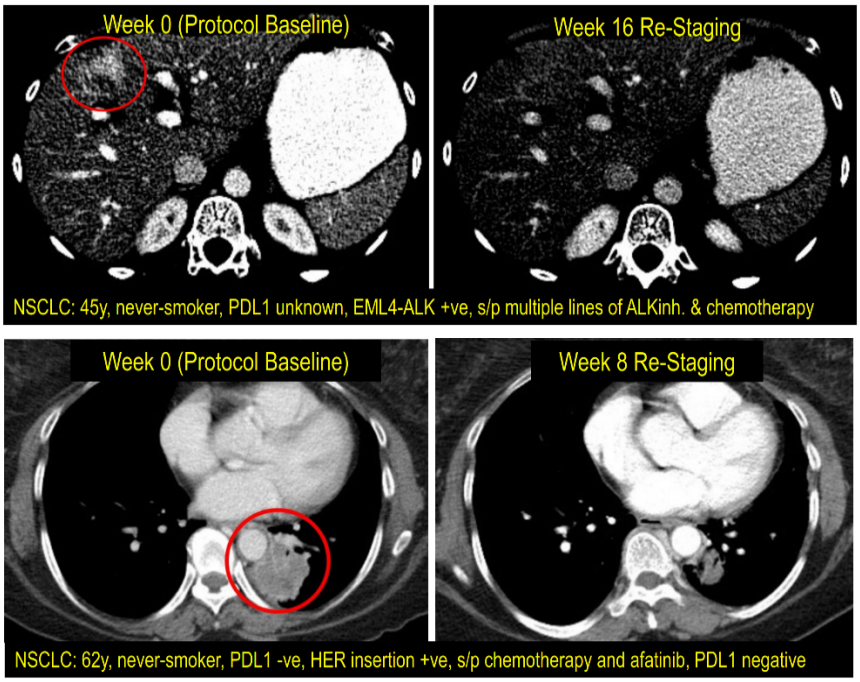


Figure 25: CT Images of Lung Cancer Patients Before and After DAC-THU/Nivolumab Therapy.

1.2.11.2 DAC-THU and Pembrolizumab

Four patients were treated on this protocol before enrollment was voluntarily halted (without formal suspension) to allow for revision of the dosing regimen. Briefly, patient #1 with medically inoperable, treatment naïve, synchronous primary NSCLC commenced oral DAC-THU (0.22 mg/kg) 3 days per week on 4/16/2018. During Week 4 she developed precipitous Grade 4 neutropenia after receiving a total DAC dose of 300 mg (2.7 mg/kg). She progressed to complete agranulocytosis several days later and was transferred to the intensive care unit for neutropenic fever and commenced broad-spectrum intravenous antibiotics. Due to ongoing agranulocytosis as well as mild anemia and thrombocytopenia, a bone marrow aspirate was performed which demonstrated hematopoietic stem cells with erythrocyte and platelet differentiation but no myeloid precursors. Although these findings were suggestive of anticipated reprogramming of hematopoietic stem cell differentiation (Section **1.2.9**), the magnitude of the granulocytopenia in the context of the investigational treatment regimen raised the possibility of autoimmune agranulocytosis. As such, the patient received IVIG, high-dose steroids, cyclosporine, and granulocyte infusions, ultimately recovering her blood counts approximately three weeks later. She commenced a gradual taper of her prednisone and cyclosporine, and was removed from treatment on 5/31/2018, and removed from study on 6/8/2018. Subsequently, she received stereotactic XRT and cryoablation for local control of her cancers. She remains alive with no clinical evidence of active disease as of June 2019.

Patient #2 with inoperable, treatment naïve NSCLC also received DAC-THU (0.22 mg/kg) 3 days per week, commencing on 5/7/2018. When patient #1 developed severe neutropenia, DAC-THU was held for 1 week (Week 3; Cycle 1) for patient #2 despite normal blood counts. The following week, patient #2 received DAC-THU for 2 days instead of three without hemato-toxicity. She developed Grade 4 ANC during Week 5 after receiving a total DAC dose of 160 mg (1.80 mg/kg). Because her clinical picture was similar to that of patient #1, she was hospitalized for observation; bone marrow aspirate revealed hematopoietic stem cells with very few myeloid precursors but preservation of erythrocyte and platelet lineages. Once again, given concerns regarding possible autoimmune agranulocytosis and ongoing neutropenia in patient #1, patient #2 received IVIG, high-dose steroids, and cyclosporine, without granulocyte infusions with resolution of her Grade 4 neutropenia within 1 week. She continued her steroid taper for approximately six weeks until she was removed from treatment and taken off protocol on 6/21/2018. Thereafter, she received XRT for new brain metastases and initiated standard of care palliative chemotherapy at an outside institution, ultimately succumbing to her disease on 2/9/2019.

As a result of the aforementioned hematologic toxicities, no patients were accrued to the trial until we had a better understanding of their etiology, and subsequent patients received DAC-THU at dose level -1 per protocol. On 1/7/2019, patient #3 with treatment naïve stage IV NSCLC commenced DAC-THU (0.2 mg/kg) twice-weekly. During Week 6 after receiving a total DAC dose of 150 mg (2.01 mg/kg), the patient developed Grade 4 neutropenia. Bone marrow aspirate demonstrated significantly decreased myeloid precursors with preservation of hematopoietic stem cells and erythroid and platelet lineages. IVIG, steroids, and cyclosporine were initiated. Four days later, the patient developed febrile neutropenia with pseudomonal bacteremia. He commenced a prolonged course of IV antibiotics due to the bacteremia and associated ecthyma gangrenosum involving the dorsum of the right hand. Within 8 days of initiating immunosuppression, the Grade 4 neutropenia had resolved to Grade 2. Steroids and cyclosporine were tapered over the course of two months. CT scans obtained as part of the evaluation for sepsis demonstrated a major response to therapy (**Figure 26**). Following consultation with the CCR-SD and immunotherapy experts at the NCI, Cleveland Clinic, and NYU Medical Center, the patient

resumed pembrolizumab without DAC-THU. He now continues pembrolizumab q3 weeks per protocol with ongoing major response as of May 2019.

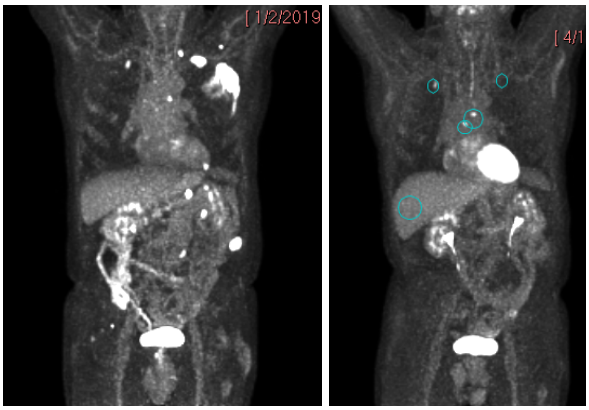


Figure 26: Baseline PET scan (left) and PET scan following two cycles of DAC-THU/ Pembrolizumab therapy (right) in a patient with NSCLC.

Patient #4 who had received chemotherapeutic pleurodesis but no systemic therapy for stage IV NSCLC commenced twice weekly DAC/THU on 1/28/2019. Because of her small size (59.7 kg) and lymphopenia requiring intermittent interruptions in drug administration, the patient's DAC dose (0.17 mg/kg) was less than the other patients, and she only received a total dose of 70 mg (1.17 mg/kg) during the 9-week course of therapy. She experienced only one Grade 3 ANC, and developed thrombocytosis requiring ASA prophylaxis suggestive of reprogramming of hematopoietic stem cells due to DNMT1 depletion. At treatment evaluation, staging studies demonstrated modest disease progression. Molecular profiling of tissue obtained from a post-treatment excisional biopsy suggested the presence of an atypical EGFR mutation not detected by NGS of her pretreatment biopsy tissue; Sanger sequencing confirmed the mutation. The patient was removed from treatment on 4/3/2019 and is now experiencing a major response to osimertinib evidenced by resolution of palpable chest wall masses and complete cessation of narcotics. Staging studies have not as yet been performed. These findings are provocative given observations that a patient with EGFR mutant NSCLC refractory to EGFR TKI had a near CR after resuming TKI therapy following treatment with DAC-THU and nivolumab on protocol NCT02664181.

In light of the severe neutropenias observed in the first three patients, and following notification of the CCR Director of Clinical Sciences, accrual to this trial was voluntarily (but not formally) suspended in order to revise the DAC-THU dosing as well as the window for assessing DLT. Data pertaining to the aforementioned patients are summarized in **Table 5**. *The severe granulocytopenias observed in these patients have now been attributed to reprogramming of hematopoietic stem cells due to DNMT1 depletion rather than autoimmunity, suggesting that the PD effect has been occurring albeit too dramatically following DAC-THU therapy. Consistent with this experience all eight patients treated with DAC-THU and Nivolumab on NCT NCT02664181 experienced Grade 3 or greater neutropenia; 5 patients experienced Grade 4 neutropenia, including one patient who was hospitalized for neutropenic fever and Grade 4 pneumonia/pneumonitis.* Consequently, the dose regimen is now modified such that drug will be administered on two consecutive days (preferably Tuesday and Wednesday to facilitate bloodwork on Mondays and Thursdays) two out of every three weeks (instead of every week) for 9 weeks. Extrapolation of data from **Table 5**, demonstrates that the same doses of DAC (and THU) over 9

vs 4-6 weeks would result in > 30% decreases in intensity of DAC-THU dosing. Additionally, the revised DAC dosing regimen will be based on the total amount of DAC (mg/kg) administered per course. As such, the revised target of 2.5 mg/kg DAC over a 9-week course will be the new DL1, which corresponds to a 54% reduction from the dose initially attempted in this protocol and a 15% reduction from the exposures in SCD patients (108). The dosing per weight has been tightened (i.e.; q 10 kg rather than q 20 kg) to avoid relative under- or overdosing of patients at the extremes of each weight range. Lastly, the window for assessment of DLT is now revised to include Cycles 1 and 2, rather than only Cycle 1 of Course 1 to better monitor toxicities, and modify drug dosing, accordingly, while optimizing epigenetic priming for immune checkpoint blockade.

Patient	Weight (kg)	BSA (M2)	Number of Caps (5 mg each pill)	DAC per Day (mg)	Days per Week	Total Number of DAC Days	Total DAC Dose (mg)	Total DAC (mg/kg)	DAC per BSA (mg/M2)
1	112.8	2.19	5	25	3	12	300	2.66	15.2
2	88.8	1.99	4	20	3	8	160	1.8	17.3
3	74.3	1.93	3	15	2	10	150	2.01	11.5
4	59.7	1.59	2	10	2	7	70	1.17	20.9

Table 5: Summary of DAC Administered with THU and Pembrolizumab.

Cumulative DAC Exposures over Nine-Week Treatment				
	Dose/day (mg/kg)	# Days	Total Dose (mg/kg)	% Change
DAC-THU/Pembro	0.2	27	5.4	
DAC-THU/Nivo	0.2	18	3.6	-33
DAC-THU (SCD)	0.16	18	2.9	-47
DAC/THU/Pembro (rev)	0.21	12	2.5	-54

Table 6: Summary of DAC Exposures in Various Protocols.

Two additional patients have been treated on the protocol since the dosing was amended. The first patient with extensive tobacco and ethanol exposure and underlying CAD presented initially with potentially resectable stage III distal esophageal adenocarcinoma. Restaging after induction chemo-XRT revealed new cervical lymph node disease that was biopsy proven to be EsC. He commenced DAC-THU and Pembro at the modified dose and exhibited no treatment related toxicities. Restaging after the first course of treatment revealed regression of disease not reaching PR. He commenced his second course of therapy and prior to his first DAC infusion he experienced a non-STEMI, which was deemed to be unrelated to DAC-THU or pembrolizumab. He underwent uneventful multivessel stenting. Because of the MI, he was no longer eligible to continue therapy.

The second patient was a 35 year old male with stage IV esophageal cancer who was referred for study after progressing on standard chemotherapy and Herceptin. He had disease within the esophagus extending from the thoracic inlet to the cardia with bulky nodal disease in the neck and mediastinum. Due to bulk of disease and stricture, a pediatric endoscope could barely pass through

the esophagus. He also tolerated the first course of therapy well with no significant treatment related toxicities. Staging studies following Course 1 revealed stable disease. Endoscopy revealed a more pliable and distensible esophagus allowing easy passage of an adult scope. The patient was now able to eat solid foods. Because of incapaciting pain, he received a palliative course of XRT to the cervical nodes. Repeat imaging demonstrated complete resolution of the left cervical lymphadenopathy (**Figure 27**). Although the regression of the lymphadenopathy could have been attributable to priming of the microenvironment by the palliative radiation that enhanced the efficacy of the immune checkpoint blockade, the magnitude of the response (CR in the neck) exceeded that which one would have anticipated from sublethal XRT and pembrolizumab alone. Furthermore, consistent with the endoscopic findings, there was less FDG uptake in the esophagus. Additionally, there was metabolic response in the retroperitoneal lymph nodes.

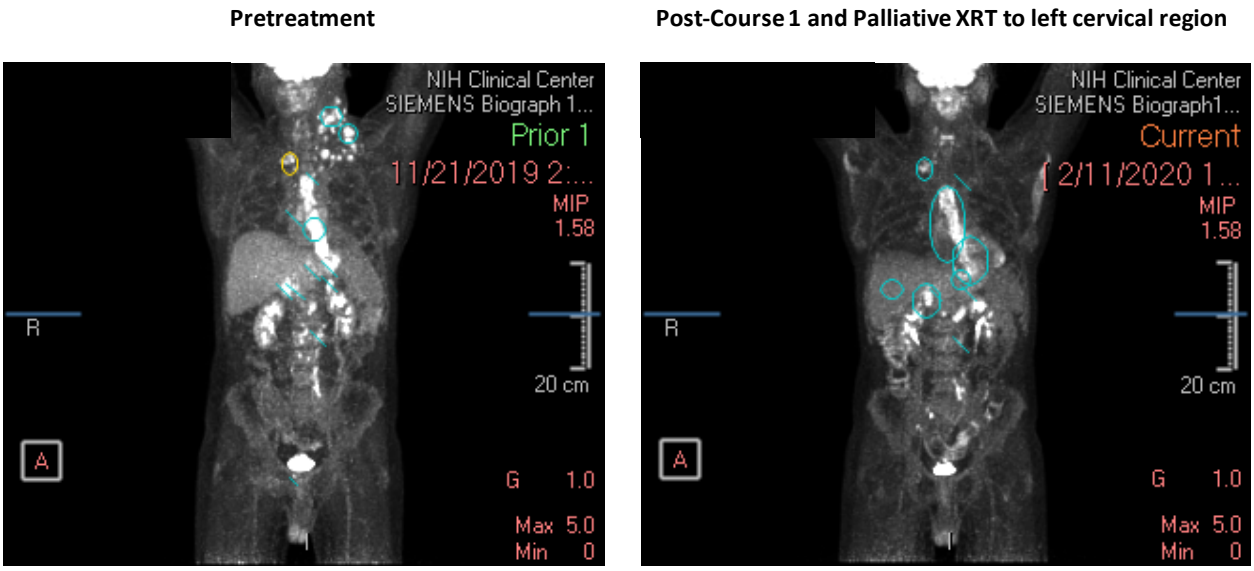


Figure 27: PET/CT scan images at Baseline and Post Course 1 of DAC-THU/Pembro and palliative XRT to left cervical region.

While at home in West Virginia as the COVID-19 pandemic started to hit the US, he developed line sepsis. His port had been used for all blood draws at home as well as his pembro infusions. The port was removed, and he commenced IV antibiotics. Since he could not undergo TEE to rule out endocarditis, Infectious Disease consultants mandated six weeks of IV antibiotics. Because this would have significantly impacted his ability to receive investigational therapy and in light of the evolving restrictions regarding patient care at the NIH Clinical Center, he was removed from study so that he could commence cytotoxic chemotherapy while completing his antibiotic regimen.

2 ELIGIBILITY ASSESSMENTAND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Histologically or cytologically confirmed, inoperable or unresectable locally advanced, or metastatic NSCLC, esophageal cancers including Seiwert-Stein Type I and Type II gastro-esophageal junction (GEJ) carcinomas, or MPM.

2.1.1.2 NSCLC patients with no prior systemic treatment, or those with prior first line treatment including an immune checkpoint inhibitor, are eligible for study.

2.1.1.3 Patients with esophageal and gastro-esophageal junction (GEJ) cancers are potentially eligible for study if they have received or refused first line standard of care cytotoxic therapy, and subsequent targeted therapy if appropriate.

2.1.1.4 Patients with MPM are eligible for study if they have received, refused or are ineligible for first line chemotherapy.

2.1.1.5 Patients who received DNA demethylating agents or PD-1/PD-L1 inhibitors for another malignancy may be eligible for study if there were no dose-limiting immune related events, and there has been either no clinical evidence of disease or minimal residual disease that has been stable for at least three years.

2.1.1.6 Patients must have analysis of PD-L1 expression in cancer cells quantitated by immunohistochemistry analysis.

2.1.1.6.1 Patients in Cohort 1 (Dose Escalation) may have any level of expression.

2.1.1.6.2 Patients in Cohort 2 (Dose Expansion: NSCLC with high PD-L1) must have ≥ 50% expression in cancer cells.

2.1.1.6.3 Patients in Cohort 3 (Dose Expansion: NSCLC with low PD-L1) must have 0-49% expression. Note: Patients in this cohort must have been offered and refused standard of care platinum-based chemotherapy.

2.1.1.6.4 Patients in Cohort 4 (Dose Expansion: EsC) may have any level of expression.

2.1.1.6.5 Patients in Cohort 5 (Dose Expansion: MPM) may have any level of expression.

2.1.1.7 Measurable disease, per RECIST 1.1. (see Section **6.3.3** for the evaluation of measurable disease).

2.1.1.8 Willingness to undergo tumor biopsies if safely accessible per PI discretion before and after treatment.

2.1.1.9 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of Decitabine (DAC) and Tetrahydrouridine (THU) in combination with Pembrolizumab in patients < 18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.1.10 ECOG performance status of ≤ 2 (see **Appendix 1**).

2.1.1.11 Patients must be without evidence of unstable or decompensated myocardial disease; and must have adequate pulmonary reserve evidenced by FEV1 and DLCO ≥ 35% predicted; oxygen saturation equal to or greater than 90% on room air by pulse oximetry or ABG (to be drawn if pulse oximetry < 90% on room air).

2.1.1.12 No immunosuppressive medications except non-systemic corticosteroids.

2.1.1.13 Patients must have normal organ and marrow function as defined below:

– leukocytes	≥ 3,000/mcL
– absolute neutrophil count	≥ 1,500/mcL (without transfusion or cytokine support)
– absolute lymphocyte count	> 800/mcL
– platelets	≥ 100,000/mcL
– PT	no more than 2 seconds above the ULN
– total bilirubin	< 1.5 X institutional upper limit of normal
	OR
– direct bilirubin	≤ ULN for patients with total bilirubin > 1.5 ULN
– serum albumin	≥ 2.0 mg/dL
– AST(SGOT)/ALT(SGPT)	≤ 2.5 X institutional ULN
– creatinine	≤ 1.6 mg/ml
	OR
– creatinine clearance (eGFR)	≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal at the time DAC-THU and pembrolizumab treatment commences.

2.1.1.14 Patients with history of brain metastases except those with meningeal carcinomatosis or leptomeningeal disease may be eligible for treatment a minimum of 1 week following completion of gamma knife or whole brain radiotherapy, or 4 weeks following surgical resection of brain metastasis provided post-treatment MR scan reveals no evidence of active disease, and no ongoing need for systemic steroids.

2.1.1.15 Patients with laboratory evidence of autoimmune disease (e.g., positive ANA or lupus anticoagulant) without associated symptoms; vitiligo, or mild autoimmunity not impacting the function of organs, such as Hashimoto or psoriasis may be eligible for study.

2.1.1.16 The effects of DAC-THU and pembrolizumab on the developing human fetus are unknown. For this reason and because antineoplastic agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men who engage in sexual activity must agree to use 2 forms of contraception at least 1 of which must be highly effective (intrauterine device [IUD], hormonal, tubal ligation; not highly effective includes barrier method) prior to study entry, for the duration of study participation and for 60 days after completion of the study treatment. Should a woman become pregnant, or suspect she is pregnant, while she or her partner is participating in this study the study participant should inform the study physician immediately.

2.1.1.17 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients with cancers harboring targetable mutations for which there is approved first or second line therapy, unless standard of care therapy refused.

2.1.2.2 Clinically significant cardiovascular / cerebrovascular disease as follows: cerebral vascular accident / stroke (< 6 months prior to enrollment), myocardial infarction (< 6

months prior to enrollment), unstable angina, congestive heart failure (New York Heart Association Classification Class ≥ II), serious cardiac arrhythmia, clinically significant bleeding or clinically significant pulmonary embolism.

2.1.2.3 Active Hepatitis A, Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

2.1.2.4 Human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness due to unknown effects of DAC-THU on systemic immunity.

2.1.2.5 Other active infection requiring systemic therapy.

2.1.2.6 Pregnant women are excluded from this study because DAC-THU may have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with DAC-THU, breastfeeding should be discontinued if the mother is treated with DAC-THU. These potential risks may also apply to other agents used in this study.

2.1.2.7 Other severe acute or chronic medical or psychiatric conditions or laboratory abnormality that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, and in the judgment of the investigator would make the patient inappropriate for entry into this study.

2.1.2.8 Patients who are receiving systemic corticosteroids.

2.1.2.9 Patients with history of or active autoimmune disease including thyroiditis, colitis, nephritis, neuropathy or pneumonitis.

2.1.2.10 Patients receiving another investigational agent.

2.1.2.11 An 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 or anal cancer, or ductal carcinoma in-situ.

2.1.2.12 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.

2.1.2.13 Psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

2.1.2.14 Thrombocytosis defined as platelet count > 1,200,000/mcL.

2.1.3 Recruitment Strategies

This study will be posted on NIH websites and on NIH social media forums. Participants may also be recruited through self-referrals, physician referrals, and referrals from the NIH Clinical Center (CC) Office of Patient Recruitment.

Patients may also be referred to this study based on confirmation of histology (done by the Laboratory of Pathology) under protocol 06C0014.

2.2 SCREENING EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol 06C0014 OR the consent for study 01C0129 (provided the procedure is permitted on that study) per PI discretion. Assessments and procedures to confirm study eligibility should be completed as follows:

Within 4 Weeks Prior to Protocol Enrollment:

a. Medical history and physical exam including vital signs and performance status.

b. Confirmation of histology (archival or fresh tissue if archival tissue is insufficient), PD-L1 expression and mutation status (EGFR and ALK) by the Laboratory of Pathology (any time prior to enrollment). This confirmation will be done under protocol 06C0014 (patients will be co-enrolled).

c. Blood tests:

   i. CBC with differential

   ii. Acute Care Panel: Sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, eGFR

   iii. Hepatic Panel: Alkaline phosphatase, ALT, AST, total and direct bilirubin

   iv. PT/PTT

d. Viral Markers (anti-HIV, anti-HCV, HBsAg, anti-HAV IgM); HCV RNA if needed.

e. 12-lead EKG. Patients with EKG evidence of cardiac ischemia (ST depression/elevation greater than or equal to 2 mm), or arrhythmia will undergo cardiology evaluation to determine eligibility.

f. Echocardiogram

g. X-rays, contrast enhanced CT scans of chest, abdomen, pelvis, as well as brain MR, and fused PET-CT scans to evaluate the status of disease (all imaging must be obtained within 4 weeks prior to starting therapy).

h. Pulmonary function tests (PFTs) with and/or without bronchodilators.

i. Arterial Blood Gas test (ABG) on room air (to be drawn if pulse oximetry < 90% on room air).

j. Lupus Anticoagulant and ANA

Within 3 Days Prior to Protocol Enrollment:

a. Women of child-bearing potential will have a urine or serum βhCG pregnancy test.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2: CCR Participant Registration & Status Updates found here.

2.3.1 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Dose-Escalation	Subjects enrolled to dose escalation cohorts
2	Dose Expansion: NSCLC with high PD-L1	NSCLC subjects with high PD-L1 tumor expression enrolled at the MTD after the MTD is established
3	Dose Expansion: NSCLC with low PD-L1	NSCLC subjects with low PD-L1 tumor expression enrolled at the MTD after MTD is established
4	Dose Expansion: EsC	EsC subjects enrolled at the MTD after MTD is established
5	Dose Expansion: MPM	MPM patients enrolled at the MTD after MTD is established

Arms

Number	Name	Description
1	Dose Escalation	DAC-THU + pembrolizumab at escalating doses
2	Dose Expansion	DAC-THU + pembrolizumab at the dose established in Arm 1

Arm Assignment

Subjects in Cohort 1 will be assigned to Arm 1.

Subjects in Cohort 2, Cohort 3, Cohort 4 and Cohort 5 will be assigned to Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This trial is unique in that it is intended to establish the rationale and conditions for the use of oral DAC-THU and pembrolizumab therapy for NSCLC, EsC, and MPM. The goal is to establish the basis for subsequent evaluation of this treatment regimen in combination with other agents targeting MDSC, or adoptive immunotherapy regimens in patients with thoracic malignancies.

Patients potentially eligible for trial will be evaluated, and undergo subsequent eligibility assessment in the Thoracic Surgery Clinic/NCI. Patients meeting eligibility criteria will undergo baseline (if fresh tumor biopsy was not performed at screening) and post treatment tumor biopsies either by percutaneous image-guided FNA techniques in Interventional Radiology (Clinical Center/NIH) or endoscopic/thoracoscopic techniques in the CC operating room by Thoracic Surgery staff using standard of care practice guidelines. Tumor biopsies may be omitted at the discretion of the PI if risks outweigh potential research benefits.

The Phase I component will be a standard 3+3 design starting with oral DAC-THU on two consecutive days (preferably Tuesday and Wednesday) for two weeks out of three weeks for 9

weeks with incremental dose escalation as shown in Section **3.1.3**. Patients with high PD-L1 tumor expression will be combined with patients with low PD-L1 tumor expression to minimize accrual numbers required for the Phase I component of the study. Pembrolizumab will be administered on Wednesday, Thursday or Friday at a fixed intravenous dose of 200 mg q 3 weeks. One cycle is three weeks; one course is 9 weeks. Treatment evaluation including repeat tumor biopsy will be performed after one course of therapy (Week 10 +/- one week). Those patients exhibiting disease progression or unacceptable toxicities will be removed from study. Patients exhibiting stable disease or disease regression will be offered an additional course of therapy followed by treatment evaluation (without mandatory biopsy). Treatment will continue in this manner until off-study criteria have been met.

Once the MTD for DAC-THU has been identified, the MTD dose level will be expanded by 4 patients to confirm safety. Then, including these 10 patients at the MTD, a total of 10 NSCLC patients with high intratumoral PD-L1 expression and 10 NSCLC patients with low intra-tumoral PD-L1 expression will be evaluated in the first stage of two separate Phase II cohorts each using a Simon optimal design (Phase II; Stage I). If 5 or more of 10 NSCLC patients with high PD-L1 expression respond to treatment, the cohort will be expanded to 23 patients (Phase II; Stage II) to determine ORR at the MTD. If 2 or more of 10 patients with low PD-L1 expression respond to treatment, the cohort will be expanded to 29 patients (Phase II; Stage II) to determine ORR at the MTD. Up to 10 EsC patients including any patients treated in the expansion cohort of the Phase I component of the trial will be accrued to a separate cohort at the MTD. If 2 or more of these 10 EsC patients respond to therapy, the protocol may be amended to enable further evaluation of the investigational treatment regimen in EsC patients. Similarly, up to 10 MPM patients including any patients treated in the expansion cohort of the Phase I component of the trial will be accrued to a separate cohort at the MTD. If 2 or more of these 10 MPM patients respond to therapy, the protocol may be amended to enable further evaluation of the investigational treatment regimen in these patients.

3.1.1 Study Schema

DAC-THU/Pembro Treatment: Course 1 (and subsequent courses if indicated)							
Week	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	Day 0	1	2	3	4	5	6
2	7	8	9	10	11	12	13
3	14	15	16	17	18	19	20
4	21	22	23	24	25	26	27
5	28	29	30	31	32	33	34
6	35	36	37	38	39	40	41
7	42	43	44	45	46	47	48
8	49	50	51	52	53	54	55
9	56	57	58	59	60	61	62
10	63	64	65	66	67	68	69
		DAC/THU					
		Pembro					
		Treatment Evaluation					

Treatment evaluation including staging studies and biopsy will be performed on Days 64 and 65 +/- 7 days. DAC-THU will be given on two consecutive days as indicated, with Tuesday/Wednesday optimal but not absolute. Pembrolizumab may be administered on Wednesday, Thursday, or Friday for administrative convenience.

3.1.2 Definition of Dose Limiting Toxicity (DLT)

DLT is defined as:

- Any Grade 3 or greater toxicity that cannot be attributed to a cause other than study treatment during the first two cycles of Course 1 of DAC-THU and/or pembrolizumab therapy such as disease progression or intercurrent illness.
- Any toxicity that cannot be attributed to a cause other than study treatment (DAC-THU and/or Pembrolizumab) that results in study treatment discontinuation.

3.1.3 Dose Escalation

The revised dosing regimen selected for this trial is based on pharmacodynamic evidence of systemic hypomethylation without significant systemic toxicities mediated by oral DAC-THU in patients with sickle cell disease in a recent Phase 1 clinical trial (108), as well as experience with DAC-THU/Pembrolizumab in NSCLC (Section **1.2.11.2**). The frequency of DAC-THU dosing will increase using a conventional 3+3 Phase I study design attempting to maximize systemic DNMT1 depletion while avoiding Grade 3 or greater systemic toxicities (primarily neutropenia/thrombocytopenia). *Due to the uncertainty regarding cumulative toxicities, no intra-patient escalation will be allowed*.

Dose escalation will proceed as indicated in **Table 7** using three patients per dose level (irrespective of Tumor PD-L1 expression) until DLT within the first 6 weeks (two cycles) of DAC-THU therapy is observed. If one of three patients at any given dose level experiences DLT, up to three additional patients will be treated at this dose level. If only one of six patients exhibit DLT, subsequent patients will be enrolled into the next higher dose level. As soon as two patients at any given dose level develop DLT, no additional patients will be entered at that level. Subsequent patients will be accrued into the preceding dose level; if DLT is observed in less than two of six patients treated at this lower level, this dose will represent maximum tolerated dose (MTD).

Table 7: DAC-THU Dosing Schema for High (50% or Greater) and Low (0-49%) PD-L1 Expression Groups

Dose Level	DAC mg/kg/course	DAC mg/kg/day*	THU mg/kg/course	THU mg/kg/day*	Frequency^ wk 1,2,4,5,7,8
-1	2.0	0.17	120	10	T;W
1	2.5	0.21	120	10	T;W
2	3.0	0.25	120	10	T;W
*on days administered					
^ T: Tuesday; W: Wednesday					

3.2 DRUG ADMINISTRATION

3.2.1 DAC-THU

***Timing between THU and decitabine**: Oral THU capsules should be ingested within two hours prior to or two hours after a meal followed 60 minutes later by oral decitabine capsules.* The capsules are to be ingested twice weekly on consecutive days as indicated in **Table 7** ('Smart Caps' that can remind patients about timing of administration should be considered for use).

3.2.1.1 THU Dosing

Weight (kg)	**THU (caps/dose)**
40-59	2
60-79	3
80-99	4
100-119	5
120-139	6

3.2.1.2 DAC Dosing

Dose Level -1: DAC 2 mg/kg/course										
					DAC Pills/week					
Mass (kg)	**Total # Pills**	**wk1 (T;W)**	**wk2 (T;W)**	**wk3(T;W)**	**wk4(T;W)**	**wk5(T;W)**	**wk6(T;W)**	**wk7(T;W)**	**wk8(T;W)**	**wk9(T;W)**
40-49	**18**	**2;1**	**2;1**	**none**	**2;1**	**2;1**	**none**	**2;1**	**2;1**	**none**
50-59	**22**	**2;2**	**2;2**	**none**	**2;2**	**2;1**	**none**	**2;2**	**2;1**	**none**
60-69	**26**	**3;2**	**2;2**	**none**	**3;2**	**2;2**	**none**	**2;2**	**2;2**	**none**
70-79	**30**	**3;2**	**3;2**	**none**	**3;2**	**3;2**	**none**	**3;2**	**3;2**	**none**
80-89	**34**	**3;3**	**3;3**	**none**	**3;3**	**3;2**	**none**	**3;3**	**3;2**	**none**
90-99	**38**	**4;3**	**3;3**	**none**	**4;3**	**3;3**	**none**	**3;3**	**3;3**	**none**
100-109	**42**	**4;3**	**4;3**	**none**	**4;3**	**4;3**	**none**	**4;3**	**4;3**	**none**
110-119	**46**	**4;4**	**4;4**	**none**	**4;4**	**4;3**	**none**	**4;4**	**4;3**	**none**
120-129	**50**	**5;4**	**4;4**	**none**	**5;4**	**4;4**	**none**	**4;4**	**4;4**	**none**
130-139	**54**	**5;5**	**5;4**	**none**	**5;4**	**5;4**	**none**	**5;4**	**4;4**	**none**
Dose Level 1: DAC 2.5 mg/kg/course										
					DAC Pills/week					
Mass (kg)	**Total # Pills**	**wk1 (T;W)**	**wk2 (T;W)**	**wk3(T;W)**	**wk4(T;W)**	**wk5(T;W)**	**wk6(T;W)**	**wk7(T;W)**	**wk8(T;W)**	**wk9(T;W)**
40-49	**22**	**2;2**	**2;2**	**none**	**2;2**	**2;1**	**none**	**2;2**	**2;1**	**none**
50-59	**27**	**3;2**	**2;2**	**none**	**3;2**	**2;2**	**none**	**3;2**	**2;2**	**none**
60-69	**32**	**3;3**	**3;3**	**none**	**3;3**	**3;3**	**none**	**3;2**	**3;2**	**none**
70-79	**37**	**4;3**	**3;3**	**none**	**3;3**	**3;3**	**none**	**3;3**	**3;3**	**none**
80-89	**42**	**4;3**	**4;3**	**none**	**4;3**	**4;3**	**none**	**4;3**	**4;3**	**none**
90-99	**47**	**4;4**	**4;4**	**none**	**4;4**	**4;4**	**none**	**4;4**	**4;3**	**none**
100-109	**52**	**5;4**	**5;4**	**none**	**5;4**	**4;4**	**none**	**5;4**	**4;4**	**none**
110-119	**57**	**5;5**	**5;4**	**none**	**5;5**	**5;4**	**none**	**5;5**	**5;4**	**none**
120-129	**62**	**6;5**	**5;5**	**none**	**6;5**	**5;5**	**none**	**5;5**	**5;5**	**none**
130-139	**67**	**6;6**	**6;5**	**none**	**6;6**	**6;5**	**none**	**6;5**	**5;5**	**none**
Dose Level 2: DAC 3.0 mg/kg/course										
					DAC Pills/week					
Mass (kg)	**Total # Pills[#]**	**wk1 (T;W)**	**wk2 (T;W)**	**wk3(T;W)**	**wk4(T;W)**	**wk5(T;W)**	**wk6(T;W)**	**wk7(T;W)**	**wk8(T;W)**	**wk9(T;W)**
40-49	**27**	**3;2**	**2;2**	**none**	**3;2**	**2;2**	**none**	**3;2**	**2;2**	**none**
50-59	**33**	**3;3**	**3;2**	**none**	**3;3**	**3;2**	**none**	**3;3**	**3;2**	**none**
60-69	**39**	**4;3**	**3;3**	**none**	**4;3**	**3;3**	**none**	**4;3**	**3;3**	**none**
70-79	**45**	**4;4**	**4;3**	**none**	**4;4**	**4;3**	**none**	**4;4**	**4;3**	**none**
80-89	**51**	**5;4**	**4;4**	**none**	**5;4**	**4;4**	**none**	**5;4**	**4;4**	**none**
90-99	**57**	**5;5**	**5;4**	**none**	**5;5**	**5;4**	**none**	**5;5**	**5;4**	**none**
100-109	**63**	**6;5**	**5;5**	**none**	**6;5**	**5;5**	**none**	**6;5**	**5;5**	**none**
110-119	**69**	**6;6**	**6;5**	**none**	**6;6**	**6;5**	**none**	**6;6**	**6;5**	**none**
120-129	**75**	**7;6**	**6;6**	**none**	**7;6**	**6;6**	**none**	**7;6**	**6;6**	**none**
130-139	**81**	**7;7**	**7;6**	**none**	**7;7**	**7;6**	**none**	**7;7**	**7;6**	**none**

Note: Dose is to be adjusted based on weight changes during the treatment only if patients fall into a different weight category bracket.

3.2.2 Pembrolizumab

Pembrolizumab will be administered as a 30 minute IV infusion every three weeks at a fixed dose of 200 mg/infusion. Subjects may be dosed no less than 12 days from the previous dose of drug. There are no pre-medications recommended for pembrolizumab on the first cycle.

Treatment of Pembrolizumab Related Infusion Reactions:

Since pembrolizumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic, and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to the study sponsor as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI CTCAE (Version 5.0) guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines, as appropriate:

Table 8: Treatment Modification for Pembrolizumab

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

NCI-CTCAE Grade	Treatment Modification for Pembrolizumab
- Once the pembrolizumab infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. - If the subject has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, the infusion should be stopped and the subject should be removed from study treatment. - If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice.	

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

3.2.3 Additional Modifications for Subjects with Grade 2 Infusion-Related Reactions

If, in the event of a Grade 2 infusion-related reaction that does not improve or worsens after implementation of the modifications indicated in **Table 8** (including reducing the infusion rate by 50%), the investigator may consider treatment with corticosteroids and the infusion should be stopped for that day. At the next cycle, the investigator may consider the addition of H2-blocker antihistamines (e.g., famotidine), in addition to the mandatory premedication, for select subjects. However, prophylactic steroids are NOT permitted.

3.2.4 Severe Hypersensitivity Reactions and Flu-like Symptoms

A. Symptoms
- Impaired airway
- Decreased oxygen saturation (< 92%)
- Confusion
- Lethargy
- Hypotension
- Pale/clammy skin
- Cyanosis

B. Management
1. Epinephrine injection and dexamethasone infusion;
2. Patient should be placed on monitor immediately;
3. Alert intensive care unit (ICU) for possible transfer if required.

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

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

For Grade 2 Symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-

inflammatory drugs, narcotics, corticosteroids, IV fluids]; prophylactic medications indicated for 24 hours): Stop the pembrolizumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms to Grade 1 or less. Resume infusion at 50% rate.

3.2.3 CCR Self –Administered Study Drugs Policy

All oral self-administered investigational agents will be properly accounted for, handled, and disposed in accordance with existing federal regulations and principles of Good Clinical Practice. All oral study drugs will be recorded in the patient diary found in **Appendix 2**. This will be used as a memory aide for subjects. The clinical research team will maintain the primary source record.

Subjects should be asked to bring the diary as well as unused study agent and empty containers with them at each study visit. If a subject goes off study while at home, the research nurse will ensure and document the return of the unused oral investigational agents from the participant.

Unused investigational study agents will be disposed and destroyed per CC pharmacy SOPs.

3.3 DOSING DELAYS AND MODIFICATIONS

This study is intended to evaluate the safety and potential efficacy of oral DAC-THU and pembrolizumab as epigenetic immunotherapy in patients with NSCLC, EsC and MPM. The proposed oral DAC dose of ~0.17 mg/kg to be combined with THU ~10 mg/kg administered 60 minutes before oral DAC, is based on non-human primate studies and the Phase 1 clinical trial in SCD which had an identical pharmacologic objective of DAC distribution through tissues with high CDA expression (intestines, liver etc.), and the wide concentration-time profile desired for non-cytotoxic DNMT1-depletion. This dose of DAC administered orally is 10-20% of the FDA-approved dosages administered intravenously and is < 50% of the dose of oral decitabine administered together with another CDA-inhibitor (a THU-analogue) in clinical trials by Astex/Otsuka.

Although it is anticipated that this therapy will be well tolerated at the revised doses, myelosuppression resulting from reprogramming of hematopoietic stem cell differentiation may become dose limiting (50). If dose limiting myelosuppression is observed during the first 2 cycles of Course 1, DAC-THU will be held until toxicities have resolved to Grade 2 or less, and DAC-THU therapy will recommence at the next lower dose, or at the SAME dose if deemed appropriate by the PI, as indicated in the dose escalation schema (**Table 7**). Only one dose reduction will be allowed.

3.3.1 General Guidance for Dose Modifications

3.3.1.1 Patients may continue to receive therapy provided the following criteria are met on Day 1 of each cycle:

- Non-hematologic toxicity recovered to < Grade 2 (or tolerable Grade 2 or baseline).
- No evidence of progressive disease.

3.3.1.2 In the event of an adverse event at least possibly related to the investigational agent, the dose of the investigational agent should be adjusted according to the parameters articulated above and guidelines listed in the Dose Delays / Dose Modifications tables shown below (**Table 9** and **Table 10**). If an adverse event is not covered in such tables, doses may be held at the discretion of the investigator for the subject's safety.

3.3.1.3 If patients fail to recover to CTCAE Grade 0-1, tolerable Grade 2 (or within 1 grade of starting values for pre-existing laboratory abnormalities), or baseline from a treatment-related adverse event attributable to DAC-THU within 42 days (2 cycles), they cannot receive additional DAC-THU. Patients may continue pembrolizumab monotherapy at the investigator discretion provided that discontinuation criteria for pembrolizumab toxicity have not been met.

3.3.2 Treatment Delays and Modifications for Medical Needs

Brief interruptions and delays up to 3 weeks may be required due to patients experiencing complications of their disease or other medical illness not attributable to disease progression, or protocol therapy. A patient that interrupts therapy for more than 3 weeks will be taken off treatment.

3.3.3 Dose Reductions

No dose reductions of Pembrolizumab are permitted on this study.

3.3.4 Dosage Modification and Management of Immune-Related Adverse Events

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

- Grade 1 to 2: treat symptomatically or with moderate dose steroids, more frequent monitoring.
- Grade 1 to 2 (persistent): manage similar to high grade AE (Grade 3 to 4).
- Grade 3 to 4: treat with high dose corticosteroids.

Treatment of irAEs should follow accepted guidelines set forth in **Table 10**.

3.3.5 Dose Modification of DAC-THU

Table 9 lists standard laboratory values that should trigger dose modifications of the investigational drug.

Table 9: Trigger Values that Result in Dose Modification

General Adverse Events	Action
Non-hematological, Grade 1 or 2 (excluding hemorrhage)	• Continue DAC-THU therapy at full dose prescribed. • Apply maximum supportive care recommendations. • If prolonged duration of Grade 2 adverse event (≥ 7 days) is affecting quality of life, start the next treatment cycle at DL-1. • If event persists and continues to affect quality of life for ≥ 7 more days following initial dose reduction, discontinue DAC-THU.
Grade 3 ALT and AST	• Hold DAC-THU therapy until recovery to Grade ≤ 1. • If ALT and AST resolve to Grade ≤ 1 within 10 days, maintain current dose level. • If ALT and AST do NOT resolve to Grade ≤ 1 within 10 days, dose reduce 1 level. • If Grade 3 ALT or AST occurs after dose reduction, discontinue DAC-THU.

General Adverse Events	Action
Grade 4 ALT and AST	• Hold DAC-THU therapy until recovery to Grade ≤ 1. • Resume DAC-THU at DL-1. • If Grade 4 toxicities occur after dose reduction, discontinue DAC-THU.
Non-hematological, Grade 3 or 4 (excluding ALT, AST, hemorrhage and QT prolongation)	• Apply maximum supportive care recommendations. Hold DAC-THU until recovery to Grade ≤ 1 and resume DAC-THU at the next lower dose level. • If symptoms continue to persist at Grade 3 or 4, following dose reduction, discontinue drug.
QT prolongation, Grade 3 or 4	• Cardiology consultation will be obtained to determine whether any cardiac functional assessment is warranted or if therapy should be delayed or discontinued. When warranted, ECGs will subsequently be reviewed by the NCI consulting cardiologist. • If therapy is continued, dose reduce 1 level for Grade 4 events. • If Grade 3 or 4 toxicities occur after dose reduction, discontinue DAC-THU.
Thrombocytosis	• If platelet count > 500/ mcL but < 1.2M/mcL, continue DAC-THU at current dose. Begin ASA 325mg PO daily. • If platelet count 1.2M /mcL or more, hold DAC-THU until platelet count < 1.2 M/mcL, begin ASA 325 mg PO daily and resume DAC-THU at DL-1 if within the first two Cycles of Course 1; **or** at SAME dose or DL-1 per PI discretion in subsequent cycles.
Hemoglobin	• Hgb > 18 g/dL: hold DAC-THU until Hgb WNL. Resume DAC-THU at DL-1.
Hematological Adverse Events	
Grades 1 and 2 events	• Continue DAC-THU therapy at full dose prescribed. Apply maximum supportive care recommendations.
Grade 3 events	• Apply supportive care. • If toxicities occur in the first two cycles of therapy and resolve to Grade 1 or less, in 10 days, resume DAC-THU at current dose. • If toxicities occur in the first two cycles of therapy and resolve to Grade 1 or less > 10 days following drug hold, resume DAC-THU at next lower dose. • If toxicities occur in subsequent cycles and resolve to Grade 1 or less in 21 days following drug hold, resume DAC-THU at SAME dose. If toxicities recur within two cycles of resuming DAC-THU, hold until toxicities resolve to Grade 1 or less, and resume DAC-THU at next lower dose. • If toxicities occur in subsequent cycles and resolve to

General Adverse Events	Action
	Grade 1 or less > 21 days following drug hold, resume DAC-THU at next lower dose. • If toxicities recur within two cycles after maximal dose reduction, discontinue DAC-THU.
Grade 4 events	• Apply maximum supportive care recommendations. • Hold DAC-THU therapy until patient meets hematologic criteria for retreatment and resume DAC-THU at next lower dose. • If recurrence of hematological adverse event after drug hold/ interruptions is observed with treatment at DL-1, in the setting of maximum supportive care measures applied, hold drug once again until patient meets hematologic criteria for retreatment, further dose reduce DAC-THU if possible or discontinue DAC-THU per PI discretion.

3.3.6 Dose Delay of Pembrolizumab

No dose modification for pembrolizumab is allowed. Because of the potential for clinically meaningful pembrolizumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories. Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to pembrolizumab). If AE is felt to be immune related and attributable to pembrolizumab, the patient can continue DAC-THU while pembrolizumab is held. Dose delay criteria apply for all drug-related AEs. Pembrolizumab must be delayed until treatment can resume.

3.3.6.1 Pembrolizumab Administration Should be Delayed for the Following:

Table 10: Management of Immune-Related Adverse Events

Gastrointestinal irAEs		
Severity of Diarrhea/Colitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Diarrhea: < 4 stools/day over Baseline Colitis: asymptomatic	Continue Pembrolizumab Symptomatic treatment (e.g. loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2, 3 or 4.

Gastrointestinal irAEs		
Severity of Diarrhea/Colitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 2 Diarrhea: 4 to 6 stools per day over Baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Withhold Pembrolizumab Symptomatic treatment	If improves to Grade ≤ 1: Resume Pembrolizumab therapy If persists > 5-7 days or recurs: Treat as Grade 3 or 4.
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 h; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Withhold Pembrolizumab for Grade 3. Permanently discontinue Pembrolizumab for Grade 4 or recurrent Grade 3. 1.0 to 2.0 mg/kg/day prednisone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves: Continue steroids until Grade ≤ 1, then taper over at least 1 month; resume Pembrolizumab therapy following steroids taper (for initial Grade 3). If worsens, persists > 3 to 5 days, or recurs after improvement: Add infliximab 5mg/kg (if no contraindication). Note: infliximab should not be used in cases of perforation or sepsis.

Dermatological irAEs		
Grade of Rash (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 to 2 Covering ≤ 30% body surface area	Continue Pembrolizumab Symptomatic therapy (for example, antihistamines, topical steroids)	If persists > 1 to 2 weeks or recurs: Withhold Pembrolizumab therapy Consider skin biopsy Consider 0.5-1.0 mg/kg/day prednisone or equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume Pembrolizumab therapy following steroids taper.

Dermatological irAEs		
Grade of Rash (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
		If worsens: Treat as Grade 3 to 4.
Grade 3 to 4 Grade 3: Covering > 30% body surface area; Grade 4: Life threatening consequences	Withhold Pembrolizumab for Grade 3. Permanently discontinue for Grade 4 or recurrent Grade 3. Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections	If improves to Grade ≤ 1: Taper steroids over at least 1 month; resume Pembrolizumab therapy following steroids taper (for initial Grade 3).

Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Radiographic changes only	Consider withholding Pembrolizumab therapy Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	Re-assess at least every 3 weeks If worsens: Treat as Grade 2 or Grade 3 to 4.
Grade 2 Mild to moderate new symptoms	Withhold Pembrolizumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily; consider hospitalization 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	Re-assess every 1 to 3 days If improves: When symptoms return to Grade ≤ 1, taper steroids over at least 1 month, and then resume Pembrolizumab therapy following steroids taper If not improving after 2 weeks or worsening:

Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
		Treat as Grade 3 to 4.
Grade 3 to 4 Grade 3: Severe new symptoms; New/worsening hypoxia; Grade 4: Life-threatening	Permanently discontinue Pembrolizumab therapy. Hospitalize. Pulmonary and Infectious Disease consults. 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to Grade ≤ 1: Taper steroids over at least 1 month If not improving after 48 hours or worsening: Add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)

Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin > ULN to 1.5 x ULN	Continue Pembrolizumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3 to 4.
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or total bilirubin > 1.5 to ≤ 3 x ULN	Withhold Pembrolizumab therapy Increase frequency of monitoring to every 3 days.	If returns to Grade ≤ 1: Resume routine monitoring; resume Pembrolizumab therapy. If elevation persists > 5 to 7 days or worsens: Treat as Grade 3 to 4.
Grade 3 to 4 AST or ALT > 5 x ULN and/or total bilirubin > 3 x ULN	Permanently discontinue Pembrolizumab therapy Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day prednisone or equivalent	If returns to Grade ≤ 1: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds:

Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
	Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist/ hepatologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	Add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines.

Renal irAEs		
Grade of Creatinine Increased (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 1 Creatinine increased > ULN to 1.5 x ULN	Continue Pembrolizumab therapy	Continue renal function monitoring If worsens: Treat as Grade 2 to 3 or 4.
Grade 2 to 3 Creatinine increased > 1.5 and ≤ 6 x ULN	Withhold Pembrolizumab therapy Increase frequency of monitoring to every 3 days and consider Nephrology consult 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy	If returns to Grade ≤ 1: Follow Nephrology recommendations if consult obtained; alternatively, taper steroids over at least 1 month, and resume Pembrolizumab therapy following steroids taper. If worsens: Treat as Grade 4.
Grade 4 Creatinine increased > 6 x ULN	Permanently discontinue Pembrolizumab therapy Monitor creatinine daily 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections Consider renal biopsy Nephrology consult	If returns to Grade ≤ 1: Follow Nephrology recommendations; alternatively, taper steroids over at least 1 month.

Cardiac irAEs		
Myocarditis	**Initial Management**	**Follow-up Management**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g., troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Withhold Pembrolizumab therapy. Hospitalize. In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Guideline based supportive treatment as per cardiology consult. * Consider myocardial biopsy if recommended per cardiology consult.	If symptoms improve and immune-mediated etiology is ruled out, re-start Pembrolizumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue Pembrolizumab. Guideline based supportive treatment as appropriate as per cardiology consult. * 1.0 to 2.0 mg/kg/day prednisone or equivalent. Add prophylactic antibiotics for opportunistic infections.	Once improving, taper steroids over at least 1 month. If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A).
*Local guidelines, or e.g., ESC or AHA guidelines ESC guidelines website: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines AHA guidelines website: http://professional.heart.org/professional/GuidelinesStatements/searchresults.jsp?q=&y=&t=1001		

Endocrine irAEs		
Endocrine Disorder	**Initial Management**	**Follow-up Management**
Grade 1 or Grade 2 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Continue Pembrolizumab therapy Endocrinology consult if needed Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for Type I diabetes mellitus) as appropriate.	Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.

Endocrine irAEs		
Endocrine Disorder	**Initial Management**	**Follow-up Management**
	Rule-out secondary endocrinopathies (i.e., hypopituitarism / hypophysitis)	
Grade 3 or Grade 4 endocrinopathies (hypothyroidism, hyperthyroidism, adrenal insufficiency, type I diabetes mellitus)	Withhold Pembrolizumab therapy Consider hospitalization Endocrinology consult Start thyroid hormone replacement therapy (for hypothyroidism), anti-thyroid treatment (for hyperthyroidism), corticosteroids (for adrenal insufficiency) or insulin (for type I diabetes mellitus) as appropriate. Rule-out secondary endocrinopathies (i.e. hypopituitarism / hypophysitis)	Resume Pembrolizumab once symptoms and/or laboratory tests improve to Grade ≤ 1 (with or without hormone replacement/suppression). Continue hormone replacement/suppression and monitoring of endocrine function as appropriate.
Hypopituitarism/Hypophysitis (secondary endocrinopathies)	If secondary thyroid and/or adrenal insufficiency is confirmed (i.e. subnormal serum FT4 with inappropriately low TSH and/or low serum cortisol with inappropriately low ACTH): • Refer to endocrinologist for dynamic testing as indicated and measurement of other hormones (FSH, LH, GH/IGF-1, PRL, testosterone in men, estrogens in women) • Hormone replacement/suppressive therapy as appropriate • Perform pituitary MRI and visual field examination as indicated **If hypophysitis confirmed:** • Continue Pembrolizumab if mild symptoms with normal MRI. Repeat the MRI in 1 month	Resume Pembrolizumab once symptoms and hormone tests improve to Grade ≤ 1 (with or without hormone replacement). In addition, for hypophysitis with abnormal MRI, resume Pembrolizumab only once shrinkage of the pituitary gland on MRI/CT scan is documented. Continue hormone replacement/suppression therapy as appropriate.

Endocrine irAEs		
Endocrine Disorder	**Initial Management**	**Follow-up Management**
	• Withhold Pembrolizumab if moderate, severe or life-threatening symptoms of hypophysitis and/or abnormal MRI. Consider hospitalization. Initiate corticosteroids (1 to 2 mg/kg/day prednisone or equivalent) followed by corticosteroids taper during at least 1 month. • Add prophylactic antibiotics for opportunistic infections.	

Other irAEs (not described above)		
Grade of other irAEs (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
Grade 2 or Grade 3 clinical signs or symptoms suggestive of a potential irAE	Withhold Pembrolizumab therapy pending clinical investigation	If irAE is ruled out, manage as appropriate according to the diagnosis and consider re-starting Pembrolizumab therapy If irAE is confirmed, treat as Grade 2 or 3 irAE.
Grade 2 irAE or first occurrence of Grade 3 irAE	Withhold Pembrolizumab therapy 1.0 to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month and resume Pembrolizumab therapy following steroids taper.
Recurrence of same Grade 3 irAEs	Permanently discontinue Pembrolizumab therapy to 2.0 mg/kg/day prednisone or equivalent Add prophylactic antibiotics for opportunistic infections Specialty consult as appropriate	If improves to Grade ≤ 1: Taper steroids over at least 1 month.
Grade 4	Permanently discontinue Pembrolizumab therapy to 2.0 mg/kg/day	If improves to Grade ≤ 1: Taper steroids over at least 1 month

Other irAEs (not described above)		
Grade of other irAEs (NCI-CTCAE v5)	**Initial Management**	**Follow-up Management**
	prednisone or equivalent and/or other immunosuppressant as needed Add prophylactic antibiotics for opportunistic infections Specialty consult.	
Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks for reasons other than hormonal replacement for adrenal insufficiency **Persistent Grade 2 or 3 irAE lasting 12 weeks or longer**	Permanently discontinue Pembrolizumab therapy Specialty consult	

ADL=activities of daily living, ALT=alanine aminotransferase, AST=aspartate aminotransferase, irAE=immune-related adverse event, IV=intravenous, LLN=lower limit of normal, MRI=magnetic resonance imaging, NCI-CTCAE=National Cancer Institute-Common Terminology Criteria for Adverse Event, NSAIDs=nonsteroidal anti-inflammatory drugs, T4=thyroxine, TSH=thyroid-stimulating hormone, ULN=upper limit of normal, IVIG=intravenous immunoglobulins, CPK=creatine phosphokinase.

Note: Subjects who require delay of pembrolizumab should be re-evaluated closely as clinically indicated and resume pembrolizumab dosing when re-treatment criteria are met.

3.3.6.2 Criteria to Resume Pembrolizumab Treatment

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

- Subjects may resume treatment in the presence of Grade 2 fatigue.
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity.
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed. Subjects with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the next scheduled time point will be delayed until dosing resumes.
If treatment is delayed or interrupted for > 6 weeks, the subject must be permanently discontinued from study therapy unless there is evidence (stable disease or disease regression) of clinical benefit.

3.4 ON STUDY PROTOCOL EVALUATION (APPENDIX 3: STUDY CALENDAR)

3.4.1 Baseline Assessments

Note: Baseline assessments do not need to be repeated if performed during screening in the designated time frame.

Any Time Prior to Initiation of Treatment:

a. HLA class I and class II analysis of PBMC.
b. Tumor biopsy if fresh sample from previous biopsy is not available. Note: Samples collected on 06C0014 or any other NCI protocol in which the patient is co-enrolled can be used.

Within 10 Days Prior to Commencing DAC-THU/Pembrolizumab Therapy:

a. Physical examination including assessment of vital signs and ECOG status.
b. Chemistries
    i. Acute Care Panel - Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN),
    ii. Mineral Panel - Albumin, Calcium total, Magnesium total (Mg), Phosphorus
    iii. Hepatic Panel - Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin
    iv. LD, Total Protein, Total CK, Uric Acid
c. Fetal hemoglobin
d. CBC, differential, platelet count, PT, PTT
e. T, B, NK cell subsets
f. Urinalysis and culture if indicated
g. Pulmonary Function Test
h. Arterial Blood Gas on room air (to be drawn if pulse oximetry <90% on room air).
i. Research Labs (refer to **Appendix 4** for details).

Within 48 Hours Prior to Initiation of DAC-THU/Pembrolizumab Therapy:

a. Women of child-bearing potential will have a urine or serum βhCG pregnancy test.

3.4.2 Treatment Evaluation

Twice Weekly During First Course of Therapy, and Weekly Thereafter Unless Clinically Indicated:

a. CBC with differential

b. Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid

c. PT/PTT

Note: The patient may have lab work obtained through his or her local physician.

Every Three Weeks During Therapy:

a. T, B, NK cell subsets

Performed After Each Course:

a. Physical examination including assessment of vital signs and ECOG status.

b. Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid

c. CBC, differential, platelet count, PT, PTT

d. Thyroid Function Tests

e. T, B, NK cell subsets

f. Urinalysis and culture if indicated

g. Biopsy of index lesion (only on first treatment evaluation).

3.4.3 Research Evaluations (Refer to Appendix 4, Unless Otherwise Indicated Below)

- Pharmacokinetic (PK) Studies
- Research Labs
- Radiological Assessments (refer to **Appendix 3**)
- Tumor Biopsies (see **Appendix 3**)

3.4.4 Follow-Up Evaluations

Patients who are removed from treatment due to disease progression or unacceptable, dose-limiting toxicities or who voluntarily withdraw from treatment will be monitored weekly until treatment related toxicities have resolved to baseline at which time they will be removed from study.

Toxicity Evaluation:

- Laboratory evaluation: CBC, Chemistries (Sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, albumin, total calcium, ionized calcium, magnesium, phosphorus, alkaline phosphatase, ALT/SGPT, AST/SGOT, total

bilirubin, direct bilirubin, lactate dehydrogenase, total protein, creatine kinase, uric acid), PT/PTT

3.5 COSTS AND COMPENSATION

3.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by their insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.5.2 Compensation

Participants will not be compensated on this study.

3.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from protocol, effort must be made to have all subjects complete a safety visit approximately 30 days after the last dose of study therapy.

3.6.1 Criteria for Removal from Protocol Therapy

- Patient refusal of further treatments
- It is deemed in the best interest of the patient
- A patient who develops a concurrent serious medical condition that might preclude or contraindicate administration of DAC-THU/pembrolizumab therapy will be removed from treatment
- A patient who becomes pregnant will be immediately taken off therapy
- Progressive disease
- Failure to meet criteria for additional treatment as defined in Sections **3.3.2** and **3.3.6**
- Delay of treatment for three weeks

Note: Patients will be followed until toxicities related to experimental therapy resolve to baseline.

3.6.2 Off-Study Criteria

- Voluntary withdrawal for any reason, or noncompliance with protocol requirements
- Completion of the follow up period (see Section **3.4.4**)
- Progressive disease unless the patient is being followed for a serious adverse event related to the research. The event must resolve to baseline prior to removing from the study

- If the investigator determines that it is in the best interest of the patient to discontinue follow up
- Lost to follow up
- Death
- PI decision to close the study

3.6.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 5 business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls/video calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 HEMATOLOGIC AND BLOOD PRODUCT SUPPORT

Blood product support should be provided to maintain platelets > 20,000 cells/mcl, Hgb > 8.0 gm/dl and as clinically indicated. Growth factor support is not permitted with exception of administration of filgrastim. Filgrastim will not be administered prophylactically, but may be administered if a patient experiences neutropenic sepsis, or if clinically indicated.

4.2 ANTIDIARRHEALS

Antidiarrheal agents will be prescribed using standard clinical practice guidelines at the preference of the investigator.

4.3 ELECTROLYTE REPLACEMENT

Electrolyte replacement will be provided to maintain serum levels within normal limits.

4.4 PROTON PUMP INHIBITORS

Given the uncertainties regarding effects of proton pump inhibitors on absorption of DAC-THU, proton pump inhibitors will not be used except on days when DAC-THU is not administered.

5 CORRELATIVE STUDIES

5.1 BIOSPECIMEN COLLECTION AND PROCESSING FOR RESEARCH

5.1.1 Pharmacokinetic (PK) Sampling

Detailed plasma pharmacokinetic (PK) sampling of DAC and THU will be collected as outlined in **Appendix 4**. PK analysis will be performed at the NCI, Genitourinary Malignancies Branch (GMB). Samples will be stored in the Thoracic Epigenetics Lab (TEL) and batched and later transferred to the Figg Lab on dry ice for analysis.

5.1.2 Studies on Tumor Tissue and Peripheral Blood

Portions of biopsy materials will be sent for frozen section or permanent section confirmation of malignancy and percent viable tumor cells. Tissue will be processed for focused gene, endogenous retroviral (ERV) and microRNA expressions, and DNA methylation signatures using quantitative RT-PCR, nanostring, pyrosequencing and digital droplet PCR techniques. Blood from EDTA and red top tubes (see **Appendix 4**) will be used to isolate plasma circulating tumor DNA and serum for focused methylation analysis and immune response to treatment. Remaining tissue samples will be snap frozen in liquid nitrogen for subsequent isolation of RNA and DNA for qRT-PCR and nanostring experiments. If sufficient tissue is available, another portion will be imbedded in paraffin for subsequent immunostaining experiments, focusing on expression of genes focusing on those proteins encoded by genes that have been identified to be clearly activated by epigenetic therapy. If sufficient materials are present, additional more comprehensive analyses including multiplex IHC analysis of tumor microenvironment may be performed with the focus of materials from patients treated at the MTD. All of the analyses, which are predicated on acquisition of sufficient materials, will be performed in the Thoracic Epigenetics Lab under direction of the PI or submitted to sequencing cores at the NCI.

5.1.3 Immune Subset Analyses

Peripheral blood mononuclear cells (PBMC) will be assessed using multiparameter flow cytometry for immune subsets including but not necessarily limited to Tregs, MDSC, effector and exhausted CD4+ or CD8+ T-cells, and CD14+ monocytes. Assessment will include functional markers, i.e. PD-1, PD-L1, Tim-3, CTLA-4, HLA-DR and/or CD40. Samples will be drawn (as indicated in **Appendix 4**) and picked up by the Trepel Lab (see Section **5.2.4**) for analysis. The Trepel Lab will prepare the samples for staining, stain and run the samples by multiparametric flow cytometry (MACSQuant, Miltenyi Biotec, Bergisch Gladbach, DE), and analyze the data using FlowJo (FlowJo LLC, Ashland, OR) software.

5.1.4 Circulating Tumor Cells

Peripheral blood will be collected to correlate changes in circulating tumor cells with clinical response. CTCs will be assessed using ferrofluidic enrichment and multi-parameter flow cytometric detection. Samples will be drawn (as indicated in **Appendix 4**) and picked up by the Trepel Lab (Section **5.2.4**) for analysis. The Trepel Lab will process the samples, stain and run one tube immediately. The other tube will be processed, 2D barcoded and viably stored for potential future analysis. The samples will be analyzed by multiparametric flow cytometry (MACSQuant, Miltenyi Biotec, Bergisch Gladbach, DE) and FlowJo (FloJo LLC, Ashland, OR) software.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered and tracked through Clinical Trial Data Management System. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.2.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.2.1.1 BPC Contact Information

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, send request to NCIBloodcore@mail.nih.gov.

5.2.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.1.3 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested). The PI will record any loss

or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

Sample bar-codes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.2.2 Procedures for Storage of Tissue Specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissue that is not embedded in paraffin is stored in formalin for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.2.3 Thoracic Epigenetics Laboratory

This study will be conducted within the Thoracic Surgery Branch, NCI. Sample collection and initial processing will be performed in the Thoracic Epigenetics Laboratory (TEL). Samples will be stored in designated monitored freezers (at least -20°C). All samples obtained on this study will be tracked using Labmatrix. Pharmacokinetic samples will be analyzed in the Figg Lab, GMB. Samples will be identified and tracked using unique identifiers linked to each subject's unique patient number (study number). Codes linking personal identifiable information to the unique identifier will be stored in secure, computer servers with limited coded access or locked file cabinets in the Thoracic Surgery Branch, with access limited to the PI or study coordinator. Gene expression, microRNA, and DNA methylation signatures, as well as methylation status of circulating tumor DNA and serologic responses to treatment will be analyzed in Dr. David Schrump's laboratory.

5.2.4 Trepel Laboratory

Contact the Trepel Lab by email (Dr. Jane Neckers (Trepel): trepel@helix.nih.gov; Min-Jung Lee: leemin@mail.nih.gov; Akira Yuno: akira.yuno@nih.gov and Sunmin Lee: lees@pop.nci.nih.gov) when the patient is scheduled and by phone as soon as the blood is drawn at 240-760-6330.

A lab member will come to pick up the blood. Please keep blood at ambient temperature. Members of the lab will enter the samples into a secure password protected patient's sample tracking database (Translational Pharmacodynamics Research Group Patient Sample Management System) and process the samples.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol. It is critical that the sample remains coded and linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate with these variables.

Blood samples will be stored initially in the Trepel Lab in the Magnuson Clinical Center. If, at any time, a subject withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested). When a patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation (refer to Section **5.2.5**). Reporting will be per the requirements of Section **7.2**.

5.2.5 Sample Destruction, End of Protocol Procedures

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues.

Following completion of this study, if the subject has co-enrolled on 06C0014, samples will be transferred to the Tissue Procurement protocol and remain in storage in the Blood Processing Core (BPC) as detailed within 06C0014. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If the subject has not co-enrolled on the Tissue Procurement protocol, the samples will be destroyed.

If the patient withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

For the purposes of the research sample analyses and correlation with clinical outcomes, demographic information, histology, operative and peri-operative interventions, pathologic findings, laboratory and imaging parameters (performed as part of routine or protocol specified patient care) may be collected on this study. The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of Study Procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or Destruction of Data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.1.1 Routine Data Collection

Following registration, all adverse events will be described in the source documents, reviewed by the designated research nurse, and captured.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention, Study Day 1, through 30 days after the last administration of study therapy. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

6.1.2 Exclusions to Routine Data Collection

6.1.2.1 Adverse Events

The following events will be captured only in the source documents and will not be recorded:

- All Grade 1 events.
- Grade 2 events that are not related to the treatment.
- Grade 1, 2, and 3 Lab values drawn outside of the protocol specified time points that are not assessed as clinically significant by the PI or his designee and are not associated with an adverse event.
- Events related to vascular access devices (occlusion, thrombi, hospitalizations for insertion or removal).

Note: Events that result in a hospitalization for convenience will not be reported.

6.1.2.2 Concomitant Medications/Measures

All concomitant medications and measures will be captured in the source documents. Only those medications that the patient is taking at baseline on a routine basis or medications that cause an AE will be captured (e.g., onetime medications, PRN medications, supportive medications, electrolyte replacement and medications given to treat adverse events will not be captured).

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center).
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository, clinicaltrials.gov, dbGaP.
- BTRIS (automatic for activities in the Clinical Center).
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients will be re-evaluated for response every 10 weeks (± 1 week). In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Objective response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) [(Version 1.1)(109)]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

6.3.1 Confirmatory Measurement/Duration of Response

- Confirmation
  To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met.
- Duration of Overall Response
  The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

  The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

6.3.2 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with DAC/THU/pembrolizumab. Dose limiting toxicities will be assessed during the first 3 weeks of DAC-THU/pembrolizumab therapy.

Evaluable for objective response: Patients who have measurable disease present at baseline, have received at least 9 weeks of DAC-THU/pembrolizumab therapy (one full course), and have had their disease re-evaluated will be considered evaluable for response provided: (1) the patient demonstrates progressive disease or death while on protocol therapy; (2) the tumor is not removed surgically prior to the time complete response or partial response is confirmed; or (3) the patient demonstrates a complete or partial response as confirmed according to protocol criteria. These patients will have their response classified according to the definitions stated below. [Note: Patients who electively terminate therapy before completing 9 weeks of DAC-THU/pembrolizumab therapy and do not expire within 14 days from start of treatment will be replaced. Patients who experience disease progression after 3 weeks of drug therapy but before

completing the minimum of 9 weeks of drug therapy will be taken off-treatment and will only be evaluable for toxicity (not evaluable for response)].

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least 8 weeks of DAC-THU/pembrolizumab therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.3 Disease Parameters

6.3.3.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under as ≥10 mm
    - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

6.3.3.2 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.

6.3.3.3 Non-Measurable Disease

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 patient, these are preferred for selection as target lesions.

6.3.3.4 Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest

lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Progressive disease by RECIST criteria (109) noted after the first re-staging scan may represent disease that was not detected on the pre-study scan, and a confirmatory scan will be required at the next scheduled re-staging evaluation unless clinically not indicated. If confirmed, progression should be dated by the initial time when the lesions are first detected. If progressive disease by RECIST criteria is seen after Cycle 3, but not confirmed on subsequent restaging scan, the scans from after Cycle 3 would serve as the baseline scan to evaluate for disease progression (110).

6.3.3.5 Non-Target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.4 Methods for Evaluation of Measurable Disease

6.3.4.1 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

6.3.4.2 Metastatic Bone Lesions

Disease progression is considered if a minimum of two new lesions is observed on bone scan. New lesions seen by the end of Cycle 3 (with the first re-staging bone scan) may represent disease that was not detected on the pre-study scan, and a confirmatory scan will be required at the next scheduled re-staging bone scan unless clinically not indicated. If confirmed, progression should be dated by the initial time when the lesions are first detected. If new lesions are seen after Cycle 3, but no additional lesions are seen on confirmatory scans, the scans from post-Cycle 3 would serve as the baseline scan to evaluate for disease progression (109).

6.3.4.3 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

6.3.4.4 Methods of Measurement

Chest X-ray - Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

CT and MRI - CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. For this study helical Multi-detector CT will be performed with cuts of 5 mm in slice thickness for chest, abdomen and pelvis lesions and 2-3 mm thickness for head and neck lesions.

6.3.4.5 Additional Response Evaluation Using Volumetric Analysis

In addition, the utility of volumetric tumor measurement in patients with measurable disease will be prospectively evaluated and compared to 1D and 2D measurements.

6.3.5 Response Criteria for Radiographic Studies

6.3.5.1 Measuring of Soft Tissue Disease

a. Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR)

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Note: For this study, determination of PD will not be made prior to the Day 60 evaluation.

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

b. Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: Tumor markers alone cannot be used to assess response. If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD (Stable Disease, SD)

Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD)

Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

c. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 4 wks. Confirmation**
CR	Non-CR Non-PD	No	PR	≥ 4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR Non-PD Not evaluated	No	PR	
SD	Non-CR Non-PD Not evaluated	No	SD	Documented at least once ≥ 4 wks. from baseline**
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

d. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

e. In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesions be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to the Clinical Director/designee at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet weekly when patients are being actively treated on the trial to discuss each patient in detail. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a clinical associate investigator in a timely manner. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

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

- Death.
- A life-threatening adverse event (see Section **8.1.3**).
- Inpatient hospitalization or prolongation of existing hospitalization.
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.

- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the Sponsor with the exception of any listed in Section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in Section **8.4**.

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to CCR

Death or Hospitalization that is deemed to be due to disease progression, and not attributable to the intervention will not be reported as an SAE. The event, and the assessment that it was caused by disease progression will be documented in the medical records. The causality assessment of Hospitalization will be re-evaluated any time when new information is received. If the causality assessment changes from disease progression to related to the study intervention, SAE report will be sent to the Sponsor immediately **in an expedited manner according to Section 8.3**. If there is any uncertainty whether the intervention is a contributing factor to the event, the event should be reported as AE or SAE as appropriate.

8.5 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions.

8.5.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 60 days after the last dose of DAC-THU and pembrolizumab.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 60 days after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the Sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good

Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

The primary objectives of this trial are to determine the objective response rates of DAC/THU plus pembrolizumab in patients with NSCLC, EsC, and MPM who have intra-tumoral PD-L1 expression of at least 50% and those who do not. Approximately 30% of NSCLC exhibit PD-L1 expression in 50% or more cancers cells, another 30-50% have PD-L1 expression in 1-49% of cancer cells, and the remaining NSCLC have no PD-L1 expression by IHC (25, 111-113). In contrast, 20-40% of gastroesophageal cancers exhibit PD-L1 expression (25, 111, 112); in the majority of these cancers, PD-L1 is expressed at low levels, and tends not to be expressed on the cancer cells (112). Despite these differences regarding PD-L1 expression, present data indicate that responses to immune checkpoint inhibitors in EsC are comparable to NSCLC with low PD-L1 expression (114). Approximately 25%-30% of MPM exhibit some level of PD-L1 expression, with the expression being primarily on tumor-associated immune cells (115). PD-L1 expression tends to be higher on sarcomatoid MPM and lower on the more common epithelioid subtype. Response rates in MPM following PD-1 blockade are comparable to NSCLC with similar levels of PD-L1 expression (99, 100).

Patients will be initially enrolled onto a multi-dose level dose escalation portion of the trial following a 3+3 design. Patients will not be stratified for the Phase I component, which will accelerate our ability to define MTD. Thus, for the dose escalation phase, up to 6 patients per dose level may be enrolled, with a total of 12 patients required with a maximum of 2 dose levels. At the MTD, an additional 4 patients will be enrolled to further estimate the safety of the combination.

Since published data have demonstrated a response rate of 45% for first-line pembrolizumab alone in NSCLC patients with PD-L1 expression of 50% or greater (95), (98), this trial will try to demonstrate if a response rate in excess of 45% may be realized in NSCLC patients with ≥ 50% PD-L1 expression by the addition of DAC/THU. Briefly, NSCLC patients with high intratumoral (50% or greater) PD-L1 expression will be evaluated for response as part of a Simon optimal two-stage Phase II trial design (116) in order to rule out an unacceptably low PR+CR rate of 35% (p0=0.35) in favor of an improved response rate of 60% (p1=0.60). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta=0.20 (probability of rejecting a good treatment=0.20), the first stage will determine the response rate in 10 evaluable patients, including those NSCLC patients among the 10 who are treated at the MTD from the Phase I cohort who have high intra-tumoral PD-L1 expression. If 0 to 4 of the 10 patients have a clinical response, then no further patients will be accrued. If 5 or more of these 10 patients have a response, then accrual would continue until a total of 23 evaluable patients have been treated at the MTD. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 5 to 10 patients with a response out of 23 patients, this would be an uninterestingly low response rate. If there were 11 or more of 23 (47.8%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (35% response rate), the probability of early termination is 75.2%.

NSCLC patients with low (0-49%) intratumoral PD-L1 expression will be evaluated for response using a Simon optimal two-stage Phase II trial design (116) in order to rule out an unacceptably low PR+CR rate of 10% (p0=0.10) in favor of an improved response rate of 30% (p1=0.30). Variable mutational burdens among NSCLC with low PD-L1 expression could affect responses to immune checkpoint inhibitors, potentially making this cohort more heterogeneous than the NSCLC high PD-L1 expresser cohort. For example, NSCLC patients without activating mutations in EGFR, HER2 or ALK with PD-L1 tumor proportion score (TPS) < 1% have an expected response rate of less than 10%, whereas those with activating mutations involving these genes have response rates of 5% or less; patients with PD-L1 TPS of 1-49% have a 17% response rate (25, 103, 104, 113). Therefore, with alpha=0.05 (probability of accepting a poor treatment=0.05) and beta = 0.20 (probability of rejecting a good treatment=0.20), the first stage will determine the response rate in 10 evaluable patients, including those among the 10 who are treated at the MTD from the Phase I cohort who have low intra-tumoral PD-L1 expression, and if 0 to 1 of the 10 have a clinical response, then no further patients will be accrued. If 2 or more of these 10 patients have a response, then accrual would continue until a total of 29 evaluable patients have been treated in this cohort. As it may take up to several months to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 to 5 patients with a response out of 29 patients, this would be an uninterestingly low response rate. If there were 6 or more of 29 (20.7%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (10% response rate), the probability of early termination is 73.6%.

While accrual of NSCLC patients is underway, up to 10 EsC patients including any from the Phase I component after the MTD has been identified, will be accrued to examine responses in EsC patients treated with DAC-THU at the MTD and pembrolizumab. Since it is anticipated that most if not all of these 10 patients will have low or no PD-L1 expression in their tumor tissues, and because response rates to immune checkpoint inhibitors in EsC appear to be comparable to NSCLC with low PD-L1 expression, criteria similar to those reported for the NSCLC low PD-L1 cohort

will be used to identify a potential signal of activity. Specifically, if there are 2 or more responses in these 10 patients, this might warrant amendment of the current protocol to enable expansion of this cohort with appropriate statistical criteria, or to justify a larger, focused Phase II trial.

Additionally, up to 10 MPM patients including any from the Phase I component after the MTD has been identified, will be accrued to examine responses in MPM patients treated with DAC-THU at the MTD and pembrolizumab. Since it is anticipated that most if not all of these 10 patients will have low PD-L1 expression in their tumor tissues, and because response rates to immune checkpoint inhibitors in MPM patients appear to be comparable to NSCLC with low PD-L1 expression, criteria similar to those reported for the NSCLC low PD-L1 cohort will be used to identify a potential signal of activity. Specifically, if there are 2 or more responses in these 10 patients, this might warrant amendment of the current protocol to enable expansion of this cohort with appropriate statistical criteria, or to justify a larger, focused Phase II trial.

It is expected that approximately 2-4 patients per month may enroll onto this trial. Thus, it is expected that 24-30 months may be required in order to enroll up to 78 evaluable patients. Assuming two dose levels will be used to define MTD, the Phase I component of the trial will require 16 patients. The Phase II portion of the trial may require 29 NSCLC patients for the PD-L1 high, and 23 NSCLC patients for the PD-L1 low cohorts, plus 10 EsC patients, plus 10 MPM patients, less 10 patients treated at the MTD in the Phase I portion of the protocol; hence 16+29+23+10+10-10=78 patients In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at **85** patients, acknowledging that the trial can be completed with 60 or fewer patients if response goals for each cohort are not met.

Twelve months after Amendment E has been implemented, accrual will be assessed. If the trial is not on track for accrual targets, e.g., if fewer than 12 patients have been accrued within 12 months, the protocol will either be terminated, or the trial will be opened at an additional site to accelerate patient accrual, and ensure timely completion of this trial.

Pharmacokinetic analysis will be conducted using non-compartmental methods. Pharmacodynamic analyses, as well as analyses of molecular endpoints in tumor tissues and PBMC before and after treatment will be performed, with the primary focus being with respect to results at the MTD. For pharmacodynamic endpoints, descriptive statistics will be used for each endpoint. Briefly, pharmacodynamic and molecular endpoints in target tissues will be assessed before and after DAC-THU/pembrolizumab treatment, with the primary focus being comparison of microarray, qRT-PCR and IHC results relative to profiles identified in our preclinical studies. It is anticipated that non-parametric statistical methods will be used to compare paired results before and after treatment (but not between groups), as well as to perform comparisons or correlations among parameters. As the exact set of comparisons and analyses to be performed will be determined following completion of the trial and will be based on limited numbers of patients, the analyses will be considered exploratory and hypothesis generating rather than definitive.

10.1 METHODS OF ANALYSIS

Response rates will be calculated as the percent of patients whose best response is a CR or PR. Toxicity information recorded will include the type, severity, time of onset, time of resolution, and the probable association with the study regimen. Tables will be constructed to summarize the observed incidence by severity and type of toxicity.

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered on this protocol have advanced NSCLC, EsC, or MPM and have limited life expectancies. This population was selected because of the unknown outcome of this treatment in terms of its effectiveness. The experimental treatment has a chance to provide clinical benefit although this is unknown. Subjects of both genders and all racial/ethnic groups are eligible. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with gender or ethnic identity are noted, accrual may be expanded, or a follow-up study may be written to investigate these differences more fully. One strategy for recruitment may be to distribute an IRB approved protocol recruitment letter to General and Oncology physicians and nurses.

11.2 PARTICIPATION OF CHILDREN

It is anticipated that most children with cancer will not have disease that is appropriate for study. Because the effects of DAC-THU have not been evaluated in children, individuals <18 will be excluded from this study until more data are available.

11.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **11.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **11.5.1** for consent procedure.

11.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

11.4.1 Risks

11.4.1.1 Study Drug Risks

The risks to the patient participating in this trial are anticipated to be small and are primarily the risks associated with administering the DAC-THU with pembrolizumab. These potential risks are addressed in Sections **13.1.9**, **13.2.8** and **13.3.6**. There is the potential risk of not responding to the investigational treatment, and being unfit to receive and potentially benefit from standard of care cytotoxic chemotherapy. Risks and benefits will be carefully discussed with the patient at the

time consent is obtained. Patients will be monitored throughout the study in order to limit the consequences of any adverse events.

11.4.1.2 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

11.4.1.3 Urine Collection

There is no physical risk involved with urine collection.

11.4.1.4 Biopsies

The risks associated with biopsies are pain and small amounts of bleeding at the biopsy site, and rarely, a small infection at the site. Biopsy sites usually heal very well and with very little scarring. In order to minimize pain, local anesthesia will be used; side effects of the local anesthesia may include a mild burning sensation when the numbing medicine is injected into the skin.

11.4.1.5 Conscious Sedation

The common side effects of conscious sedation include drowsiness, delayed reflexes, hypotension, headache, and nausea. These are generally mild and last no more than a few hours.

11.4.1.6 Pulmonary Function Tests

These tests are safe and side effects are unlikely, but may include brief light headedness or slight soreness of the chest.

11.4.1.7 Scans and Contrast

The most common discomfort is the length of time a patient must lay still during a scan. Patients may also become uncomfortable with the closed space of the machines.

There is a small risk of reaction in scans involving contrast. Common reactions include pain in the vein where the contrast was given, a metallic or bitter taste in the mouth, headache, nausea and a warm or flushing feeling that lasts from 1-3 minutes. In very rare cases, severe reactions that affect breathing, heart rhythm or blood pressure have occurred.

An IV catheter may need to be inserted for administration of the contrast agent or anesthetic, which may cause pain at the site where the IV is placed and there is a small risk of bruising or infection, or inflammation of the skin and vein with pain and swelling.

11.4.1.8 Risks of Exposure to Ionizing Radiation

This research study involves the potential for 5 CT CAP scans, 5 PET/CT scans, 5 brain CT scans as well as 2 CT-guided biopsies over the course of the first year on study. This radiation exposure is not required for medical care and is for research purposes only. Subjects will be exposed to approximately 13.6 rem. This amount of radiation is above the guideline of 5 rem per year and will expose the subject to the roughly the same amount of radiation as 45.3 years of background radiation.

11.4.2 Benefits

There may be some direct benefit to patients who participate in this trial since it is anticipated that DAC-THU with pembrolizumab may cause tumor stabilization or some tumor regression. The greatest benefit will be the information regarding the feasibility, toxicity and dosing of DAC-THU with pembrolizumab administration, as well as the information on changes in the epigenome of lung and gastroesophageal cancers and systemic immunity to up-regulated CTAs.

11.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (Non-Electronic) Signature on Electronic Document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found here.

11.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.5**.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or

termination, will be provided by the suspending or terminating party to investigators, funding agencies, the Investigational New Drug (IND) sponsor and regulatory authorities, as applicable. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 DECITABINE

13.1.1 Synonyms

DAC

deoxyazacytidine

NSC 127716

Dacogen®

13.1.2 Description

Decitabine is an analog of 2'-deoxycytidine in which the carbon at position 5 in the pyridine ring has been replaced by nitrogen. In acid media, decitabine undergoes cleavage at the glycosidic bond to yield 5-azacytosine and deoxyribose. In acid media, a rapid, reversible opening of the 5-azacytosine ring occurs between positions 1 and 6, producing N-(formylamidino)-N'-D-2'-deoxyribofuranosylurea which decomposes with the loss of a formyl group.

Decitabine is enzymatically phosphorylated in sequence by deoxycytidine kinase, deoxycytidylic kinase, and nucleoside diphosphonucleoside kinase to the active forms, 5-Aza-dCMP, 5-Aza-dCDP, 5-Aza-dCTP, respectively. The latter form is incorporated into DNA. Decitabine is enzymatically deaminated by cytidine deaminase; phosphorylated decitabine nucleoside (5-Aza-dCMP) is catabolized by deoxycytidylic deaminase.

13.1.2.1 Mode of Action

Inhibition of DNA methylation, and induction of cellular differentiation.

13.1.3 Chemical Names

(1) 1-(2'-deoxy-D-ribofuranosyl)-5-azacytosine

(2) 3,5-Triazin-2(1H)-one,4-amino-l-(2-deoxy-ß-D-erythro-pentofuranosyl)-

(3) 4-Amino-l-(2-deoxy-ß-D-erythro-entofuranosyl)-1,3,5-triazin-2(1H)-one

(4) 5-aza-2'-deoxycytidine

13.1.4 Chemical Identification

Empiric formula $C_8H_{l2}N_40_4$

Molecular weight 228.21 daltons

CAS registry No. 2353-33-5 pertains to free base.

13.1.5 Formulation

Decitabine is manufactured by IriSys LLC (San Diego, CA) and supplied by DCTD as a freeze-dried powder in 20 mL-capacity vials containing DAC 50 mg with 68 mg potassium phosphate, and NaOH. Decitabine will be encapsulated by IriSys LLC under contract with the NIH CC Pharmacy per federal Good Manufacturing Practice guidelines. In this formulation decitabine is supplied as capsules containing 5 mg of decitabine per capsule, in bottles containing 30 capsules, together with a drying agent. The label on the bottles includes the following information: the (i) name of the drug; (ii) quantity of drug per capsule; (iii) date of packaging; (iv) recommended storage temperature; (v) lot number of bulk drug used to generate the capsules

13.1.6 Stability

Powder: The glass bottles containing decitabine should be stored at -20°C. The drug substance has been tested after 18 months of storage at -20°C and no change in appearance, assay or impurities was observed. Decitabine is stable as a dry powder, however, degrades in a pH dependent manner in aqueous solution (optimal pH for stability is 7.0). Decitabine is hydrolyzed in aqueous solution at pH 7.4 at a rate of approximately 2.5% every 7 hours at 4°C.

Capsules: Shelf life stability testing of the intact bottles is on-going.

13.1.7 Storage

Powder: Containers should be tightly closed and stored in the freezer at -20°C.

Capsules: Bottles should be tightly closed and stored at refrigerated temperatures (2-8°C).

13.1.8 Administration

Please see Section **3.2.1**.

13.1.9 Toxicities

There is substantial information regarding the toxicity of decitabine in humans from clinical trials in patients with MDS and AML, including relapsed or poor prognosis cases. Some of this information is summarized in the package insert for this FDA approved drug. In almost all studies, the patients received doses much higher than the decitabine doses planned in this study. Leukopenia was a major toxicity and nausea, or vomiting were common non-hematologic toxicities. A better guide to anticipated side-effects in this clinical trial are observations from clinical studies of decitabine repositioned for non-cytotoxic DNMT1-depletion for sickle cell disease (SCD), β-thalassemia intermedia and MDS. In these clinical trials there were no episodes of non-hematologic CTCAE Grade 2 or higher toxicity from decitabine. In the SCD and β-thalassemia trials, consistent with a non-cytotoxic mechanism of action, the main side-effect was an increase in the platelet count (as opposed to the usual side-effect of thrombocytopenia seen with cytotoxic treatments). Cytotoxicity/DNA damage assays based on bone marrow morphological examination, bone marrow DNA content analysis, VDJ recombination assay, erythrocyte micronucleus assay and gamma-H2AX assay were also negative. These assays did not reveal evidence of DNA damage or cytotoxicity. The increase in the platelet count was not associated with any clinical adverse events. Other common side effects are fatigue, fever, nausea, cough, petechiae, constipation, diarrhea, and hyperglycemia.

Expected side effects in current trial:

1. Thrombocytosis: Decitabine-induced shifts in differentiation include increased erythropoiesis and megakaryopoiesis (60, 61). Therefore, increases in platelet counts are expected, especially in patients who are hyposplenic (e.g., patients with SCD) or asplenic (many patients with β-thalassemia). Although it is intuitive to expect an association between platelet count and thrombosis risk, this has not been evident in clinical observations and studies, whereas qualitative RBC defects from underlying disease can be pro-thrombotic (60, 117-124). Hence, in a SCD study, SC decitabine-induced improvement in multiple indices of RBC pathology was accompanied by improvement in multiple markers of coagulation pathway activity, despite concurrent platelet count increases to > $800x10^9$/L (60). Thus, it is possible that decitabine-induced improvements in RBC phenotype could reduce thrombophilia despite concurrent platelet count increases. Coagulation pathway evaluation (for example by measurement of D-dimer levels) should probably be a component of studies with decitabine to treat hemoglobinopathies.

2. Neutropenia: Decitabine induced shifts in differentiation include favoring erythroid-megakaryocyte progenitor production over granulocyte-monocyte progenitor production by hematopoietic stem cells (60, 61). Hence, even at low, non-cytotoxic concentrations, a downward shift in neutrophil counts is expected. Of note, the current standard of care for symptomatic SCD is DNA damaging, cytotoxic doses of HU. Consequently, in SCD subjects treated with HU,

hematologic toxicities are pancellular, with neutrophil recovery taking up to 2 weeks (AHFS Drug Information®, 2000).

3. Lymphopenia: Decitabine induced shifts in hematopoietic differentiation may also reduce lymphocyte production.

3. Testicular toxicity: Pre-clinical studies suggest an effect on sperm-counts can occur even with the intended low, non-cytotoxic concentrations of decitabine. This toxicity appears reversible. Of note, the current standard of care for symptomatic SCD with HU also causes testicular toxicity (AHFS Drug Information®, 2000).

4. Teratogenicity: Pre-clinical data indicates that there is a teratogenic risk even at low doses of decitabine. Therefore, all clinical studies with decitabine must take into account, and incorporate precautions, to address a risk for teratogenicity. Current standard therapy for SCD with either HU or stem cell transplantation also has teratogenic risks (pregnancy category D).

13.2 TETRAHYDROURIDINE

13.2.1 Names

Chemical Name: 2(1H)-pyrimidinone, tetrahydro-4-hydroxy-1.beta.-D-ribofuranosyl-

Other Names: THU, H_4U

CAS Registry Number: 18771-50-1

Molecular Formula: $C_9H_{16}N_2O_6$ M.W.: 248

Approximate Solubility: The drug is highly soluble in water, up to 200 mg/mL.

13.2.2 Mode of Action:

Cytidine deaminase inhibitor

13.2.3 How Supplied:

Tetrahydrouridine is manufactured by KP Pharmaceutical Technology Inc (Bloomington, IN, USA) and will be purchased from the Cleveland Clinic. THU is supplied as capsules containing 250 mg of THU per capsule, in bottles containing 8 capsules per bottle together with a drying agent. The label on the bottles includes the following information: the (i) name of the drug; (ii) quantity of drug per bottle; (iii) date of manufacture; (iv) recommended storage temperature; (v) manufacturer's lot number.

13.2.4 Storage:

Capsules: Bottles should be tightly closed and stored at refrigerated temperatures 2°-8°C (35-46°F)

13.2.5 Stability:

Capsules: Shelf life stability testing of the intact bottles is on-going.

13.2.6 Route of Administration:

Oral

13.2.7 Method of Administration:

Please see Section **3.2.1**.

13.2.8 Toxicities:

Most reports discuss the use of THU co-administered with a cytidine analogue in order to inhibit the metabolism of that cytidine-based therapeutic, and the side-effects are that of the active agent cytidine analogue therapeutic. The single dose toxicology of THU was evaluated in dogs and Rhesus monkeys at intravenous doses of up to 1000 mg/kg: with the exception of local inflammation at the injection site, no notable toxic effects were attributed to the administration of THU (125). Although there is only limited non-clinical toxicology data with THU alone, the data that exists suggests that THU by itself does not pose a risk for toxicity, even at doses considerably higher than the dose in the planned study.

13.3 PEMBROLIZUMAB

(Refer to FDA-approved package insert for complete product information)

13.3.1 Description:

Pembrolizumab is a humanized monoclonal IgG4 antibody directed against the human cell surface receptor, programmed death-1, (PD-1). Following administration, pembrolizumab binds to PD-1 and blocks the interaction between PD-1 and its ligands.

13.3.2 How Supplied:

Keytruda (pembrolizumab) will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Pembrolizumab is supplied in a single-use 50 mg vial as pembrolizumab lyophilized powder and as a single use 25 mg/mL ready-to-use solution containing 4 mL (100 mg/vial).

13.3.3 Reconstitution/Dose Preparation:

The lyophilized powder is reconstituted with 2.3 mL Sterile Water for injection, USP, and the resultant concentration is 25 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Let contents sit for up to 5 minutes to allow all bubbles to clear. Do not shake.

The ready-to-use solution or reconstituted lyophilized powder must be further diluted prior to administration. It may be diluted in 0.9% Sodium Chloride injection, USP or 5% Dextrose Injection, USP. The final concentration of the dilution solution should be between 1 mg/ml to 10 mg/ml.

13.3.4 Stability/Storage:

The reconstituted lyophilized power is stable for up to 6 hours at room temperature or up to 24 hours at when stored under refrigeration (2-8°C or 36-46°F).

The diluted solution is stable for up to 6 hours at room temperature or up to 24 hours at when stored under refrigeration (2-8°C or 36-46°F).

13.3.5 Administration:

Pembrolizumab will be given at a fixed dose of 200 mg as an intravenous infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.

13.3.6 Toxicities:

This protocol uses commercial supply of pembrolizumab; please refer to current FDA-approved package insert.

14 REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.
2. Rajan A, and Schrump DS. Precision Therapy for Lung Cancer: Tyrosine Kinase Inhibitors and Beyond. *Seminars in thoracic and cardiovascular surgery.* 2015;27(1):36-48.
3. Schrump DS, Carter D, Kelsey CR, Marks LB, and Giaccone G. In: DeVita V, Lawrence TS, and Rosenberg SA eds. *Cancer Principles and Practice of Oncology*. Philadelphia: Lippincott Williams Wilkins; 2011:799-847.
4. Zheng M. Classification and Pathology of Lung Cancer. *Surgical oncology clinics of North America.* 2016;25(3):447-68.
5. Pietanza MC, Zimmerman S, Peters S, and Curran WJ, Jr. Seeking New Approaches to Patients With Small Cell Lung Cancer. *American Society of Clinical Oncology educational book / ASCO American Society of Clinical Oncology Meeting.* 2016;35:e477-82.
6. Siegel RL, Miller KD, and Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68(1):7-30.
7. Huang FL, and Yu SJ. Esophageal cancer: Risk factors, genetic association, and treatment. *Asian journal of surgery.* 2018;41(3):210-5.
8. Domper Arnal MJ, Ferrandez Arenas A, and Lanas Arbeloa A. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. *World journal of gastroenterology.* 2015;21(26):7933-43.
9. Bononi A, Napolitano A, Pass HI, Yang H, and Carbone M. Latest developments in our understanding of the pathogenesis of mesothelioma and the design of targeted therapies. *Expert Rev Respir Med.* 2015;9(5):633-54.
10. Hassan R, Morrow B, Thomas A, Walsh T, Lee MK, Gulsuner S, et al. Inherited predisposition to malignant mesothelioma and overall survival following platinum chemotherapy. *Proc Natl Acad Sci U S A.* 2019.
11. Betti M, Aspesi A, Ferrante D, Sculco M, Righi L, Mirabelli D, et al. Sensitivity to asbestos is increased in patients with mesothelioma and pathogenic germline variants in BAP1 or other DNA repair genes. *Genes, chromosomes & cancer.* 2018;57(11):573-83.
12. Panou V, Gadiraju M, Wolin A, Weipert CM, Skarda E, Husain AN, et al. Frequency of Germline Mutations in Cancer Susceptibility Genes in Malignant Mesothelioma. *J Clin Oncol.* 2018;36(28):2863-71.
13. Odgerel CO, Takahashi K, Sorahan T, Driscoll T, Fitzmaurice C, Yoko OM, et al. Estimation of the global burden of mesothelioma deaths from incomplete national mortality data. *Occupational and environmental medicine.* 2017;74(12):851-8.
14. Furuya S, Chimed-Ochir O, Takahashi K, David A, and Takala J. Global Asbestos Disaster. *International journal of environmental research and public health.* 2018;15(5).
15. de Gooijer CJ, Baas P, and Burgers JA. Current chemotherapy strategies in malignant pleural mesothelioma. *Transl Lung Cancer Res.* 2018;7(5):574-83.
16. Berzenji L, and Van Schil P. Multimodality treatment of malignant pleural mesothelioma. *F1000Res.* 2018;7.
17. Domen A, Berzenji L, Hendriks JMH, Yogeswaran SK, Lauwers P, Van Meerbeeck JP, et al. Extrapleural pneumonectomy: still indicated? *Transl Lung Cancer Res.* 2018;7(5):550-5.

18. Bueno R, and Opitz I. Surgery in Malignant Pleural Mesothelioma. *J Thorac Oncol.* 2018;13(11):1638-54.
19. Topalian SL, Drake CG, and Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell.* 2015;27(4):450-61.
20. Du L, Herbst RS, and Morgensztern D. Immunotherapy in Lung Cancer. *Hematology/oncology clinics of North America.* 2017;31(1):131-41.
21. Kelly RJ. Immunotherapy for Esophageal and Gastric Cancer. *American Society of Clinical Oncology educational book / ASCO American Society of Clinical Oncology Meeting.* 2017;37:292-300.
22. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015;348(6230):124-8.
23. Hellmann M, Rizvi N, Wolchok JD, and Chan TA. Genomic profile, smoking, and response to anti-PD-1 therapy in non-small cell lung carcinoma. *Molecular & cellular oncology.* 2016;3(1):e1048929.
24. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science.* 2016;351(6280):1463-9.
25. Koemans WJ, Chalabi M, van Sandick JW, van Dieren JM, and Kodach LL. Beyond the PD-L1 horizon: In search for a good biomarker to predict success of immunotherapy in gastric and esophageal adenocarcinoma. *Cancer Lett.* 2018;442:279-86.
26. Baylin SB, and Jones PA. Epigenetic Determinants of Cancer. *Cold Spring Harbor perspectives in biology.* 2016.
27. Shukla S, and Meeran SM. Epigenetics of cancer stem cells: Pathways and therapeutics. *Biochim Biophys Acta.* 2014;1840(12):3494-502.
28. Easwaran H, Johnstone SE, Van NL, Ohm J, Mosbruger T, Wang Q, et al. A DNA hypermethylation module for the stem/progenitor cell signature of cancer. *Genome Res.* 2012;22(5):837-49.
29. Jones PA. At the tipping point for epigenetic therapies in cancer. *J Clin Invest.* 2014;124(1):14-6.
30. De Smet C, and Loriot A. DNA hypomethylation and activation of germline-specific genes in cancer. *Adv Exp Med Biol.* 2013;754:149-66.
31. Whitehurst AW. Cause and consequence of cancer/testis antigen activation in cancer. *Annu Rev Pharmacol Toxicol.* 2014;54:251-72.
32. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, and Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* 2005;5(8):615-25.
33. Zendman AJ, Ruiter DJ, and Van Muijen GN. Cancer/testis-associated genes: identification, expression profile, and putative function. *J Cell Physiol.* 2003;194(3):272-88.
34. Caballero OL, and Chen YT. Cancer/testis (CT) antigens: potential targets for immunotherapy. *Cancer Sci.* 2009;100(11):2014-21.
35. Podberezin M, Wen J, and Chang CC. Cancer stem cells: a review of potential clinical applications. *Arch Pathol Lab Med.* 2013;137(8):1111-6.
36. Yamada R, Takahashi A, Torigoe T, Morita R, Tamura Y, Tsukahara T, et al. Preferential expression of cancer/testis genes in cancer stem-like cells: proposal of a novel sub-category, cancer/testis/stem gene. *Tissue Antigens.* 2013;81(6):428-34.

37. Yin B, Zeng Y, Liu G, Wang X, Wang P, and Song Y. MAGE-A3 is highly expressed in a cancer stem cell-like side population of bladder cancer cells. *Int J Clin Exp Pathol.* 2014;7(6):2934-41.
38. Singh V, Sharma P, and Capalash N. DNA methyltransferase-1 inhibitors as epigenetic therapy for cancer. *Curr Cancer Drug Targets.* 2013;13(4):379-99.
39. Patel K, Dickson J, Din S, Macleod K, Jodrell D, and Ramsahoye B. Targeting of 5-aza-2'-deoxycytidine residues by chromatin-associated DNMT1 induces proteasomal degradation of the free enzyme. *Nucleic Acids Res.* 2010;38(13):4313-24.
40. Ghoshal K, Datta J, Majumder S, Bai S, Kutay H, Motiwala T, et al. 5-Aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. *Mol Cell Biol.* 2005;25(11):4727-41.
41. Pathania R, Ramachandran S, Elangovan S, Padia R, Yang P, Cinghu S, et al. DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nat Commun.* 2015;6:6910.
42. Liu F, Killian JK, Yang M, Walker RL, Hong JA, Zhang M, et al. Epigenomic alterations and gene expression profiles in respiratory epithelia exposed to cigarette smoke condensate. *Oncogene.* 2010;29(25):3650-64.
43. Schrump DS. Targeting epigenetic mediators of gene expression in thoracic malignancies. *Biochim Biophys Acta.* 2012;1819(7):836-45.
44. Hong JA, Kang Y, Abdullaev Z, Flanagan PT, Pack SD, Fischette MR, et al. Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells. *Cancer Res.* 2005;65(17):7763-74.
45. Weiser TS, Guo ZS, Ohnmacht GA, Parkhurst ML, Tong-On P, Marincola FM, et al. Sequential 5-Aza-2 deoxycytidine-depsipeptide FR901228 treatment induces apoptosis preferentially in cancer cells and facilitates their recognition by cytolytic T lymphocytes specific for NY-ESO-1. *J Immunother.* 2001;24(2):151-61.
46. Wargo JA, Robbins PF, Li Y, Zhao Y, El-Gamil M, Caragacianu D, et al. Recognition of NY-ESO-1+ tumor cells by engineered lymphocytes is enhanced by improved vector design and epigenetic modulation of tumor antigen expression. *Cancer Immunol Immunother.* 2009;58(3):383-94.
47. Rao M, Chinnasamy N, Hong JA, Zhang Y, Zhang M, Xi S, et al. Inhibition of histone lysine methylation enhances cancer-testis antigen expression in lung cancer cells: implications for adoptive immunotherapy of cancer. *Cancer Res.* 2011;71(12):4192-204.
48. Chinnasamy N, Wargo JA, Yu Z, Rao M, Frankel TL, Riley JP, et al. A TCR Targeting the HLA-A*0201-Restricted Epitope of MAGE-A3 Recognizes Multiple Epitopes of the MAGE-A Antigen Superfamily in Several Types of Cancer. *J Immunol.* 2011;186(2):685-96.
49. Guo ZS, Hong JA, Irvine KR, Chen GA, Spiess PJ, Liu Y, et al. De novo induction of a cancer/testis antigen by 5-aza-2'-deoxycytidine augments adoptive immunotherapy in a murine tumor model. *Cancer Res.* 2006;66(2):1105-13.
50. Schrump DS, Fischette MR, Nguyen DM, Zhao M, Li X, Kunst TF, et al. Phase I study of decitabine-mediated gene expression in patients with cancers involving the lungs, esophagus, or pleura. *Clin Cancer Res.* 2006;12(19):5777-85.

51. Van Groeningen CJ, Leyva A, O'Brien AM, Gall HE, and Pinedo HM. Phase I and pharmacokinetic study of 5-aza-2'-deoxycytidine (NSC 127716) in cancer patients. *Cancer Res.* 1986;46(9):4831-6.
52. Saunthararajah Y, Triozzi P, Rini B, Singh A, Radivoyevitch T, Sekeres M, et al. p53-Independent, normal stem cell sparing epigenetic differentiation therapy for myeloid and other malignancies. *Semin Oncol.* 2012;39(1):97-108.
53. Saunthararajah Y. Key clinical observations after 5-azacytidine and decitabine treatment of myelodysplastic syndromes suggest practical solutions for better outcomes. *Hematology Am Soc Hematol Educ Program.* 2013;2013:511-21.
54. Wongtrakoongate P. Epigenetic therapy of cancer stem and progenitor cells by targeting DNA methylation machineries. *World J Stem Cells.* 2015;7(1):137-48.
55. Eliopoulos N, Cournoyer D, and Momparler RL. Drug resistance to 5-aza-2'-deoxycytidine, 2',2'-difluorodeoxycytidine, and cytosine arabinoside conferred by retroviral-mediated transfer of human cytidine deaminase cDNA into murine cells. *Cancer Chemother Pharmacol.* 1998;42(5):373-8.
56. Chabner BA, Johns DG, Coleman CN, Drake JC, and Evans WH. Purification and properties of cytidine deaminase from normal and leukemic granulocytes. *J Clin Invest.* 1974;53(3):922-31.
57. Wentworth DF, and Wolfenden R. On the interaction of 3,4,5,6-tetrahydrouridine with human liver cytidine deaminase. *Biochemistry.* 1975;14(23):5099-105.
58. Terse P, Engelke K, Chan K, Ling Y, Sharpnack D, Saunthararajah Y, et al. Subchronic oral toxicity study of decitabine in combination with tetrahydrouridine in CD-1 mice. *Int J Toxicol.* 2014;33(2):75-85.
59. Lavelle D, Vaitkus K, Ling Y, Ruiz MA, Mahfouz R, Ng KP, et al. Effects of tetrahydrouridine on pharmacokinetics and pharmacodynamics of oral decitabine. *Blood.* 2012;119(5):1240-7.
60. Saunthararajah Y, Hillery CA, Lavelle D, Molokie R, Dorn L, Bressler L, et al. Effects of 5-aza-2'-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. *Blood.* 2003;102(12):3865-70.
61. Milhem M, Mahmud N, Lavelle D, Araki H, DeSimone J, Saunthararajah Y, et al. Modification of hematopoietic stem cell fate by 5aza 2'deoxycytidine and trichostatin A. *Blood.* 2004;103(11):4102-10.
62. Neil GL, Moxley TE, Kuentzel SL, Manak RC, and Hanka LJ. Enhancement by tetrahydrouridine (NSC-112907) of the oral activity of 5-azacytidine (NSC-102816) in L1210 leukemic mice. *Cancer chemotherapy reports.* 1975;59(3):459-65.
63. Ho DH, Bodey GP, Hall SW, Benjamin RS, Brown NS, Freireich EJ, et al. Clinica, pharmacology of tetrahydrouridine. *J Clin Pharmacol.* 1978;18(5-6):259-65.
64. Kreis W, Woodcock TM, Gordon CS, and Krakoff IH. Tetrahydrouridine: Physiologic disposition and effect upon deamination of cytosine arabinoside in man. *Cancer treatment reports.* 1977;61(7):1347-53.
65. Appleton K, Mackay HJ, Judson I, Plumb JA, McCormick C, Strathdee G, et al. Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. *J Clin Oncol.* 2007;25(29):4603-9.
66. DeSimone J, Heller P, Molokie RE, Hall L, and Zwiers D. Tetrahydrouridine, cytidine analogues, and hemoglobin F. *Am J Hematol.* 1985;18(3):283-8.

67. Riccardi R, Chabner B, Glaubiger DL, Wood J, and Poplack DG. Influence of tetrahydrouridine on the pharmacokinetics of intrathecally administered 1-beta-D-arabinofuranosylcytosine. *Cancer Res.* 1982;42(5):1736-9.
68. Kreis W, Budman DR, Chan K, Allen SL, Schulman P, Lichtman S, et al. Therapy of refractory/relapsed acute leukemia with cytosine arabinoside plus tetrahydrouridine (an inhibitor of cytidine deaminase)--a pilot study. *Leukemia.* 1991;5(11):991-8.
69. Dover GJ, Charache S, Boyer SH, Vogelsang G, and Moyer M. 5-Azacytidine increases HbF production and reduces anemia in sickle cell disease: dose-response analysis of subcutaneous and oral dosage regimens. *Blood.* 1985;66(3):527-32.
70. Wong PP, Currie VE, Mackey RW, Krakoff IH, Tan CT, Burchenal JH, et al. Phase I evaluation of tetrahydrouridine combined with cytosine arabinoside. *Cancer Treat Rep.* 1979;63(8):1245-9.
71. Beumer JH, Parise RA, Newman EM, Doroshow JH, Synold TW, Lenz HJ, et al. Concentrations of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine (FdCyd) and its cytotoxic metabolites in plasma of patients treated with FdCyd and tetrahydrouridine (THU). *Cancer Chemother Pharmacol.* 2008;62(2):363-8.
72. Marsh JH, Kreis W, Barile B, Akerman S, Schulman P, Allen SL, et al. Therapy of refractory/relapsed acute myeloid leukemia and blast crisis of chronic myeloid leukemia with the combination of cytosine arabinoside, tetrahydrouridine, and carboplatin. *Cancer Chemother Pharmacol.* 1993;31(6):481-4.
73. Newman EM, Morgan RJ, Kummar S, Beumer JH, Blanchard MS, Ruel C, et al. A phase I, pharmacokinetic, and pharmacodynamic evaluation of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine, administered with tetrahydrouridine. *Cancer Chemother Pharmacol.* 2015.
74. Olivieri NF, Saunthararajah Y, Thayalasuthan V, Kwiatkowski J, Ware RE, Kuypers FA, et al. A pilot study of subcutaneous decitabine in beta-thalassemia intermedia. *Blood.* 2011;118(10):2708-11.
75. Saunthararajah Y, Sekeres M, Advani A, Mahfouz R, Durkin L, Radivoyevitch T, et al. Evaluation of noncytotoxic DNMT1-depleting therapy in patients with myelodysplastic syndromes. *J Clin Invest.* 2015.
76. Morikawa H, and Sakaguchi S. Genetic and epigenetic basis of Treg cell development and function: from a FoxP3-centered view to an epigenome-defined view of natural Treg cells. *Immunol Rev.* 2014;259(1):192-205.
77. Kehrmann J, Tatura R, Zeschnigk M, Probst-Kepper M, Geffers R, Steinmann J, et al. Impact of 5-aza-2'-deoxycytidine and epigallocatechin-3-gallate for induction of human regulatory T cells. *Immunology.* 2014;142(3):384-95.
78. Wu CJ, Yang CY, Chen YH, Chen CM, Chen LC, and Kuo ML. The DNA methylation inhibitor 5-azacytidine increases regulatory T cells and alleviates airway inflammation in ovalbumin-sensitized mice. *Int Arch Allergy Immunol.* 2013;160(4):356-64.
79. Singer BD, Mock JR, Aggarwal NR, Garibaldi BT, Sidhaye VK, Florez MA, et al. Regulatory T Cell DNA Methyltransferase Inhibition Accelerates Resolution of Lung Inflammation. *Am J Respir Cell Mol Biol.* 2014.
80. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc Natl Acad Sci U S A.* 2014;111(32):11774-9.

81. Brahmer JR, and Pardoll DM. Immune checkpoint inhibitors: making immunotherapy a reality for the treatment of lung cancer. *Cancer immunology research.* 2013;1(2):85-91.
82. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012;366(26):2455-65.
83. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366(26):2443-54.
84. Marrone KA, and Brahmer JR. Immune Checkpoint Therapy in Non-Small Cell Lung Cancer. *Cancer J.* 2016;22(2):81-91.
85. Wrangle J, Wang W, Koch A, Easwaran H, Mohammad HP, Vendetti F, et al. Alterations of immune response of Non-Small Cell Lung Cancer with Azacytidine. *Oncotarget.* 2013;4(11):2067-79.
86. Li H, Chiappinelli KB, Guzzetta AA, Easwaran H, Yen RW, Vatapalli R, et al. Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. *Oncotarget.* 2014;5(3):587-98.
87. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, et al. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell.* 2015;162(5):974-86.
88. Roulois D, Loo Yau H, Singhania R, Wang Y, Danesh A, Shen SY, et al. DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. *Cell.* 2015;162(5):961-73.
89. Ehrlich P. Über den jetzigen Stand der Chemotherapie. *Berichte der deutschen chemischen Gesellschaft.* 1909;42(1):17-47.
90. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res.* 1970;13:1-27.
91. Muenst S, Laubli H, Soysal SD, Zippelius A, Tzankov A, and Hoeller S. The immune system and cancer evasion strategies: therapeutic concepts. *J Intern Med.* 2016.
92. Pardoll D. Cancer and the Immune System: Basic Concepts and Targets for Intervention. *Semin Oncol.* 2015;42(4):523-38.
93. Sul J, Blumenthal GM, Jiang X, He K, Keegan P, and Pazdur R. FDA Approval Summary: Pembrolizumab for the Treatment of Patients With Metastatic Non-Small Cell Lung Cancer Whose Tumors Express Programmed Death-Ligand 1. *Oncologist.* 2016;21(5):643-50.
94. Gettinger SN, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, et al. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol.* 2015;33(18):2004-12.
95. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2016;375(19):1823-33.
96. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial. *JAMA Oncol.* 2018;4(5):e180013.

97. Mehta R, Shah A, and Almhanna K. Pembrolizumab for the treatment of patients with recurrent locally advanced or metastatic gastric or gastroesophageal junction cancer: an evidence-based review of place in therapy. *OncoTargets and therapy.* 2018;11:6525-37.
98. Shitara K, Ozguroglu M, Bang YJ, Di Bartolomeo M, Mandala M, Ryu MH, et al. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. *Lancet.* 2018;392(10142):123-33.
99. Alley EW, Lopez J, Santoro A, Morosky A, Saraf S, Piperdi B, et al. Clinical safety and activity of pembrolizumab in patients with malignant pleural mesothelioma (KEYNOTE-028): preliminary results from a non-randomised, open-label, phase 1b trial. *Lancet Oncol.* 2017;18(5):623-30.
100. Metaxas Y, Rivalland G, Mauti LA, Klingbiel D, Kao S, Schmid S, et al. Pembrolizumab as Palliative Immunotherapy in Malignant Pleural Mesothelioma. *J Thorac Oncol.* 2018;13(11):1784-91.
101. Popat S C-FA, Polydoropoulou V, et al. A multicenter randomized phase III trial comparing pembrolizumab (P) vs single agent chemotherapy (CT) for advanced pre-treated malignant pleural mesothelioma (MPM)—results from the European Thoracic Oncology Platform (ETOP 9-15) PROMISE-meso trial. ESMO Congress. Abstract LBA91. 2019.
102. Chu GJ, van Zandwijk N, and Rasko JEJ. The Immune Microenvironment in Mesothelioma: Mechanisms of Resistance to Immunotherapy. *Front Oncol.* 2019;9:1366.
103. Malhotra J, Jabbour SK, and Aisner J. Current state of immunotherapy for non-small cell lung cancer. *Transl Lung Cancer Res.* 2017;6(2):196-211.
104. Lee CK, Man J, Lord S, Links M, Gebski V, Mok T, et al. Checkpoint Inhibitors in Metastatic EGFR-Mutated Non-Small Cell Lung Cancer-A Meta-Analysis. *J Thorac Oncol.* 2017;12(2):403-7.
105. Munari E, Zamboni G, Marconi M, Sommaggio M, Brunelli M, Martignoni G, et al. PD-L1 expression heterogeneity in non-small cell lung cancer: evaluation of small biopsies reliability. *Oncotarget.* 2017;8(52):90123-31.
106. Melosky B, Chu Q, Juergens R, Leighl N, McLeod D, and Hirsh V. Pointed Progress in Second-Line Advanced Non-Small-Cell Lung Cancer: The Rapidly Evolving Field of Checkpoint Inhibition. *J Clin Oncol.* 2016;34(14):1676-88.
107. Takada K, Toyokawa G, Shoji F, Okamoto T, and Maehara Y. The Significance of the PD-L1 Expression in Non-Small-Cell Lung Cancer: Trenchant Double Swords as Predictive and Prognostic Markers. *Clin Lung Cancer.* 2017.
108. Molokie R, Lavelle D, Gowhari M, Pacini M, Krauz L, Hassan J, et al. Oral tetrahydrouridine and decitabine for non-cytotoxic epigenetic gene regulation in sickle cell disease: A randomized phase 1 study. *PLoS medicine.* 2017;14(9):e1002382.
109. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45(2):228-47.
110. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol.* 2008;26(7):1148-59.

111. Yagi T, Baba Y, Ishimoto T, Iwatsuki M, Miyamoto Y, Yoshida N, et al. PD-L1 Expression, Tumor-infiltrating Lymphocytes, and Clinical Outcome in Patients With Surgically Resected Esophageal Cancer. *Annals of surgery.* 2017.
112. Jin Z, and Yoon HH. The promise of PD-1 inhibitors in gastro-esophageal cancers: microsatellite instability vs. PD-L1. *Journal of gastrointestinal oncology.* 2016;7(5):771-88.
113. Reck M. Pembrolizumab as first-line therapy for metastatic non-small-cell lung cancer. *Immunotherapy.* 2017.
114. De Mello RA, Lordick F, Muro K, and Janjigian YY. Current and Future Aspects of Immunotherapy for Esophageal and Gastric Malignancies. *American Society of Clinical Oncology educational book / ASCO American Society of Clinical Oncology Meeting.* 2019;39:237-47.
115. Chapel DB, Stewart R, Furtado LV, Husain AN, Krausz T, and Deftereos G. Tumor PD-L1 expression in malignant pleural and peritoneal mesothelioma by Dako PD-L1 22C3 pharmDx and Dako PD-L1 28-8 pharmDx assays. *Human pathology.* 2019;87:11-7.
116. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials.* 1989;10(1):1-10.
117. Chuncharunee S, Archararit N, Ungkanont A, Jootar S, Angchaisuksiri P, Bunyaratavej A, et al. Etiology and incidence of thrombotic and hemorrhagic disorders in Thai patients with extreme thrombocytosis. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet.* 2000;83 Suppl 1:S95-100.
118. Hathirat P, Mahaphan W, Chuansumrit A, Pintadit P, Sasanakul W, and Isarangkura P. Platelet counts in thalassemic children before and after splenectomy. *The Southeast Asian journal of tropical medicine and public health.* 1993;24 Suppl 1:213-5.
119. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, et al. Splenectomy and thrombosis: the case of thalassemia intermedia. *Journal of thrombosis and haemostasis : JTH.* 2010;8(10):2152-8.
120. van der Bom JG, Heckbert SR, Lumley T, Holmes CE, Cushman M, Folsom AR, et al. Platelet count and the risk for thrombosis and death in the elderly. *Journal of thrombosis and haemostasis : JTH.* 2009;7(3):399-405.
121. Tefferi A. Platelet count in essential thrombocythemia: the more the better? *Blood.* 2008;112(8):3526; author reply -7.
122. Habib A, Kunzelmann C, Shamseddeen W, Zobairi F, Freyssinet JM, and Taher A. Elevated levels of circulating procoagulant microparticles in patients with beta-thalassemia intermedia. *Haematologica.* 2008;93(6):941-2.
123. Gladwin MT, and Kato GJ. Hemolysis-associated hypercoagulability in sickle cell disease: the plot (and blood) thickens! *Haematologica.* 2008;93(1):1-3.
124. Ataga KI, Moore CG, Hillery CA, Jones S, Whinna HC, Strayhorn D, et al. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. *Haematologica.* 2008;93(1):20-6.
125. Goldenthal EI, Cookson KM, Geil RG, and Wazeter FX. Preclinical toxicologic evaluation of tetrahydrouridine (NSC-112907) in beagle dogs and rhesus monkeys. *Cancer chemotherapy reports Part 3.* 1974;5(1):15-6.
126. Scanlan MJ, Simpson AJ, and Old LJ. The cancer/testis genes: review, standardization, and commentary. *Cancer Immun.* 2004;4:1.

15 APPENDICES AND SUPPLEMENTARY TABLES

15.1 SUPPLEMENTARY TABLES

15.1.1 Supplementary Table 1: Cancer/Testis Genes and Their Chromosomal Locations (Partial List)

Gene family	Family members	CT identifier	Chromosomal localization
MAGEA	MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA5, MAGEA6, MAGEA8, MAGEA9, MAGEA10, MAGEA11, MAGEA12	CT1	Xq28
BAGE	BAGE	CT2	21p11.1
MAGEB	MAGEB1, MAGEB2, MAGEB3, MAGEB4, MAGEB5, MAGEB6	CT3	Xp21.3
GAGE	GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GAGE7, GAGE8	CT4	Xp11.23
SSX	SSX1, SSX2, SSX3, SSX4	CT5	Xp11.23-p11.22.
NY-ESO-1	CTAG1B, CTAG2	CT6	Xq28
MAGEC1	MAGEC1	CT7	Xq26
SYCP1	SYCP1	CT8	1p12-p13
BRDT	BRDT	CT9	1p22.1
MAGEC2	MAGEC2	CT10	Xq27
SPANX	SPANXA1, SPANXB1, SPANXC, SPANXD	CT11	Xq27.1
XAGE	XAGE1	CT12	Xp11.22
HAGE	DDX43	CT13	6q12-q13.
SAGE	SAGE1	CT14	Xq26.
ADAM2	ADAM2	CT15	8p11.2.
PAGE-5	PAGE5	CT16.1	Xp11.23
LIPI	LIPI	CT17	21q11.2
NA88A pseudogene	VENTXP1	CT18	Xp21.3.
IL13RA	IL13RA2	CT19	Xq13.1-q28
TSP50	TSP50	CT20	3p12–14
CTAGE-1	CTAGE1	CT21.1	18p11.2
SPA17	SPA17	CT22	11q24.2
ACRBP	ACRBP	CT23	12p12-p13.
CSAG	CSAG1, CSAG2	CT24	Xq28.
MMA1	DSCR8	CT25	21q22.2.
CAGE/DDX53	DDX53	CT26	Xp22.11
BORIS	CTCFL	CT27	20q13.31
HOM-TES-85	LUZP4	CT28	Xq23
AF15q14	CASC5	CT29	15q14
HCA661	TFDP3	CT30	Xq26.2
JARID1B	JARID1B	CT31	1q32.1.
LDHC	LDHC	CT32	11p15.3-p15.5

Gene family	Family members	CT identifier	Chromosomal localization
MORC	MORC1	CT33	3q13
SGY-1	DKKL1	CT34	19q13.33
SPO11	SPO11	CT35	20q13.2-q13.3
TPX1	CRISP2	CT36	6p21-qter
NY-SAR-35	FMR1NB	CT37	Xq27.3-q28.
FTHL17	FTHL17	CT38	Xp21
NXF2	NXF2	CT39	Xq22.1
TAF7L	TAF7L	CT40	Xq22.1
TDRD1	TDRD1	CT41	10q25.3.
TEX15	TEX15	CT42	8p12
FATE	FATE1	CT43	Xq28
TPTE	TPTE	CT44	21p11
CT45	CT45-1, CT45-2, CT45-3, CT45-4, CT45-5, CT45-6	CT45	Xq26.3
HORMAD1	HORMAD1	CT46	1q21.2.
CT47	CT47-1, CT47-2, CT47-3, CT47-4, CT47-5, CT47-6, CT47-7, CT47-8, CT47-9, CT47-10, CT47-11, CT47-12	CT47	Xq24
SLCO6A1	SLCO6A1	CT48	5q21.1
TAG	TAG	CT49	Na
LEMD1	LEMD1	CT50	1q32.1
HSPB9	HSPB9	CT51	1p36.2-p35
CCDC110	CCDC110	CT52	4q35.1
ZNF165	ZNF165	CT53	6p21.3
SPACA3	SPACA3	CT54	17q11.2
CXorf48	CXorf48	CT55	Xq26.3
THEG	THEG	CT56	19pter-p13
ACTL8	ACTL8	CT57	1p36.2-p35
NLRP4	NLRP4	CT58	1p36.2-p35
COX6B2	COX6B2	CT59	19q13.42
LOC348120	LOC348120	CT60	15q11.2
CCDC33	CCDC33	CT61	15q24.1
LOC196993	LOC196993	CT62	15q23
PASD1	PASD1	CT63	Xq28
CT64	CT64/BX103208	CT64	3q26.1.
TULP2	TULP2	CT65	19q13.1.
CT66	CT66/AA884595	CT66	7q11.22.
KLKBL4	KLKBL4	CT67	16q21
RBM46	RBM46	CT68	4q32.1
CT69	CT69/BC040308	CT69	6q23.2
CT70	CT70/BI818097	CT70	Na

Gene family	Family members	CT identifier	Chromosomal localization
SPINLW1	SPINLW1	CT71	20q12-q13.2
TSSK6	TSSK6	CT72	19p13.11
ADAM29	ADAM29	CT73	4q34.
CCDC36	CCDC36	CT74	3p21.31
LOC440934	LOC440934	CT75	2q36.1
SYCE1	SYCE1	CT76	10q26.3
CPXCR1	CPXCR1	CT77	Xq21.3
TSPY1	TSPY1	CT78	Yp11.2
TSGA10	TSGA10	CT79	2q11.2.
PIWIL2	PIWIL2	CT80	8p21.3
ARMC3	ARMC3	CT81	10p12.31
AKAP3	AKAP3	CT82	12p13.3.
CXorf61	Cxorf61	CT83	Xq23
PBK	PBK	CT84	8p21.2
C21orf99	C21orf99	CT85	21q11.2
OIP5	OIP5	CT86	15q15.1
CEP290	CEP290	CT87	12q21.32
CABYR	CABYR	CT88	18q11.2
SPAG9	SPAG9	CT89	17q21.33.
MPHOSPH1	MPHOSPH1	CT90	10q23.31
ROPN1	ROPN1	CT91	3q21.1.
PLAC1	PLAC1	CT92	Xq26
CALR3	CALR3	CT93	19p13.1
PRM	PRM1/PRM2	CT94.1	16p13.2
CAGE1	CAGE1	CT95	6p24.3
CT96	TTK	CT96	6q13-q21
LY6K	LY6K	CT97	8q24.3
IMP-3	IMP-3	CT98	7p11.
AKAP4	AKAP4	CT99	Xp11.2
DPPA2	DPPA2	CT100	3q13.13
KIAA0100/MLAA-22	KIAA0100	CT 101	7q11.2
TCC52	TCC52	CT102	9p13.3
SEMG1	SEMG1	CT103	20q12-q13.2
POTE	POTE21, POTE2, POTE8, POTE14, POTE15, POTE18, POTE22	CT104	2q21.1
FLJ36144/MAD-CT2	MAD-CT2	CT105	15q11.2.
NUF2/CDCA1	CDCA1	CT106	1q23
RHOXF2/PEPP2	PEPP2	CT107	Xq24

Gene family	Family members	CT identifier	Chromosomal localization
OTOA	OTOA	CT108	16p12.2
CCDC62	CCDC62	CT109	12q24.31
GPATCH2	GPATCH2	CT110	1q41

Na: not available

15.1.2 Supplementary Table 2: Frequency of CT Gene Expression in Various Human Cancers as Determined by Semi-Quantitative RT-PCR

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Bladd	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
MAGEA1/CT1.1	22	-	18	2	53	29	28	80	0	49	48	28	-	15	0	14
BAGE1/CT2.1	15	-	10	0	-	-	8	-	0	4	26	15	-	0	0	6
MAGEB1/CT3.1	0	0	17	0	-	0	0	-	0	14	22	-	-	0	0	9
GAGE/CT4.1	12	-	9	0	-	-	19	38[b]	1	19	28	31	-	10	0	25
SSX2/CT5.2	44	6	7	12	-	-	35	9[b]	36	16	35	-	-	40	5	50
NY-ESO-1/CT6.1	80	0	30	0	-	0	-	29	0	17	34	25	0	25	9	0
MAGEC1/CT7.1	44	-	30	10	-	-	36	-	-	33	70	-	-	-	-	60
SYCP1/CT8	-	47	20	0	-	7	-	28[b]	0	7	14	0	-	0	8	0
BRDT/CT9	0	-	0	0	8	-	8	-	-	25	0	-	-	-	0	-
MAGEE1/CT10	44	-	38	0	-	-	36	-	-	24	50	-	-	-	-	0
SPANXC/CT11.3	9	-	25	22	0	-	-	-	-	33	70	-	0	-	-	-
XAGE-1a/CT12.1a	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	22
HAGE/CT13	24	37	5	31	27	-	-	20	9	32	17	-	-	22	6	20
SAGE/CT14	12	0	5	0	20	-	17	-	4	22	4	-	-	0	5	5
ADAM2/CT15	-	-	0	0	-	-	-	-	-	0	0	0	-	-	12	-
PAGE-5/CT16	-	-	5	11	-	-	-	-	-	39	22	0	-	-	44	-
LIP1/CT17	-	-	5	0	-	-	-	-	-	0	0	0	-	-	25	-

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
NA88/CT18	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-
TSP50/CT20	-	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-
CTAGE-1/CT21.1	-	-	-	-	-	-	-	-	35	-	-	-	-	-	-	-
SPA17/CT22	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-
OYTES1/CT23	28	-	40	15	-	0	-	40	-	20	-	-	-	-	0	-
MMA1a/CT25.1a	-	-	0	0	0	-	-	-	-	40	26	-	0	-	-	18
CAGE/CT26	-	-	-	-	-	89	-	-	-	100	-	-	-	-	-	-
HOMTES85/CT28	-	35	0	10	-	-	-	19	-	28	36	32	-	0	-	-
D40/CT29	-	20	-	13	-	0	-	-	-	41	-	36	27	-	-	-
HCA661/CT30	0	-	-	-	-	0	0	29	-	-	20	-	-	-	-	-
PLU-1/CT31	-	-	86	-	-	-	-	-	-	-	-	-	-	-	-	-
LDHC/CT32	-	-	35	15	-	-	-	-	-	47	44	42	-	37	57	-
MORC/CT33	-	-	0	0	-	-	-	-	-	18	18	14	-	0	0	-
SGY-1/CT34	-	-	20	0	-	-	-	-	-	12	25	57	-	12	0	-
SPO11/CT35	-	-	0	0	-	-	-	-	-	0	6	0	-	0	0	-
TPX1/CT36	-	-	15	0	-	-	-	-	-	-	6	14	-	37	14	-
NYSAR35/CT37	42	-	23	0	8	-	-	-	-	17	6	8	-	-	0	8
FTHL17/CT38	22	-	14	0	0	-	10	-	0	25	0	-	-	0	0	0
NXF2/CT39	19	-	0	11	12	-	5	-	0	15	55	-	-	14	0	27

CT Family (Member)	Frequency (%) of Expression in Tumor Type															
	Blad	Brn	Brst	Col	Eso	Gas	H/N	Liver	Leuk/Lymph	Lung (NSCLC)	Mel	Ov	Pancr	Pros	Renal	Sarc
TAF7L/CT40	10	-	0	0	0	-	10	-	0	9	21	-	-	0	0	12
TDRD1/CT41.1	28	-	37	0	10	-	22	-	5	5	0	-	-	38	0	0
TEX15/CT42	21	-	0	0	20	-	11	-	0	21	27	-	-	12	33	28
FATE/CT43	-	-	-	21	-	7	-	66	-	0	-	-	-	-	-	-
TPTE/CT44	-	-	-	0	-	0	-	39	-	36	-	-	-	-	-	-

[a]Abbreviations: Blad, bladder; Brn, brain; Brst, breast; Col, colon; Gas, gastric; H/N, head and neck; Leuk, leukemia; Lymph, lymphoma, NSCLC, non-small cell lung carcinoma; Mel, melanoma; Ov, ovarian; Pancr, pancreatic; Pros, prostate; Sarc, sarcoma.

Reprinted from ref (126)

15.2 APPENDICES

15.2.1 Appendix 1: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

15.2.2 Appendix 2: Sample Medication Self-Administration Record and Patient Diary

NAME **DATE** **April 2015**

Sun	Mon	Tue	Wed	Thu	Fri	Sat
			1	2	3	4
	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____
5	6	7	8	9	10	11
_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____
12	13	14	15	16	17	18
_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____
19	20	21	22	23	24	25
_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____	_____ _____
26	27	28	29	30		
_____ _____	_____ _____	_____ _____				

Take Medications: DAC and THU once per day on assigned days. THU should be taken approximately 2 hours before or 2 hours after a meal. DAC should be taken 1 hour after THU.

GI	Dates	Treated at Home	Treated in doctor's office	Hospitalized	Comments
diarrhea					
nausea					
vomiting					
mouth sores					
neuropathy					
tingling hands and feet					
sensitivity to cold					
general					
Fever					
Infection					
swelling arms/legs					
fatigue					
Injection site					
redness/swelling					
pain					
shortness of breath					
bruising/bumps					

swollen glands near injection site					
rash					
Respiratory					
sore throat					
runny nose					
cough					
Other:					

15.2.3 Appendix 3: Study Calendar

Screening to Completion of DAC-THU/Pembrolizumab

Procedure	Screening Within 4 weeks prior to enrollment	Courses 1 and Course N+1 [Course 2 Onwards]* * Note: Couse N+1 administered only for patients who exhibit stable disease or disease regression					End of Treatment Visit[7]
		Baseline Within 10 days prior to start of DAC-THU	During Treatment		Within 3 days prior to completion of DAC-THU	At the end of course completion	
			Day 1 of DAC-THU	Day 2 of DAC-THU			
History and PE	X	X				X	X
Vital signs	X	X				X	X
Performance Score	X	X				X	
NCI LP Confirmation of Dx[1]	X						
CBC with differential; Acute Care Panel, Hepatic Panel (Sections **2.2** and **3.4.1**)	X	X	Twice weekly during Course 1, and then weekly unless clinically indicated			X	X
PT, PTT	X	X	Twice weekly during Course 1, and then weekly unless clinically indicated			X	
Thyroid Function Tests						X	
Albumin, Ca^{2+} total, Mg^{2+} total, Phosphorus, LD, Total Protein, Total CK, Uric Acid		X	Twice weekly during Course 1, and then weekly unless clinically indicated			X	X
Fetal Hemoglobin		X					
Urinalysis		X[4]				X[4]	
Anti HIV, anti HCV, HBsAg, anti-HAV IgM, and if needed, HCV RNA	X						
Urine or serum HCG in women of childbearing potential	X[3]	X[3]					
T, B, NK cell subsets		X[9]				X	

Procedure	Screening Within 4 weeks prior to enrollment	Courses 1 and Course N+1 [Course 2 Onwards]* * Note: Couse N+1 administered only for patients who exhibit stable disease or disease regression					End of Treatment Visit[7]
		Baseline Within 10 days prior to start of DAC-THU	During Treatment		Within 3 days prior to completion of DAC-THU	At the end of course completion	
			Day 1 of DAC-THU	Day 2 of DAC-THU			
HLA class I and class II analysis of PBMC	X[1]						
EKG	X						
Echocardiogram	X						
PFTs	X	X					
ABGs (to be drawn if pulse oximetry <90% on room air)	X	X					
Lupus Anticoagulant and ANA	X						
Radiological Assessments	X[2]					X[2]	
DAC-THU			Once a day on days according to dose level				
Tumor sample for research[8]		X				X	
Correlative Research Studies		X[5]	X[5]		X[5]		
PK/PD			X[6]	X[6]			
Adverse Events[10]			X →	→	→	→	
Concomitant Medications[10]		X	→	→	→	→	

1. May be obtained any time prior to initiation of study therapy. Does not need to be repeated if already done at NCI.
2. Radiological Assessments: Pre-enrollment screening assessment: X-rays, contrast enhanced CT scans of chest, abdomen, pelvis, as well as brain MR (no gadolinium contrast), and fused PET-CT scans to evaluate the status of disease. (All imaging must be obtained within 4 weeks prior to treatment.) At the end of each course completion assessment: CT scan (C/A/P) (with standard oral/IV contrast), fused PET/CT scan and brain MR without gadolinium contrast (or CT scan if MR contraindicated).
3. Must be completed within 3 days prior to enrollment or 48 hours prior to start of study drugs.
4. Urinalysis and culture if indicated.

5. See **Appendix 4** for details of Research Labs timepoints and collection details. Note: Tubes/media may be adjusted at the time of collection based upon materials available if approved by the PI/laboratory investigator.
6. See **Appendix 4** for PK sampling timepoints and collection details. **Note:** PK samples will only be drawn on Day 1 and Day 2 of Cycle 1.
7. End of treatment visit will occur approximately 30 days after the last dose of study drug. If the patient cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs (specify as needed) from a local physician or laboratory. If this is not possible, patients may be assessed by telephone/or other NIH approved remote platforms for symptoms.
8. Tumor biopsy will be performed at baseline if screening biopsy was not performed and after one course of therapy (Week 10 +/- one week).
9. Every three weeks during therapy.
10. Adverse events and concomitant medications will be assessed weekly during the dose finding portion of the trial and every three weeks prior to the administration of pembrolizumab during the expansion portion of the trial.

15.2.4 Appendix 4: Pharmacokinetic Samples and Research Bloods

15.2.4.1 Course 1 Cycle 1

TEL Research Sample # (2 red top tubes, 2 lavender top tubes)	**Immune Subset & CTC Samples # (2 CPT tubes, and 2 lavender top tubes)**	**PK Sample # (1 6 mL green top tube)**	**Hour (pre dose = within 1 hour prior to THU)**	**Target Time**	**Actual Time**	**Initials**
Baseline (post - enrollment)						
R1	**T1**	n/a	n/a	n/a	n/a	n/a
Day 1 DAC-THU						
R2 (within 1 hour prior to THU)	**T2** (within 1 hour prior to THU)	**PK 1**	pre dose			
n/a	n/a	**PK 2**	0.5h post DAC dose			
n/a	n/a	**PK 3**	1h post DAC dose			
n/a	n/a	**PK 4**	1.5h post DAC dose			
n/a	n/a	**PK 5**	2h post DAC dose			
n/a	n/a	**PK 6**	3h post DAC dose			
n/a	n/a	**PK 7**	4h post DAC dose			
n/a	n/a	**PK 8**	8h post DAC dose			
Day 2 DAC-THU						
n/a	n/a	**PK 9**	pre dose			
n/a	n/a	**PK 10**	0.5h post DAC dose			
n/a	n/a	**PK 11**	1h post DAC dose			
n/a	n/a	**PK 12**	1.5h post DAC dose			
n/a	n/a	**PK 13**	2h post DAC dose			
n/a	n/a	**PK 14**	3h post DAC dose			
n/a	n/a	**PK 15**	4h post DAC dose			
n/a	n/a	**PK 16**	8h post DAC dose			

After completion of each course of DAC-THU						
R3	**T3**	n/a	n/a	n/a	n/a	n/a

15.2.4.2 Course N+1 (Course 2 Onwards)*****

TEL Research Sample # (2 red top tubes, 2 lavender top tubes)	**Immune Subset & CTC Samples # (2 CPT tubes, and 2 lavender top tubes)**	**PK Sample # (1 6 mL green top tube)**	**Hour (pre dose = within 1 hour prior to THU)**	**Target Time**	**Actual Time**	**Initials**
Day 1 DAC-THU						
R4* (within 1 hour prior to THU)	**T4*** (within 1 hour prior to THU)	n/a	pre dose			
After completion of each course of DAC-THU						
R5*	**T5***	n/a	n/a	n/a	n/a	n/a

***Note:** Labels should list the specific collection number as appropriate; indicated collection numbers are for Course # (e.g., Course 2) draws.

15.2.4.3 **Instructions**

PK Studies (PK)
Collect 6 mL blood in 1 green top tube. Tubes should then be placed on crushed ice, and samples should be centrifuged for 15 minutes at approximately 1000 x g at 0-5 °C within 2 hours after collection. The plasma should be transferred to separate pre-labeled screw-capped polypropylene transfer tubes and stored at -80 °C in the Thoracic Epigenetics Lab (TEL) until batched and later sent to the Figg Lab on dry ice for analysis.

Note: All samples are to be placed on wet ice and transported to the Thoracic Epigenetics Lab (TEL) within one hour of draw.

Research Labs (R)

Samples will be collected in 2 red top (10 mL each) tubes, and 2 lavender top tubes (EDTA; 10 mL each) for focused methylation analysis and immune response to treatment (see Section **5.1.2**).

Note: All samples are to be placed on wet ice and transported to the Thoracic Epigenetics Lab (TEL) within one hour of draw.

Research Labs will be collected at the following timepoints (see also **Appendix 3: Study Calendar**):

- Baseline (once), see Section **3.4.1**;
- Each Course
    - Prior to DAC-THU initiation,
      and at

- DAC-THU completion.

Immune Subset and Circulating Tumor Cell Analysis (T)

Samples will be collected in 2 CPT citrate tubes (BD; 8 mL) for immune subset analysis (see Section **5.1.3**) and 2 lavender top tubes (10 mL) for CTCs analysis (Section **5.1.4**). The Trepel Lab will be notified to pick up the samples (as indicated in Section **5.2.4**) as soon as the blood is drawn for sample processing and analysis.

Samples will be drawn at the following timepoints (see also **Appendix 3**):

- Each course
  - Prior to DAC-THU initiation,
    and at
  - DAC-THU completion.

Labeling (PK and Research Labs)

Prior to the infusion, the PK /research lab sheet and labels will be placed in the patient's room and a separate set of labels will be given to the Thoracic Epigenetics Lab staff. The labels will list the patient identifiers as well as the Cycle and draw number as appropriate. As the RN (or designee) draws the bloods, the RN (or designee) will place a label on the tube and write the sample reference # on the label on the tube. When the Thoracic Epigenetics Lab personnel or fellow processes the sample, the TEL personnel will label the storage tube with the label and will transcribe the corresponding sample reference # on the label on the tube.

Tracking (PK and Research Labs)

Upon completion of the PK/Research sample draws, the research nurse will make 3 copies of the PK sheet. The original and one copy should be placed in the research binder. 2 copies should be given to the TEL personnel. TEL will retain one copy for their records and will send the other copy with the PK samples to the Figg Lab. Note: Prior to sending the PK sheets to the Figg Lab, the patient name should be crossed out.