STUDY NUMBER: CASE 3516

Protocol Date: August 10, 2018

OFFICIAL TITLE: **Phase II Evaluation of Nivolumab, an Immune Checkpoint Inhibitor, alone or in combination with Oral Decitabine/Tetrahydrouridine as Second Line Therapy for Non-Small Cell Lung Cancer**

Abbreviated Title: **Pharmacologically Rational EpigenetiC Immunotherapy for SEcond line Therapy in patients with Non-Small Cell Lung Cancer: PRECISE trial**

NCI Number: NCT02664181

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SUPPORT/FUNDING: Cleveland Clinic Taussig Cancer Institute, National Cancer Institute, American Society of Clinical Oncology

SUPPLIED AGENT***(S):*** Oral THU-decitabine, Nivolumab

IND #: [redacted]

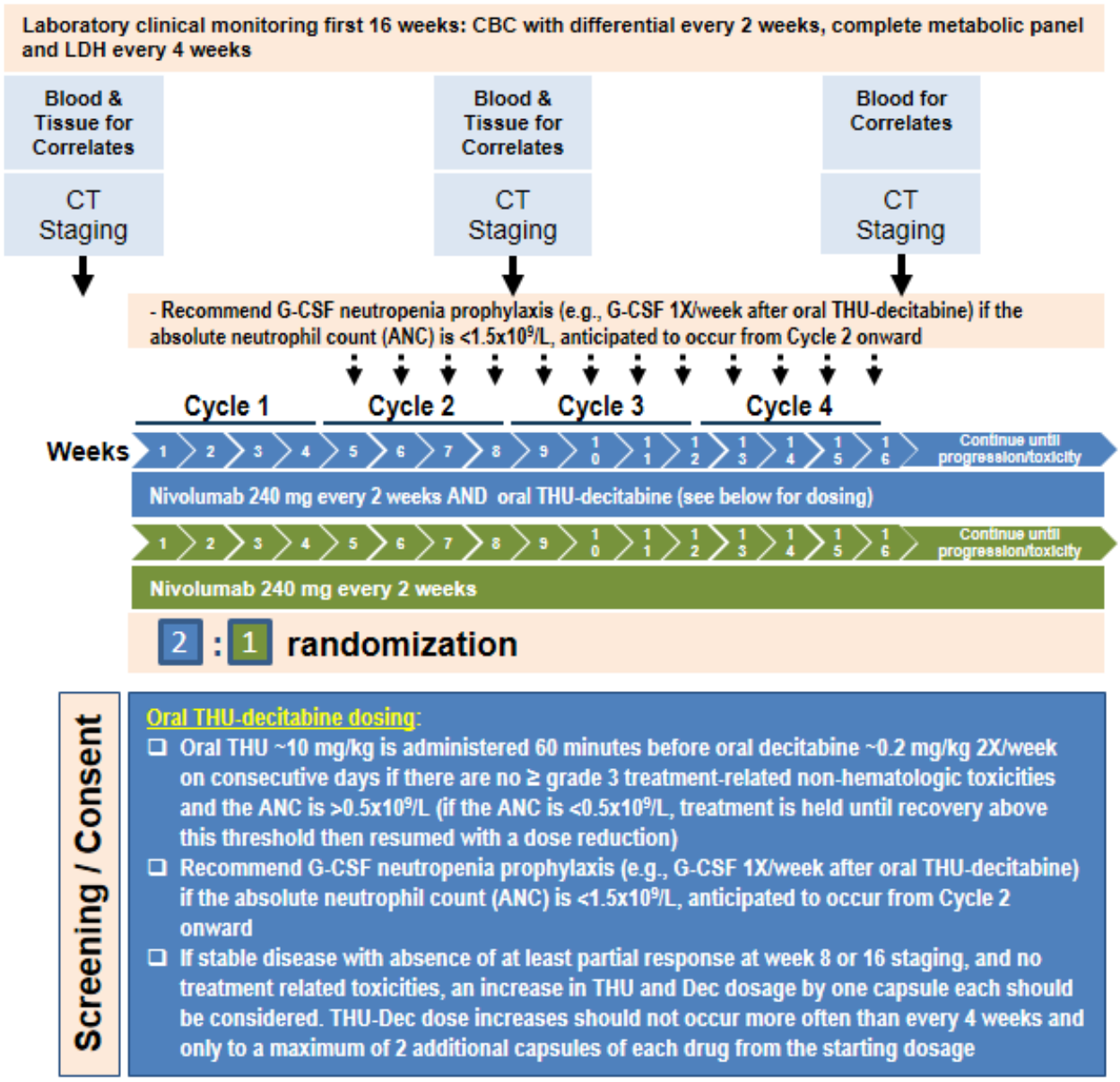
OTHER AGENT***(S):*** NA

SUMMARY OF CHANGES

Protocol Date	Change	Reason
05/31/2016	Removed option for crossover of patients in control arm	Per request from NCI co-investigators and study sponsor
05/31/2016	Updated statistics section	Incorporating comments from study sponsor and co-investigators
05/31/2016	Study calendars updated to reflect the correlative blood collections accurately	For clarity on time points for blood collection
05/31/2016	Updated safety reporting section	Administrative per Sponsor request
05/31/2016	Clarified WOCB age and requirements for contraception	Administrative per Sponsor request
05/31/2016	Clarification on days of administration of THU-dec in treatment plan	Minor clarification on treatment plan
05/31/2016	Adverse event definitions updated and clarified	Per sponsor request
05/31/2016	Nivolumab (standard of care) product information included	Per sponsor request
05/31/2016	Section 12.8, patients beyond cycle 8 continue to have CT scans every 2 cycles i.e. 8 weeks; option of doing scans every 12 weeks per investigator is now removed.	Per recommendations from NCI co-investigators
08/25/2016	Section 13.1, adding language regarding use of Overture	Per sponsor request
03/31/2017	Including response information, clarifying safety parameters, updating calendar, updating treatment plan and other administrative changes throughout document	Per recommendations from Taussig Cancer Center Quality Assurance

03/31/2017	Sections 6.1 and 9.3, Nivolumab will be given as a flat 240 mg dose	Per recommendations from Taussig Cancer Center Quality Assurance; the flat dose is standard of care
7/24/2017	Sections 4.1 and 4.2, updating inclusion and exclusion criteria	Clarification of inclusion criteria regarding pre-study biopsies and exclusion criteria regarding cardiac events, organ function, prior malignancy, and autoimmune disease
7/24/2017	Section 10.0, study calendar updated to clarify frequency of thyroid assessments	Calendar updated to more clearly indicate that thyroid assessment only need to be performed on Day 1 of applicable cycles
02/02/2018	9.1.3 Method of Administration	- Starting dose of oral THU and Dec is by weight (all subsequent doses are based on the Cycle 1 Day 1 dose with dose modifications based on toxicities and tumor response as described in the protocol):
02/02/2018	3.4 Expected Duration of Treatment and Subject Participation	Clarified the duration of treatment and the assessment of response and treatment beyond progression based on clinical benefit.
02/02/2018	4.1 inclusion criteria	Clarified "systemic therapy" criteria Clarified "biopsy requirement criteria and exception" Clarified brain met inclusion criteria
02/02/2018	4.2 Exclusion criteria	Clarified language on additional malignancies
02/02/2018	6.0 Treatment plan	Clarified dose reduction criteria and the weight based dosing.
02/02/2018	6.23 General Concomitant Medications	Clarified the use of radiation on trial.
02/02/2018	6.34 Discontinuation of Subjects from treatment	Clarified the disease progression and criteria for continuation of treatment for patients with clinical benefit.
02/02/2018	7.1 Dose reduction	Criteria for dose reduction and use of growth factors clarified
02/02/2018	8.2.1 Adverse event	AE reporting process clarified
02/02/2018	12.0 Measurement of Effect	Clarified evaluation of response criteria
9/7/2018	Removing Dr. Vamsidhar Velcheti as PI. Adding Dr. Nathan Pennell as PI.	Dr. Velcheti leaving CCF.
9/7/2018	Changing coordinator to Ben Pannell	Study was transitioned to new coordinator

STUDY SCHEMA



PROTOCOL SUMMARY

Official Title: Phase II Evaluation of Nivolumab, an Immune Checkpoint Inhibitor, alone or in combination with Oral Decitabine/Tetrahydrouridine as Second Line Therapy for Non-Small Cell Lung Cancer

Number/Title: Case 3516 Pharmacologically Rational EpigenetiC Immunotherapy for SEcond line Therapy in patients with Non-Small Cell Lung Cancer: PRECISE trial

Study Phase: Phase II

Principal Investigator: Nathan Pennell, MD, PhD

IND Holder: Yogen Saunthararajah, MD; IND # 112,914

Rationale/Hypothesis/Objective: Lung cancer is the world's leading cause of cancer death. 1st-line platinum-based cytotoxic (p53-dependent) chemotherapy for advanced non-small cell lung cancer (NSCLC) produces transient responses at best, possibly because p53-system mutation/deletion is universal in NSCLC and metastatic disease[2-10]. A recent landmark development has been approval of the immune checkpoint inhibitor (anti-PD-1) nivolumab in 2nd-line for NSCLC. Unfortunately, the objective response rate to nivolumab is only ~20%. A key factor underlying this limited response is use by cancer cells of altered epigenetics to suppress neo-antigen expression and avoid immune-recognition (reviewed in[1]). As such, we and several other groups have conducted clinical trials adding inhibitors of epigenetic repression to immunotherapy. The major class of epigenetic drugs in these trials are the DNA methyltransferase (DNMT1)-depletors (5-azacytidine, decitabine). DNMT1-depletion is appealing beyond immuno-modulatory effects because we and others have validated DNMT1 as a molecular target that cytoreduces NSCLC[11-16] and other cancers directly by p53-independent mechanisms (via upregulation of p27/CDKN1B)[11-13,15-22], offering a true alternative to cytotoxic chemotherapy.

Unfortunately, 5-azacytidine and decitabine (Dec) have fundamental pharmacologic limitations that have impeded translation of the compelling pre-clinical science: although it is possible to separate DNMT1-depletion by Dec from anti-metabolite effects/cytotoxicity that would suppress immune-effectors, plasma $t_{½}$ is <15 minutes and solid tissue distribution/oral bioavailability is negligible, because of rapid deamination/inactivation by the enzyme cytidine deaminase (CDA)[23,24]. This is a severe limitation because the DNMT1-depletion is S-phase, and hence exposure time, dependent. We recognized that this pharmacology was undermining clinical translation after conducting clinical trials with Dec alone[21,25]. Therefore, we combined very low doses of oral Dec with an inhibitor of CDA, tetrahydrouridine (THU) and showed in a Phase 1 clinical trial (IND#112,914, NCT#01685515) that the combination solves the oral bioavailability/solid-tissue-distribution problems, producing Dec low C_{max}/multi-hour T_{max} (>1 log-improvement) needed for non-cytotoxic DNMT1-depletion in solid tissues[26-28]. Thus, the hypothesis driving this clinical trial is: *Oral THU-Dec overcomes pharmacologic limitations of Dec alone to deplete DNMT1 within solid tumors to the extent necessary to increase the proportion of patients who benefit from FDA-approved immunotherapy.*

Study Design: This is a randomized two arm phase II trial of nivolumab alone or in combination with THU-Dec, in previously treated patients with stage IV NSCLC. The primary goal of this trial is to compare the efficacy of THU-Dec as a way of enhancing the anti-tumor immune response to nivolumab to that of nivolumab alone. Patients will be randomized 2:1 favoring the treatment arm with THU-Dec.

Sample Size: 60 patients

Primary objective: To determine if non-cytotoxic oral THU-Dec when combined with nivolumab can improve objective response rates of nivolumab
Secondary objectives: (i) To evaluate clinical efficacy end points and toxicity of oral THU-Dec when combined with nivolumab; (ii) evaluate the induction of a T-cell response in patients with metastatic NSCLC; (iii) To evaluate hypotheses regarding mechanisms of resistance and predictive biomarkers for response to nivolumab

Criteria for Evaluation:
-Primary end-point:
1. Objective response by Response Evaluation Criteria in Solid Tumors (RECIST v1.1)
-Secondary endpoints/scientific correlates:
1. To determine the progression-free and overall survival in patients with NSCLC receiving nivolumab with and without THU-Dec
2. To determine the safety and toxicity of the combination of THU-Dec and Nivolumab
3. To examine potential predictive biomarkers in tumor samples and peripheral blood
4. To evaluate the induction of a T-cell response in patients with metastatic NSCLC treated with THU-Dec
5. Molecular pharmacodynamics by immunohistochemical and flow cytometric assessment of DNMT1-protein in tumor tissue (where feasible) and peripheral blood buffy coat cells respectively, and by measurement of plasma CDA enzyme activity by HPLC enzyme assay
6. QRT-PCR evaluation of DCK, UCK2, CDA and KI67 in baseline and post treatment tissue where feasible

Study Population: Histologically confirmed metastatic NSCLC; progression of disease on one prior line of systemic therapy; measurable disease per RECIST v1.1; ECOG performance status 0-2.

Treatment: Patients will receive study drugs as follows:

Nivolumab 240mg IV Q2 weeks until progression per RECIST v1.1; This is the standard of care for patients with NSCLC who have progressed on prior chemotherapy.

With or without

Oral tetrahydrouridine and decitabine: THU is supplied as 250 mg/capsule, Dec as 5 mg/capsule.
- **Starting dose of oral THU-Dec is by weight (all subsequent doses are based on the Cycle 1 Day 1 dose with dose modifications based on toxicities and tumor response as described in the protocol)**:
Weight 40-60kg = 2 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules. *Weight 61-80kg* = 3 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules. *Weight 81-100kg* or higher = 4 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
- **Timing between THU and Dec**: Oral THU capsules followed 60 minutes later by oral Dec capsules. .
- **Frequency of THU and Dec ingestion:**
- THU ~10 mg/kg is administered 60 minutes before oral decitabine ~0.2 mg/kg 2X/week on consecutive days if there is no ≥ grade 3 treatment-related non-hematologic toxicity and the ANC is $>0.5x10^9$/L (if ANC is $<0.5x10^9$/L, treatment is held until recovery above this threshold then resumed with a dose reduction as per Table 1).
- G-CSF neutropenia prophylaxis is recommended 1X/week if the ANC is $<1.5x10^9$/L

Clinical Monitoring: History and examination, laboratory tests, and regular radiologic assessments. Patients will continue study treatment until disease progression by clinical and/or radiologic parameters (RECIST v1.1), or unacceptable toxicity. Efficacy will be determined by radiographic evaluation and other measures as described above. THU-Dec safety profile will be determined by incidence of treatment emergent adverse events captured from the first dose of THU. Each adverse event will be summarized by grade, causality, expected/unexpected based on the current knowledge. Further, adverse events will be summarized by body system and at the patient level (worst grade for specified adverse event).

ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
CRU	Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals
BMS	Bristol Myers Squib
Dec	Decitabine
THU	Tetrahydrouridine
SAE	Serious Adverse Event

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Poor Prognosis of Metastatic Non-small Cell Lung Cancer (NSCLC)

Lung cancer is the leading cause of cancer related mortality in the United States, with over two third of patients presenting at an advanced stage. 5-year survival for patients with advanced lung cancer is a dismal 15%.[29] For decades treatments and research has been focused on inducing apoptosis using cytotoxic chemotherapy, but with little improvement in clinical outcomes. Conventional chemotherapies have varying proximal molecular targets but the final common pathway converges onto p53 and p16/CDKN2A which are the master regulators of apoptosis. A majority of lung cancers have inactivating genetic alterations in the p53 and p16/CDKN1A genes[30,31] conferring natural resistance to conventional cytotoxic chemotherapy.[2-10] Perhaps therefore, it is therefore no surprise that decades of work focused on cytotoxic chemotherapy in lung cancer have been largely disappointing.[22] In this proposal, we evaluate a novel approach to terminate malignant proliferation that does not require master apoptosis regulators such as p53 and p16/CDKN2A and that moreover, can improve tumor antigenicity by re-expression of tumor specific antigens.[32-35] Cancers including lung cancers overexpress un-mutated forms of master differentiation driving transcription factors (e.g., CEBPA, GATA1, FOXA1, PAX2, SOX2, HNF1, MITF), that is, the "Yamanaka" factors that usually drive lineage-differentiation. Paradoxically, however, the terminal differentiation (epithelial differentiation) genes which are targets of these master transcription factors, and which usually antagonize MYC-function to terminate proliferation, are epigenetically silenced (discussed in our review[36]). This epigenetic repression is caused by mutations in co-factors for the master transcription factors, including co-activators, such that there is unbalanced activity of corepressors that create epigenetic repression instead of activation of the terminal differentiation target genes. DNMT1 is a key member of the network of corepressor proteins. Hence, depleting DNMT1 rebalances towards the action of activating chromatin modifying enzymes, and because the cancer expresses very high levels of differentiation-driving transcription factors at baseline, physiologic, differentiation-related maturation genes are activated and MYC is physiologically antagonized to terminate proliferation. This approach has a good therapeutic-index, since normal stem cell express master stem cell transcription factors, and self-renew in response to the same treatments, whereas normal progenitors differentiate, their intended fate dictated by the baseline master transcription factor context.[18]

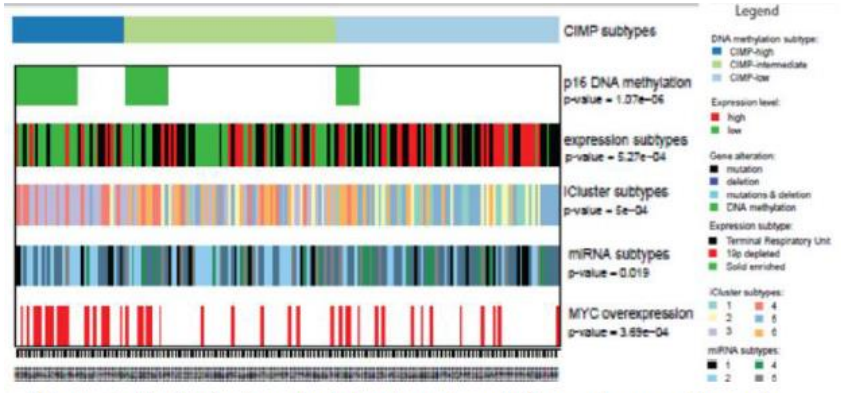


Figure 1: CpG island methylator phenotype in lung adenocarcinoma is common and is strongly associated with an aggressive phenotype.

1.2 Rationale for Use of Tetrahydrouridine (THU) and Decitabine (Dec) in Lung Cancer

DNMT1 is a validated molecular target for treating lung cancer: Hypermethylation of key apoptosis and differentiation-related tumor suppressor genes including homeobox-associated genes is nearly ubiquitous in NSCLC patients.[30,31] DNMT1 has been validated as a molecular target for therapy of NSCLC in pre-clinical in vitro and in vivo models.[11-16] Also, CpG island methylator phenotype in lung cancer is strongly associated with increased expression of MYC, the master oncoprotein driver of cell growth and division.[30,31] Recently published comprehensive molecular profiling of of 230 lung adenocarcinomas revealed several mutations in chromatin-modifying genes that are components of coactivator complexes (e.g., SMARCA4, ARID1A and SETD2), that is, chromatin-modifying enzymes that orthogonally oppose DNMT1 are genetically inactivated.[31] Epigenetic alterations are in many cases dynamic and reversible, thus are appealing targets for treatment. However, clinical translation of these scientific observations has been largely disappointing. Both Dec and 5-azacitidine (a prodrug for Dec) have been studied in several phase I/II trials in various solid tumors including lung. The objective response rates were very modest (<5%).[37] In a trial combining 5-azacitidine and entinostat (a HDAC inhibitor), a modest OR (4%) was seen, although responses when they occurred were durable.[38]

We propose that these disappointing clinical responses in lung cancer and other solid tumors are not because of erroneous pre-clinical observations, but instead, because of fundamental pharmacologic limitations of Dec and 5-azacytidine, the DNMT1-depleting drugs used for clinical translation. *Impaired tissue bioavailability of Dec is a basic problem preventing effective clinical translation:* Dec is very rapidly inactivated in solid organs: the half-life of Dec in buffer *in vitro* is over 10 hrs, however, *in vivo*, the plasma half-life is under 10 minutes, because of rapid metabolism by the enzyme cytidine deaminase (CDA), that deaminates Dec into its inactive uracil base moiety counterparts.[39] This short in vivo half-life is especially a problem since the epigenetic therapeutic effect of Dec, depletion of DNMT-1, is exquisitely S phase dependent. Stated another way, exposure times to Dec must overlap with cancer cell S-phase entries, and thus a very brief exposure time fundamentally undermines the possibility of a meaningful clinical epigenetic therapeutic effect, regardless of exciting pre-clinical observations.

This problem is not overcome by increasing Dec dose, since this produces off-target anti-metabolite and cytotoxic effects in more sensitive organs such as the bone marrow that have substantially lower CDA expression than other tissues such as the liver. Thus, to successfully use Dec to target DNMT1 in vivo to treat solid tissue cancers such as lung cancer, the pharmacologic barrier of CDA should be overcome. With the support from the National Institutes of Health Rapid Access to Interventional Development (NIH-RAID) Program, we have developed a combination therapy of oral Dec combined with an inhibitor of CDA (oral tetrahydrouridine, THU) and have an IND (#112, 914) for the novel combination therapy. Inhibiting CDA in this way produces the Dec pharmacology desired for a potent non-cytotoxic DNMT1-depleting effect: low C_{max} that avoids off-target effects/cytotoxicity and extended T_{max} with oral bioavailability and balanced distribution of Dec through the tissues of the body.[27]

The oral THU-Dec drug and trial concept are novel compared to Dec/5-azacytidine in lung cancer by overcoming the pharmacologic barriers as described above allowing (i) Substantially improved oral bioavailability[26,40]; (ii) Lower C_{max} and log-fold increase in T_{max}; (iii) Allowing meaningful distribution of Dec into solid tissues[26]; (iv) Noncytotoxic,

p53-independent mechanism of cell cycle exit that improves tolerability, safety and sustainability of therapy; (v) Preservation of immune-effectors and upregulation of neo-antigens and antigen presentation by cancer cells, creating a logical platform for combination with FDA-approved immune checkpoint inhibitor drugs.

1.3 Mechanisms of Immune-Escape in Lung Cancer that Limit the Response Rate to Newly Approved Immune Checkpoint Blockade

Traditionally melanoma and renal cell cancer were considered "immunogenic" tumors while other solid tumors were considered "non-immunogenic". Recently, however, a new class of immunotherapy agents, targeting the PD-L1/PD-1 immune-checkpoint axis, was approved in NSCLC (~20% overall response rate)[41-44]. The PD-L1/PD-1 axis (immune checkpoint) is used by self-tissue to suppress attack by adaptive immunity. Patients in whom these drugs have shown significant anti-tumor activity are those in whom the tumors appear to be "immune primed", that is, enriched in lymphocytic infiltrates requiring that the tumor use immune checkpoints to survive. Conversely, patients who do not have responses to these agents appear to have tumors that have not triggered an immune response, despite the fact that primary and secondary lung cancer is genomically very deranged, suggesting it should be susceptible to host's immune-recognition because of neo-antigens.

How then has most lung cancer evaded immune-recognition? Attack of a cancer by powerful adaptive immunity requires engagement of antigen-specific T-cell receptors (TCR) on $CD8^+$ T lymphocytes by tumor-associated antigens presented on the cancer cell in the form of MHC class I molecule-bound peptides[45], together with costimulatory/accessory molecules, e.g., CD80, CD86, and ICAM1 that engage their cognate counter-receptors on T cells. All these critical processes of antigen presentation, including MHC expression, are down-regulated in NSCLC by epigenetic changes, mainly DNA methylation (requiring DNMT1), but also histone post-translational modifications (e.g., by HDAC)[45-531](**Fig.1**). Previously, we showed that such suppression is especially a characteristic of the most advanced or aggressive NSCLC[54-56].

Accordingly, we[25,57-64] and many others have demonstrated that HDACi and DNMT1-depleting drugs 5Aza and Dec, can reverse this epigenetic silencing, promote antigen expression and induce anti-tumor immune responses in pre-clinical models[65-67]. In a Phase 1 clinical trial, we evaluated continuous infusions of Dec by a phase I dose-escalation schema (n=35)[25]. Each full course of therapy consisted of two identical 35-day cycles. Immunohistochemical techniques were used to evaluate antigen presentation cancer tissue biopsies. Twenty-five subjects were evaluable for treatment response. No objective responses were observed. Improved antigen presentation was observed in 3 subjects. Our conclusion was that the pharmacology of Dec (or 5Aza), especially the severely abbreviated *in vivo* $t_{½}$ and very poor solid tissue distribution[23,24], and not flaws in the pre-clinical science, were the cause of the limited efficacy, and that this pharmacology is likely to undermine the efficacy of several other ongoing clinical trials - in other clinical trials of Dec and 5Aza in various solid tumors including lung, objective response rates have been very modest (<5%)[37,38]. This pharmacology may similarly limit the ongoing phase 2 clinical trial evaluating 5Aza and HDACi combination with nivolumab (NCT01928576), the recently concluded phase 2 trial evaluating 5Aza and HDACi vs. observation in resected stage I NSCLC (NCT01207726), and at least 9 other epigenetic immunotherapy

cancer clinical trials ongoing with Dec or the Dec analogue guadecitabine[1]. We expect immune-priming will be even more meaningful and significant with the pharmacology of oral THU-Dec that is optimized for non-cytotoxic DNMT1-depletion.

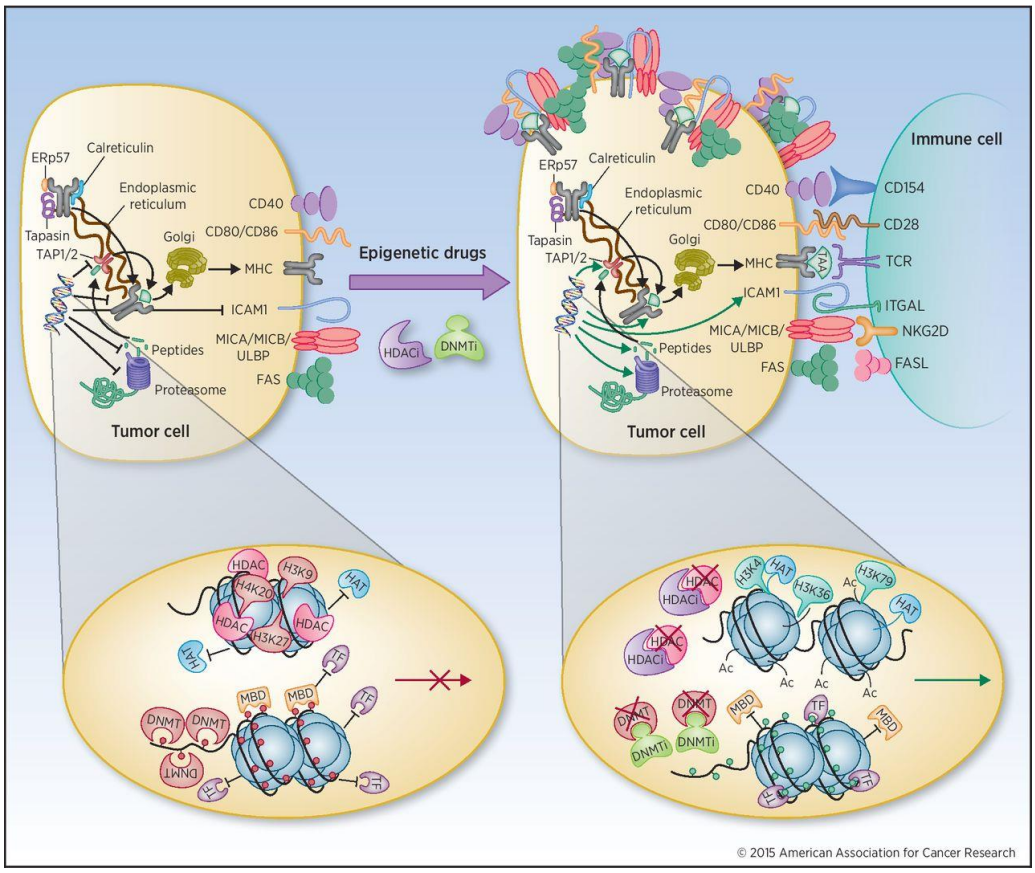
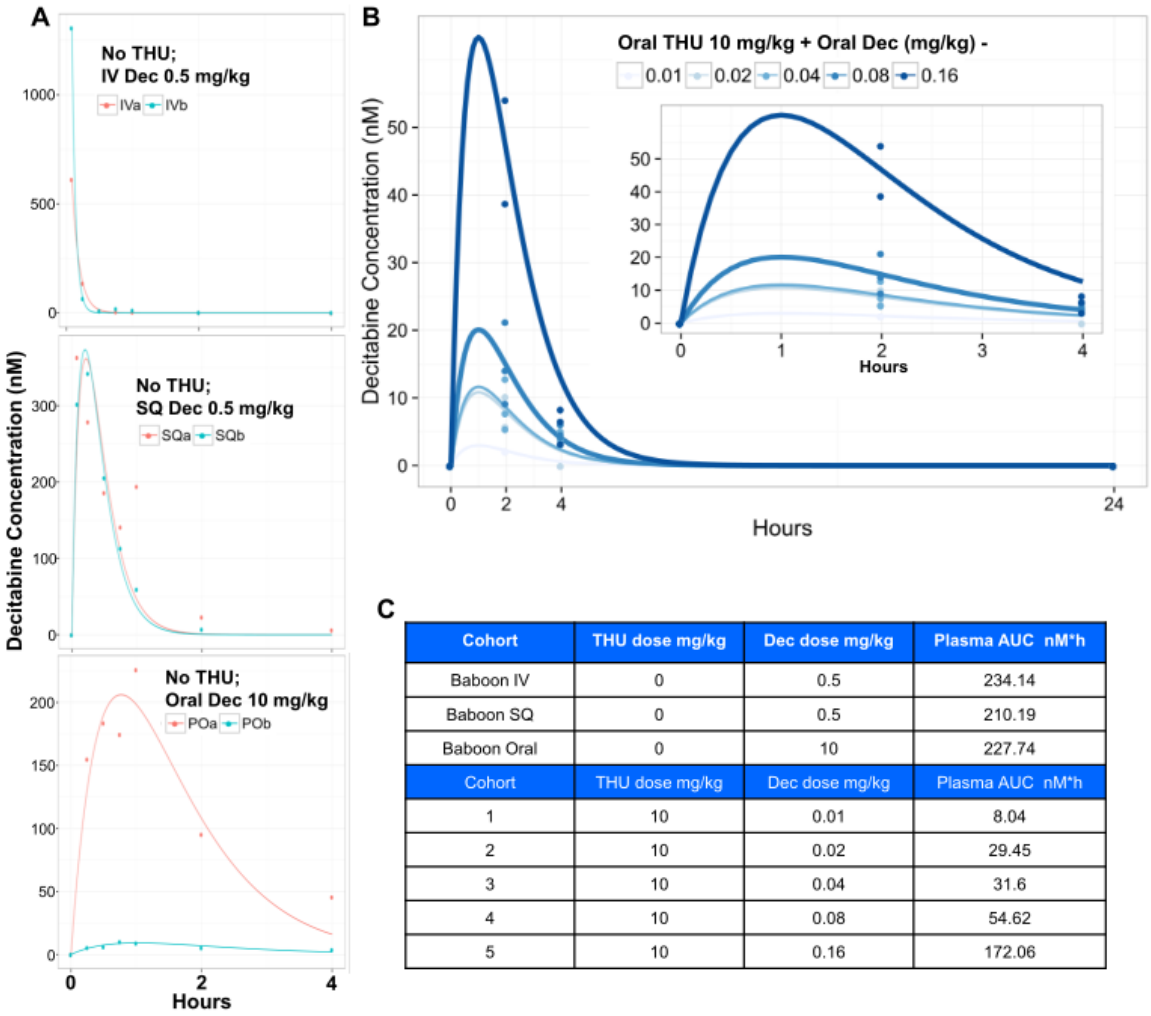


Figure 2. Epigenetics and immune-evasion, and its pharmacologic editing to enable immune-recognition of cancer cells (figure from[1]).

1.4 Background on the Drugs

1.4.1 Investigational Agent Oral THU-Dec. DNMT1-depletion requires cancer cell S-phase entries to overlap with intra-cellular exposures, which in turn depends on extra-cellular exposures. Plasma $t_{½}$ is dictated by cytidine deaminase (CDA) - CDA drastically reduces plasma $t_{½}$ to ~10 minutes from ~12 hours *in vitro*[23,24]. Moreover, we have demonstrated that CDA-enriched tissues (essentially all major solid tissue organs) provide sanctuary to malignant cells from Dec treatment-effects[23].

This pharmacology is not solved by increasing Dec dose, since off-target effects from higher C_{max} causes cytotoxicity that paradoxically decreases efficacy by restricting the feasible frequency of administration. Higher C_{max} also destroys immune-effectors, subverting the rationale for epigenetic immunotherapy. Similarly, continuous infusion produces toxic increases of Dec in some tissues while inadequate exposure remains in others (CDA expression varies widely between tissues).



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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 3: Pharmacokinetics (PK) of Dec and oral THU-Dec. A) Dec PK after intravenous (IV), subcutaneous (SQ) and oral (PO) administration to baboons. B) Dec 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 C_{max} but extended T_{max} or $t_{½}$) and Dec distribution through CDA rich organs such as the intestines and liver.

To address this severe $t_{½}$/distribution limitation, we combined lower dosages of Dec with the CDA-inhibitor THU, and have demonstrated in mice, non-human primates and in a Phase 1 clinical trial in sickle cell disease (in which the PK and pharmacodynamic objectives of non-cytotoxic DNMT1-depletion are the same as in this clinical trial), that this combination produces: *(i) Low C_{max} and log-fold increase in T_{max}* – High C_{max} produces off-target anti-metabolite effects/cytotoxicity (undesired). High T_{max} increases overlap between sufficient intracellular drug levels and S-phase entries of malignant cells, needed for DNMT1-depletion (desired). By inhibiting CDA, we have extended the plasma half-life of Dec from ~10 minutes to >4 hours, with a C_{max} ~50 nM[26-28] (**fig.3**). *(ii) Meaningful distribution of Dec into solid tissues* – Marketed Dec is able to treat myeloid (liquid) cancers such as MDS (FDA-approval), but because of the high expression of CDA in organs such as the liver etc., Dec, like all cytidine analogues, is ineffective against cancers of or in these tissues (reviewed in[23]). Oral THU-Dec addresses this basic pharmacologic factor to rationally enable treatment of solid tissue malignancies and myeloid malignancies that infiltrate solid tissues, e.g., myeloproliferative neoplasms (**fig.3**). *(iii) Oral bioavailability* - CDA enrichment in the intestines and the liver is a substantial metabolic barrier to oral bioavailability of cytidine analogues such as Dec (no cytidine analogues are FDA-approved for this route of administration)[27,28]; although oral 5Aza is currently being investigated in clinical trials, there are major difficulties, including poor AUC, significant inter-individual variability and substantial GI toxicity from high dosages administered in an attempt to overcome the intestinal CDA-barrier. We have shown that combining THU and Dec improves oral bioavailability ~10-fold (**fig.3**)[26-28]. Thus, we administer Dec orally at a dose that is <10-25% of the FDA-approved intravenous dosages[26-28]. *(iv) Decrease inter-individual variability* - Pharmacogenetic differences in CDA expression and/or sequence between individuals causes clinically significant inter-individual variability in cytidine analogue pharmacokinetics (reviewed in[39]). Inhibiting CDA decreases this inter-individual variability[27,39]. *(v) Non-cytotoxic, normal stem cell sparing, p53-independent method of use* - Dec was originally developed for the conventional oncotherapeutic objective of cytotoxicity (apoptosis), and FDA-regimens are pulse-cycled per anti-metabolite therapy, can cause anti-metabolite effects and cytotoxicity, and require anti-emetics. Underlying the proposed clinical trials are the observations that cytotoxicity is counter-productive in p53/p16-altered cancers, by selecting for the most apoptosis-resistant cancer sub-clones, by causing toxicity that limits frequency of drug administration, thereby decreasing the fraction of the malignant clone subject to S-phase dependent, p53-independent epigenetic-therapeutic effects, and by destroying immune-effectors.

When Dec is repositioned to avoid cytotoxicity and increase DNMT1-depletion as is the intent in this clinical trial, no significant clinical toxicities are anticipated. Non-cytotoxic DNMT1 depletion whether by genetic means or by Dec has been demonstrated to shift hematopoietic differentiation towards megakaryopoiesis and erythropoiesis and away from granulopoiesis and monopoiesis). The major risk with therapy therefore is of neutropenia/neutropenic fever/sepsis associated with nadir. To reduce this risk, this clinical trial excludes patients with baseline neutrophil counts of $<1.5 \times 10^9$/L. THU is not expected to cause any toxicities, based on a number of clinical trials, including trials with chronic administration at higher doses than are used in this study.

1.4.2 *Background on Nivolumab*

For a complete review of non-clinical and clinical information, please refer to the BMS-936558 Investigator Brochure (IB).[68]

a) Non-clinical: Preclinical animal models of tumors have shown that blockade of Programmed death-1 (PD-1) by monoclonal antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection. Antitumor activity by PD-1 blockade functions in PD-L1+ tumors as well as for tumors that are negative for the expression of PD-L1.[68-72] This suggests that host mechanisms (ie, expression of PD-L1 in antigen-presenting cells) limit the antitumor response. Consequently, both PD-L1 positive and negative tumors may be targeted using this approach. In humans, constitutive PD-L1 expression is normally limited to macrophage-lineage cells, although expression of PD-L1 can be induced on other hematologic cells as well, including activated T cells. However, aberrant expression of PD-L1 by tumor cells has been reported in a number of human malignancies.[72] PD-L1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor-specific T cells in vitro.[68] Moreover, the expression of PD-L1 may protect the tumor cells from the induction of apoptosis by effector T cells. Retrospective analyses of several human tumor types suggest that tumor overexpression (as measured by IHC) of PD-L1 may permit immune evasion by tumors. PD-L1 expression had been shown to be consistently associated with tumors that are more inflamed and is a major mechanism of immune evasion. [55,73-75]

Reproductive and Developmental Toxicity: Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported. The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in BMS-936558 (nivolumab) exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with BMS-936558 during pregnancy.[68]

b) Pharmacology: BMS-936558 (MDX-1106) is a fully human, IgG4 (kappa) isotype, mAb that binds PD-1. Blockade of the PD-1 pathway by BMS-936558 was studied using the mixed lymphocyte reaction (MLR). PD-1 blockade resulted in a reproducible enhancement of both proliferation and interferon release in the MLR. The effect of BMS-936558 on antigen-specific recall response was investigated using a CMV-restimulation assay with human PBMC, and was evaluated by ELISA. These data indicated that BMS-936558, versus an isotype-matched control antibody, augmented interferon secretion from CMV-specific memory T cells in a dose-dependent manner. PD-1 blockade by BMS-936558 has therefore been pursued as a promising avenue for immunotherapy of tumors.[68]

c) Clinical results: Two studies contributed to most of the clinical experience with BMS-936558 in subjects with malignancies. MDX1106-01 was a Phase 1 single-dose escalation study in subjects (N = 39) with previously treated advanced or metastatic cancer. Subjects received a single dose of BMS-936558 at 0.3, 1, 3, or 10 mg/kg with an option for re-treatment at 3 months. CA209003 (MDX1106-003) is an ongoing Phase 1 multi-dose dose escalation study in subjects with previously treated advanced or metastatic melanoma, RCC, non-small cell lung cancer (NSCLC), colorectal cancer (CRC), or hormone-refractory prostate cancer (HRPC). 169 subjects have received at least one dose

of BMS-936558 intravenously every 2 weeks at doses of 0.1, 0.3, 1, 3, or 10 mg/kg. During the dose escalation portion of the study, subjects were treated at three dose levels: 1, 3, and 10 mg/kg. Initial cohort expansions occurred in each tumor type at the highest tolerable dose evaluated (10 mg/kg) as well as in melanoma at 1 mg/kg and 3 mg/kg. The protocol was subsequently amended (Amendment 4), to include expansion cohorts in RCC at 1 mg/kg, melanoma at 0.1, 0.3, and 1 mg/kg, and NSCLC (squamous and non-squamous) at 1, 3, and 10 mg/kg.[68]

d) Safety Summary: No MTD was identified in CA209003. The maximum dose level evaluated was 10 mg/kg. The most frequent AEs were fatigue (49%), diarrhea (29%), decreased appetite (27%), nausea (26%), vomiting (22%), and rash (21%). There was no pattern in the incidence, severity or relationship of adverse events to the BMS-936558 dose level. Any grade drug-related AEs were experienced by 57% of subjects. The most common drug related AEs were fatigue (22%), rash (15%), pruritus (11%), and diarrhea (9%). Most drug-related AEs were Grade 1 or Grade 2 in severity. Similar to the overall AE profile, there was no apparent relationship in the incidence or severity of drug-related AEs to BMS-936558 dose level. There were no apparent differences in the frequency of adverse events based on subjects' tumor type. As of the clinical cut-off date (31-May-2011), 18 deaths have been reported. Death was considered secondary to disease progression and unrelated to BMS-936558 in 16 subjects. One death was considered secondary to sepsis (Grade 5)/pneumonitis (Grade 4). The fatal sepsis was considered unrelated to study drug; however the preceding pneumonitis was considered related to BMS-936558. One death was considered secondary to ischemic cardiomyopathy and considered unrelated to BMS-936558. Subsequent to the clinical cut-off date, other cases of pneumonitis, including 2 fatal cases, have been reported. Additional details regarding these cases are provided in the Investigator Brochure, Section 5.

1.5 Summary of Rationale for proposed study

A validated molecular target for therapy yet failure to translate: The epigenetic protein DNA methyltransferase 1 (DNMT1) has been extensively validated in pre-clinical studies as a molecular target for treatment of NSCLC[11-16], since inhibition of DNMT1 produces cell cycle exits that do not require p16/p53, but instead utilize physiologic, cell maturation-related MYC antagonists (e.g., p27/CDKN1B, reviewed in[22]). The extensively reproduced pre-clinical data prompted multiple clinical trials of the DNMT1-depleting drugs decitabine and 5-azacytidine to treat solid tumor malignancies, however, results have been disappointing (response rates typically <10%[32,76-81]), so Dec is not approved to treat any solid tumor malignancy, despite several clinical trials over decades. We hypothesize that this failure to translate from the lab into clinical benefit is not because of improperly executed pre-clinical science, but because of a failure to rigorously capitalize on basic pharmacologic properties of Dec.

Bridging to delayed effect of immune therapy with non-cytotoxic tumor cytoreducing therapy: Delayed effects have been reported with immune therapies like nivolumab. Non-cytotoxic, p53-independent therapy with oral THU-decitabine can potentially bridge this

gap while sparing the immune-system, thereby contrasting with traditional cytotoxic therapy.

Immune priming effect of DNMT1 inhibition: The clinical trials with Dec and 5-azacytidine showed dismal responses. However patients on these trials had durable responses to their subsequent line of therapy, particularly immune-checkpoint inhibitors. Several of these patients had an unusually prolonged duration of responses.[41,66,82-84] This raised the possibility of the epigenetic treatment "priming" the tumors for subsequent therapies.[83] In trials evaluating DAC, an increase in the expression of tumor specific cancer testis antigens (NY-ESO-1 and MAGE-A3) were noted in the sequential biopsies Thus an "immune priming" effect of epigenetic therapy has been proposed.[67 82] In in-vivo mice tumor models treatment with low dose Dec increased IFN-γ producing T lymphocytes in the tumors and resulting in an anti-tumor response.[65] In addition, pre-clinical studies show that 5-azacytidine can promote tumor infiltrating myeloid cells and promote differentiation into mature immune-stimulatory antigen presenting cells (APC) in addition to constitutively overexpressing HLA class I antigens and other co-stimulatory molecules.[85-89] Increased APCs can orchestrate an immune cascade by activating cytotoxic T-Cells resulting in an anti-tumor effect. Furthermore the negative immune checkpoints, including PD1 on T-cells appear to be tightly regulated by methylation thus influencing cytotoxic T-cell differentiation.[90] Recently, increased activation of immune evasive pathways mainly PD-1/PDL1 and CTLA4 was demonstrated in leukemia cells with treatment of Dec.[91] There is an ongoing phase II clinical trial evaluating Azacitidine and entinostat combination with nivolumab, an anti-programmed death-1, antibody (NCT01928576). In addition, there was a recently concluded phase II trial with the combination of 5azacytidine and entinostat vs. observation in resected stage I NSCLC (NCT01207726) the results of which are awaited. With the pharmacology of oral THU-Dec that is optimized for non-cytotoxic DNMT1-depletion, we suspect that such immune-priming will be even more meaningful and significant.

There are no other drugs available for clinical use that have been designed and optimized for non-cytotoxic epigenetic-differentiation therapeutic effects. Thus, this proposal constitutes a first pharmacologically rational attempt to translate extensive scientific data regarding the value of DNMT1 as a molecular target for therapy that can potentially be effective even in advanced NSCLC that is refractory to current therapies, including the latest alternative molecular targeted therapies. Moreover, by augmenting expression of neoantigens via epigenetic mechanisms, this non-cytotoxic therapy is a rational platform for increasing the response rate and effectiveness of immune-checkpoint inhibitors[41] in NSCLC.

Thus, our overall objective is to evaluate if oral THU-low dose Dec based on rational pharmacology can realize the potential for DNMT1 as a molecular target in NSCLC, with a distinctive p53/p16-independent pathway of action achieving efficacy in refractory disease with excellent safety profile and augment the efficacy of nivolumab by immune priming.

1.6 Background and rationale of correlative studies

a) Correlatives in biopsy tissue

All the tissue based immunological correlatives PD-L1 expression and other exploratory correlatives will be performed in the Translational Immuno-oncology Laboratory lab at the Yale University. This laboratory is specialized in measurement of immune biomarkers and has access to CLIA-compliant facilities.

i) Measurement of Tumor infiltrating immune cells on pre-treatment and post treatment biopsies: The automated quantitative spectral multiplexing platform for evaluation of immune biomarkers is highly novel. We have previously developed and validated ways to reproducibly measure and quantify TILs and immune biomarkers by QIF[55,73,75]. To understand the tumor immune landscape, we will characterize the immune infiltrates in the samples using a previously described and validated assay to objectively interrogate different lymphocyte subsets[75]. This assay comprises the simultaneous staining and measurement of tumor cells (cytokeratin), T cells (CD3), cytotoxic T cells (CD8) and B lymphocytes (CD20). The signal intensity and amount of cells will be measured using informatic tools as described for PD-L1. These will be done in collaboration with Tumor immunology lab at Yale to develop and validate several immune biomarker panels for potential use in the clinical setting. This collaboration will expand on our previous work.

ii) Measurement of several immune biomarkers in the tumor and stromal compartments on pre-treatment and post treatment biopsies: PD-L1 expression will be determined for all patients on pre-treatment samples per standard of care using the DAKO PD-L1 assay in the Yale CLIA lab. Positivity will be determined by the presence of any (non-nuclear) PD-L1 signal present in intact tumor and/or stromal cells within the tumor region of the tissue examined, as evaluated by a pathologist in preparations stained with chromogenic immunohistochemistry for PD-L1 and counterstained with hematoxylin. Operationally, a positivity threshold of signal present in >1% of cells will be used. An additional 3-tiered semi-quantitative categorical result will be rendered by the pathologist based in the estimation of the percentage of positive cells including 1+ (<30%), 2+ (30-<60%) and 3+ (60-100%). PD-L1 negative cases will be those with absence of any detectable signal in the sample tested and with appropriate tissue and staining controls.

In addition, to chromogenic PD-L1 IHC using novel platform we can increase specific staining of multiple tissue biomarkers (PD-L2, B7H4, TIM-3, VISTA, LAG-3, IDO-1 etc) and able to reach 6-plex and beyond in a single image retaining the morphologic context of the tumor. The incorporation of such novel approaches could positively impact the value of tissue biomarkers determination for anti-cancer immunotherapies. Finally, additional correlative studies using novel approaches with higher sensitivity and throughput such as the Luminex assay69 and CyTOF massspect based cytometry70 will be pursued once these platforms are appropriately standardized.

iii) mRNA expression profiling: We will evaluate changes in mRNA expression profiles in FFPE tumor samples obtained before and after treatment with THU-Dec using the Nanostring and Counter Pancancer panel. Specifically, we will assess variations in immune-related transcripts and changes in MYC, as well as upregulation of the terminal differentiation markers and tumor specific antigen changes (Cancer testis antigens). We will also look at changes in DNMT1, DCK, UCK2 and CDA expression to study the intended pharmacodynamics of the THU-Dec combination

iv) Next Generation Sequencing for T-cell receptor clonality: TCR sequencing and clonality quantification on pre-and post-treatment samples will be performed using ImmunoSeq assay in a multiplexed PCR method using forward primers specific to TCR Vβ gene segments and reverse primers specific to TCR Jβ gene segments. In addition, using Immune Repertoire Capture™ (IRC™) technology we will evaluate immune response enabling the identification and generation of functional human antibodies and TCRs. This delivers an unparalleled quantitation of the adaptive immune response. This will be done in collaboration with Atreca Inc.

v) Pharmacodynamic analyses: The intended molecular pharmacodynamic effect of oral THU-decitabine is depletion of DNMT1 in tumor tissue. This will be measured by QIF. DNMT1-depletion is expected to induce p53-independent cell cycle exits, with downregulation of MYC and upregulation of p27/CDKN1B. This will be measured by QIF also. Finally, the intracellular accumulation of decitabine to deplete DNMT1 depends on relative expression in the cancer cells of pyrimidine metabolism enzymes DCK, CDA and UCK2, and also the growth fraction of the malignancy, measured by surrogates such as KI67. These parameters will also be measured by QIF.

b) Correlatives in blood:

i) Phenotypic character of PBMC: To be performed by GenOptix. Multiplex T cell phenotyping flow cytometry panels: We will design a broad T-cell flow cytometry panel composed of multiple exhaustion markers, checkpoint receptors and activation markers. Non-biased automated algorithms will be used to determine T –cell signatures predictive of response and resistance to immunotherapies. The putative markers in the panel may include CD3, CD4, CD8, CD45RO, CD45RA, CCR7, CD95, CD27, CD25, CD57, CD107, CD69 CD11A, TIM3, HLA-DR, PD-1, LAG3, OX40, ICOS, CCR4, CXCR3, CCR6, BIM, EOMES, CTLA-4. In addition to T-cells, we will evaluate other immune cell subsets including DCs, monocyte populations, and NK cells. A whole blood immunophenotyping assay will quantify the absolute number and proportion of NK cells (CD56+CD3-), NKT cells (CD56+CD3+), B cells (CD19+), and monocytes (CD16+). In addition, both myeloid (CD45+HLADR+ CD11c+CD123-) and plasmacytoid (CD45+HLA-DR+CD11c-CD123+) dendritic cell (DC) frequencies will be measured in this precision-validated multiparameter flow cytometric assay. In addition, PBMCs will be cryopreserved for future potential studies using platforms such as Mass cytometry (CyTOF), IRC™ and CIBERSORT, and will be correlated to the immunologic response, clinical response, and survival data.

ii) Serum proteomic and ctDNA correlatives: Exploratory proteomic analysis using MALDI ToF mass-spectrometry and ProTS will be performed in collaboration with Biodesix and the Pandey lab at Johns Hopkins on baseline and on treatment plasma samples at time points defined in the protocol from patients in the study.

PBMCs, Serum and plasma will be banked for other potential correlatives

2.0 OBJECTIVES & HYPOTHESES

Hypothesis*:* We hypothesize that the combination of nivolumab with non-cytotoxic low dose oral Dec with THU achieves immune priming and improves clinical efficacy of nivolumab.

Primary objective: To determine if non-cytotoxic oral THU-Dec when combined with nivolumab can improve objective response rates of nivolumab

Secondary objectives:

i) To evaluate clinical efficacy end points and toxicity of oral THU-Dec in combination with nivolumab;

ii) To evaluate the Immune priming effect of THU-Dec combination therapy by functional and phenotypic circulating immune cell characterization and changes in the immune contexture in the tumor tissue

iii) To evaluate hypotheses regarding mechanisms of resistance and predictive biomarkers for response to nivolumab

3.0 STUDY DESIGN

3.1 Study design: This is a Phase 2 randomized two arm trial of nivolumab alone or in combination with THU-Dec, in previously treated patients with stage IV Non-Small Cell Lung Cancer (NSCLC). The primary goal of this trial is to compare the efficacy of oral THU-Dec as a way of enhancing the anti-tumor immune response to nivolumab to that of nivolumab alone. The primary efficacy endpoint is overall RECIST v1.1-defined response. To accomplish this goal 60 patients will be randomized 2:1 to THU-Dec plus nivolumab or nivolumab only respectively.

3.2 Statistics and Sample size justification: The primary goal of this trial is to compare the efficacy of immune priming with oral tetrahydrouridine (THU) plus decitabine (THU-Dec) and immune check-point inhibitor nivolumab (THU-Dec+nivolumab) to that of nivolumab alone. The primary efficacy endpoint is overall response. To accomplish this goal 60 patients will be randomized 2:1 to THU-Dec+nivolumab versus nivolumab alone (standard of care) respectively. The purpose of the nivolumab alone arm is to support the notion that the population studied is typical of the general population for which nivolumab has an established response rate of 20%. If the THU-Dec+nivolumab arm has a response rate of 35% or higher, we will consider it worthy of further investigation in a phase III trial. Thus, using a two-stage design (http://www.swogstat.org/stat/public/twostage.htm), with alpha = 0.1 and beta = 0.2, we compute that 20 patients will be needed in each of two stages in the THU-Dec+nivolumab arm. If, after the 1st stage of 20 patients in the THU-Dec+nivolumab arm, 2 or fewer respond, the trial will be stopped and the null hypothesis that THU-Dec+nivolumab is no better than nivolumab will be accepted, else the trial continues for an additional 20 patients, at the end of which if 13 or more respond of the 40

patients in the THU-Dec+nivolumab arm, the null hypothesis is rejected and experimental arm of THU-Dec+nivolumab is recommended for further study.

As an additional exploratory analysis we will compare response rates between treatment groups. We expect a 20% response rate in our control arm of 20 patients. The 95% confidence interval of the proportion 4/20 is 7% to 44%. It thus includes our treatment arm expected response rate of 35%. It is therefore very unlikely that our control arm will disprove our assumption that our sample population has a historical response rate of 20%. Thus, to increase the likelihood of gaining some information from our control arm, differences in proportions will be compared between arms. Using a calculator for two arm binomial studies we compute that the power to detect a difference in rates between our control arm and our treatment arm of 40 patients, assuming one-sided tests, alpha=0.10 and holding the treatment arm response rate at 35%, is, respectively, 89%, 70%, 50%, 32% and 18% for control response rates of 5%, 10%, 15%, 20%, and 25%. Thus, if our control arm rates are <15%, we have >50% chance of detecting a difference in response. In addition, we will evaluate the PD-L1 expression in both the control and treatment arm after every 20 patients. If there is >10% difference in the PD-L1 status between the groups, we will stratify randomization by PD-L1 status for additional patients to balance the PD-L1 status.

Secondary endpoints include progression-free survival (progression is defined as progression by both RECIST v1.1), overall survival, and toxicity. Progression-free and overall survival will be summarized using the Kaplan-Meier method. Adverse events will be graded using CTCAE version 4.0 criteria, categorized by organ system, and summarized using frequency counts and percentages. With 20 patients treated with nivolumab only and 40 with THU-Dec+nivolumab the probability of observing at least one toxicity of a particular type and/or grade on each arm is 64% and 87%, respectively, if the risk of such an event is 5%, i.e. $0.64 = (1-0.95^{20})$.

Methods such as Fisher's exact test, chi-square tests, logistic regression (categorical data), the log-rank test and proportional hazards models (time to event data) will be used to compare patient groups. These analyses are primarily exploratory and it is acknowledged that they will have relatively low statistical power.

3.3 Replacement of Subjects

- If a subject is withdrawn from the study before week 8 for reasons not related to toxicity of study drug, the subject will be replaced.
- If a subject does not take at least 12 of the intended 16 doses in the first 8 weeks, the subject will be replaced because he/she has not taken enough THU-Dec that analysis of the primary end-point will be meaningful.

3.4 Expected Duration of Treatment and Subject Participation

Treatment on protocol monitoring continues until (i) disease progression (defined by RECIST v1.1 with the following caveat: since it is known that there can be initial progression by RECIST v1.1 criteria with immune checkpoint blockade followed by subsequent regression and clinical benefit, if the treating clinical team and the patient judge that they have benefitted from therapy, treatment continuation is allowed even if there is disease progression by RECIST v1.1 criteria. However, patients should have confirmed stable disease or better at the time of the following scheduled imaging time-point. If there

is progression at this following re-staging, patients should be discontinued from therapy); (ii) toxicity as defined in Section 7.1; or (iii) withdrawal of consent up to a maximum of 24 months.

At the end of the protocol-specified period of active study therapy, the sponsor will not continue to supply study drug to subjects/investigators unless the sponsor chooses to extend the study, which may include continuation of nivolumab. The investigator should ensure that the subject receives appropriate standard of care, which may include continued treatment with nivolumab.

4.0 SUBJECT SELECTION

Each of the criteria in the sections that follow must be met in order for a subject to be considered eligible for this study. Use the eligibility criteria to confirm a subject's eligibility.

4.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Histologically or cytologically-proven NSCLC

2. Subjects must have received 1 or more prior systemic therapies for this disease, (this can include neo-adjuvant or adjuvant chemotherapy if administered <2years prior to study enrollment), should not have had prior treatment with immunotherapy (including immune checkpoint inhibitor drugs, or immunotherapy vaccines); Patients with EGFR or ALK alterations will need to have progressed on a TKI treatment.

3. Measurable disease per RECIST v1.1

4. Disease that can be accessed by a bronchoscopic, surgical or percutaneous biopsy, eligible for biopsy from safety perspective, and agrees to biopsy prior to study. The pre-study biopsy can be waived if (i) there is an archival biopsy specimen that was obtained after the most recent therapy or(ii) if the risks of biopsy are judged to be excessive by the study PI

5. ECOG performance status 0 – 2

6. Male or female, 18 years or older (Because no dosing or adverse event data are currently available on the use of oral THU-Dec in subjects ≤18 years of age, children are excluded from this study)

7. Adequate organ function as defined by the following criteria:

Adequate Organ Function Laboratory Values

System	Laboratory Value

Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥8.5 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance	≤1.5 X upper limit of normal (ULN) **OR** ≥30 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2 mg/dL
[a]Creatinine clearance should be calculated per institutional standard.	

8. Patients with leptomeningeal disease are not eligible but patients with brain metastases are eligible – these patients should commence treatment on study > 1 week after completion of gamma knife or whole brain radiotherapy or >4 weeks after surgical resection of brain metastasis. Patients should ideally be off steroids at the start of study treatment, however patients on steroid taper and a dose of no more than 2mg/day of dexamethasone can begin study treatment; and steroids should be tapered off as quickly as clinically feasible.
   Repeat brain MRI after radiation is not required for eligibility but is strongly recommended, to establish the pre-treatment baseline status of any brain metastases, necessary to accurately assess for response or progression.

   Patients with untreated, asymptomatic brain metastasis not requiring steroids are also eligible, however, these patients need to be discussed with the study PI prior to being registered.

9. Subjects must have the ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

The presence of <u>any</u> of the following will exclude a patient from study enrollment:

1. Within 6 months prior to study drug administration, clinically significant cardiovascular/cerebrovascular disease defined as follows: cerebral vascular accident/stroke, myocardial infarction, unstable angina, congestive heart failure (New York Heart Association Classification Class ≥ II), bleeding or pulmonary embolism or cardiac arrhythmias that was severe enough to cause hemodynamic compromise.

2. Known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness (HIV-positive subjects on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with oral THU-Dec. Appropriate studies will be undertaken in subjects receiving combination antiretroviral therapy when indicated.

3. Pregnancy or breastfeeding (pregnant or breastfeeding women are excluded from this study because oral THU-Dec has 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 oral THU-Dec, breastfeeding should be discontinued if the mother is treated with oral THU-Dec.

4. 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.

5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 2 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

6. Has had prior chemotherapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 2 or at baseline) from adverse events due to a previously administered agent and meeting the criteria for organ function described in section 4.1.7 Patients who receive palliative radiation therapy within 1 week prior to day 1 are allowed. Patients on treatment with targeted therapy (like EGFR or ALK TKIs) may start study treatment 5 days from last treatment.
   Note: Subjects with ≤ Grade 2 neuropathy or other clinically insignificant AEs as determined by the PI are an exception to this criterion and may qualify for the study following adequate pre-study documentation.
   Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

7. Receiving other investigational agent concomitantly.

8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include superficial skin cancers that are surgically removed without need for systemic therapy, in situ cervical cancer, superficial bladder cancer or localized low grade prostate cancer not requiring active treatment.

9. Has active or documented history of autoimmune disease that has required systemic treatment in the preceding 2 years (i.e. required use of disease modifying agents such as corticosteroids or other immunosuppressive drugs). Patients with psoriasis and rheumatoid arthritis with no evidence of disease flare off immunosuppression for >1year may be allowed after discussion with the study PI. Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has known history of, or any evidence of active, non-infectious pneumonitis.

11. Has an active infection requiring systemic therapy.

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

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

14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 217 days after the last dose of trial treatment. Women of childbearing potential (WOCBP)* should agree to methods of contraception described in the protocol.

    * WOCBP is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 62 must have a documented serum follicle stimulating hormone (FSH) level >40 mIU/mL.
    Women of childbearing potential (WOCBP) receiving nivolumab will be instructed to adhere to contraception for a period of 23 weeks after the last dose of investigational product. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product. These durations have been calculated using the upper limit of the half-life for nivolumab (25 days) and are based on the protocol requirement that WOCBP use contraception for

5 half-lives plus 30 days and men who are sexually active with WOCBP use contraception for 5 half-lives plus 90 days.

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION
Male condoms with spermicide
Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena® by WOCBP subject or male subject's WOCBP partner Female partners of male subjects participating in the study may use hormone based contraceptives as one of the acceptable methods of contraception since they will not be receiving study drug
Nonhormonal IUDs, such as ParaGard®
Tubal ligation
Vasectomy
Complete Abstinence*

*Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

LESS EFFECTIVE METHODS OF CONTRACEPTION
Diaphragm with spermicide
Cervical cap with spermicide
Vaginal sponge
Male Condom without spermicide
Progestin only pills by WOCBP subject or male subject's WOCBP partner
Female Condom*.

* A male and female condom must not be used together

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

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

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

18. Has received a live vaccine within 30 days of planned start of study therapy.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

4.3 Inclusion of Women and Minorities
Men, women and members of all races and ethnic groups are eligible for this trial.

5.0 REGISTRATION

All subjects who have been consented are to be registered in the OnCore™ Database. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded. All subjects will be registered through Cleveland Clinic and will be provided a study number by contacting the study coordinator listed on the cover page.

6.0 TREATMENT PLAN

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

6.1 Nivolumab: Nivolumab will be given every two weeks at a dose of 240mg IV to be administered as a 60 minute IV infusion. Subjects may be dosed no less than 12 days from the previous dose of drug. There are no pre-medications recommended for nivolumab on the first cycle. The dosing calculations should be based on the actual body weight at baseline. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the original dose, the dose must be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed. See section 7 for dose delay guidelines.

Treatment of Nivolumab Related Infusion Reactions:
Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, 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 medical monitor and reported as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI CTCAE (Version 4.0) guidelines.

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

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated): Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic 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 nivolumab administrations.
For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, iv fluids]; prophylactic medications indicated for 24 hours): Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent)

and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms.

Additional dose modifications (dose delays) of nivolumab are described in **Section 7.0**

6.2 Oral Tetrahydrouridine (THU) and Decitabine (Dec):

- **THU** is supplied as capsules containing 250 mg of THU per capsule, in bottles containing 8 capsules per bottle. See Section 9 for drug handling and storage recommendations.
- **Dec** is supplied as capsules containing 5 mg of Dec per capsule, in bottles containing 8 capsules per bottle.

- **Starting dose of oral THU and Dec is by weight (all subsequent doses are based on the Cycle 1 Day 1 dose with dose modifications based on toxicities and tumor response as described in the protocol)**:
Weight 40-60kg = 2 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 61-80kg = 3 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 81-100kg or higher = 4 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.

Subsequent dose changes should be based on hematologic parameters, non-hematologic toxicities attributed to study drug or as specified in the protocol.

- **Timing between THU and Dec**: Oral THU capsules followed ~60 minutes later by oral Dec capsules (oral Dec can be taken up to 6 hours after oral THU).

- **Frequency of THU and Dec ingestion**:
Oral THU ~10 mg/kg is administered 60 minutes before oral decitabine ~0.2 mg/kg 2X/week on consecutive days if there is no ≥ grade 3 treatment-related non-hematologic toxicity and the ANC is $>0.5x10^9$/L (if ANC is $<0.5x10^9$/L, treatment is held until recovery above this threshold then resumed with a dose reduction as per Table 1).

- Patients will be required to have the treatment on the same consecutive days every week. If patients miss a dose they need they can make up the dose the same week (±3 days) and the study team needs to be notified.

- G-CSF neutropenia prophylaxis is recommended 1X/week if the ANC is <1.5x109/L

If there is stable disease or partial response by RECIST v1.1 at the week 8 or 16 staging CTs, and there are no treatment-related ≥grade 3 toxicities, and blood count trends (trends in the absolute neutrophil, absolute lymphocyte and platelet counts) suggest that such treatment-related toxicities are not imminent, the dose of THU and Dec can be increased by one capsule each. THU-Dec dose increases should not occur

more often than every 4 weeks and only to a maximum of 2 additional capsules of each drug from the starting dosage.

-Pre-medications are not required, since nausea, vomiting and diarrhea are not anticipated with this non-cytotoxic mode of therapy.
Dose modifications (dose reductions, dose escalations) of oral THU-Dec are described in Section 7.0

Reported adverse events and potential risks of oral THU-Dec are described in Section 8.0

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's malignancy.

6.3 General Concomitant Medications

The following medications are prohibited during the study (unless utilized to treat a drug-related adverse event):

a) Immunosuppressive agents
b) Immunosuppressive doses of systemic corticosteroids

- Any concurrent antineoplastic therapy (ie, chemotherapy, hormonal therapy, immunotherapy, extensive radiation therapy, or standard or investigational agents for treatment of NSCLC Palliative and supportive care for disease related symptoms may be offered to all subjects on the trial. Palliative (limited-field) radiation therapy is permitted for subjects who have investigator assessed clinical benefit. However, radiation to target lesions is not permitted. For example patients with asymptomatic oligo-metastatic brain metastatic disease that would clinically be amenable to Gamma Knife Radiosurgeryis allowed on the protocol so long as they are not having systemic disease progression and per investigator are receiving clinical benefit from the trial.
- Inter-current illness that prevents further administration of treatment
- The investigator considers it, for safety reasons, to be in the best interest of the subject
- Unacceptable treatment related toxicity, NCI CTC AE version 4.0 Grade 3 or 4 that fails to recover to baseline or < Grade 3 in the absence of treatment within 4 weeks See section 6.1.1, 6.1.2 and 6.1.3.
- Subject decision to withdraw from treatment (partial consent) or from the study (full consent)
- Pregnancy during the course of the study for a child-bearing participant
- Death, or
- Sponsor reserves the right to temporarily suspend or prematurely discontinue this study.
- The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments

6.5 Stopping Rule

If 3 of the first 5 patients in the THU-Dec + nivolumab arm are removed from study for grade 3/4 non-hematologic toxicity or toxicities prior to cycle 2 that are likely attributable to THU-Dec, the study will be terminated.

The study incorporates a two-stage design in the experimental THU-Dec + nivolumab arm. If after the 1st stage of 20 patients in the THU-Dec+nivolumab arm, 2 or fewer respond, the trial will be stopped and the null hypothesis that THU-Dec+nivolumab is no better than nivolumab will be accepted, else the trial continues for an additional 20 patients.

6.6 Duration of Follow Up

Subjects will be followed for toxicity for 30 days after treatment has been discontinued, until start of new anti-cancer treatment or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause, unless the event exceeds 6 months in duration. Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately. All randomized subjects who discontinue study treatment must continue to be followed for collection of outcome and/or survival follow-up data every 2 months until death or subjects withdraw consent from all study related activities. The survival and toxicity follow up of the patients could be by telephone or through review of medical records.

6.7 Post Study Access to Therapy

At the conclusion of the study, subjects who continue to demonstrate clinical benefit will be eligible to receive study drug. Study drug will be provided via an extension of the study, a rollover study requiring approval by responsible health authority and ethics committee, or through another mechanism at the discretion of the sponsor. The sponsor reserves the right to terminate access to study drug if any of the following occur: a) the marketing application is rejected by responsible health authority; b) the study is terminated due to safety concerns; c) the subject can obtain medication from a government sponsored or private health program; or d) therapeutic alternatives become available in the local market.

7.0 DOSE MODIFICATION

Potential dose modifications of oral THU-Dec include dose reductions and dose escalations, described in sections below.

7.1 Dose Reduction of THU-Dec

Table 1 lists standard laboratory values that should trigger dose reduction of the investigational drug oral THU-Dec.

Table 1: Trigger Values that Result in Dose Reduction of oral THU-Dec

Measurement	Trigger value for Dose Modification	Dose Modification
Absolute neutrophil count (ANC)	$<0.5 \times 10^9$/L (non-cytotoxic DNMT1-depletion shifts normal hematopoietic differentiation away from neutrophils towards platelets and hemoglobin)	Hold treatment with THU-Dec until ANC$\geq 0.5 \times 10^9$/L, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in treatment frequency of THU-Dec to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion. Only continue treatment if it is judged to be in the best interests of the patient. Only continue treatment if it is judged to be in the best interests of the patient. G-CSF growth factor support is recommended if ANC is $<1.5 \times 10^9$/L.
Absolute lymphocyte count	$<0.5 \times 10^9$/L	Hold treatment with THU-Dec until absolute lymphocyte count $\geq 0.5 \times 10^9$/L, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in treatment frequency of THU-Dec to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion. Only continue treatment if it is judged to be in the best interests of the patient.
Platelets	$>1{,}200 \times 10^9$/L (see above, differentiation shifts with non-cytotoxic DNMT1-depletion	Withhold until platelets $\leq 1{,}200 \times 10^9$/L, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in

	can increase platelet counts)	THU-Dec frequency to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion.
Hemoglobin	Hemoglobin >17g/dl (differentiation shifts with non-cytotoxic DNMT1-depletion can increase hemoglobin)	Withhold until hemoglobin ≤17 g/dl, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in THU-Dec frequency to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion.
Serum creatinine	≥1.6 mg/dL (if pre-treatment value <0.5mg/dL) ≥1.7 mg/dL (if pre-treatment value 0.6-0.8 mg/dL) Doubling of creatinine (if pre-treatment value 0.9-2.9 mg/dL)	Neither Dec nor THU are known to be nephrotoxic. Consider other causes of worsening renal function. If no other causes are identified, hold THU-Dec until levels recover below threshold, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in THU-Dec frequency to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion.
ALT	ALT ≥ 2.5X the upper limit of normal	Neither Dec nor THU are anticipated to be hepatotoxic. Consider other causes of worsening liver function (e.g., hepatitis). If no other causes

		are identified, hold THU-Dec until levels recover below threshold, then restart with decrease in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt study drug administration until recovery from toxicity then resume with a reduction in THU-Dec frequency to 1X/week. If toxicity recurs at this dose, interrupt study drug until toxicity resolved then resume at same dose and frequency. The overall goal is to sustain regular administration, at a lower dose if necessary, rather than infrequent administration of a higher dose, however, a minimum dose is required to produce DNMT1 depletion.

Non-Hematologic Toxicity ≥ Grade 3:

Hold drug until recovery to grade 1 or baseline then resume with a reduction in dose by 1 capsule each of Dec and THU. If recurrent toxicity occurs, interrupt drug administration and after recovery from the toxicity, resume therapy with a decrease in frequency of administration to 1X/week. The overall goal is to sustain regular administration at a lower dose if necessary, rather than infrequent administration of a higher dose. Only continue treatment if it is judged to be in the best interests of the patient.

7.2 Dose Escalation of THU-Dec

Inter-individual differences in cytidine analogue metabolism (e.g., differences in CDA expression between individuals[39]) are anticipated to require the ability to increase dose if necessary in some individuals in order to better achieve the intended molecular pharmacodynamic effect of non-cytotoxic DNMT1-depletion. The following considerations guide the option of dose escalation:

If there is stable disease or partial response by RECIST v1.1 at the week 8 or 16 staging CTs, and there are no treatment-related ≥grade 3 toxicities (Table 1), and blood count trends (trends in the absolute neutrophil, absolute lymphocyte and platelet counts) suggest that such treatment-related toxicities are not imminent, then the dose of THU and Dec can be increased by one capsule each. THU-Dec dose increases should not occur more often than every 4 weeks and only to a maximum of 2 additional capsules of each drug from the starting dosage. **ALTERNATIVELY,** if the patient is receiving oral THU-Dec 1X/week because of previous frequency reduction, the frequency of administration can be increased to 2X/week at the same dose of oral THU-Dec.

7.3 Dose Modification or dose delay of Nivolumab

No Dose modification for nivolumab is suggested. Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention,

management algorithms have been developed for suspected AEs of selected categories. See Appendix III

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab). If AE is felt to be immune related and attributable to nivolumab, if the patient is on the study arm they can continue THU-Dec while nivolumab is held.

Dose delay criteria apply for all drug-related AEs. Nivolumab must be delayed until treatment can resume

Nivolumab administration should be delayed for the following:
Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:
- Grade 3 lymphopenia or leukopenia does not require dose delay.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

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

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

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- 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 timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

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

8.0 ADVERSE EVENTS AND POTENTIAL RISKS

8.1 Decitabine

Dec is manufactured by ScinoPharm (Taiwan) and formulated by KP Pharmaceutical Technologies (Bloomington, IN, USA) as per Federal Good Manufacturing Practice guidelines. Dec is supplied as capsules containing 5 mg of Dec per capsule, in bottles containing 8 capsules per bottle, together with a drying agent. Bottles should be tightly closed and stored at 2-8^{o}C in the refrigerator. 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.

Side-Effects of Dec: Dec is an intravenously-administered therapeutic which is FDA-approved for the treatment of the blood cancer myelodysplastic syndrome (MDS) under the brand name Dacogen. As such, the toxicology, pharmacology and ADME profile of Dec has been reviewed by the FDA (NDA #21,790).
There is substantial information regarding the toxicity of Dec 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 Dec doses planned in this study. Leukopoenia 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 Dec 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 NCI/CTEP grade 2 or higher toxicity from Dec. 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, and specifically, without evidence by correlative studies for an increase in thrombotic tendency.

8.2 Tetrahydrouridine

THU is a uridine analogue competitive inhibitor of CDA with a multi-decade track-record of safety in several clinical trials[92-103]. THU does not have therapeutic utility as a separate entity, instead, it has been used to increase the activity of co-administered cytidine analogues in these clinical trials[92-103]. In almost all these clinical studies, the intended pharmacodynamic effect of the co-administered cytidine analogue (e.g., cytarabine) was cytotoxicity, and toxicities were from the co-administered cytidine analogue and not from THU[28,92-103]. Therefore, this clinical protocol contrasts with these previous studies in that THU is being used to create a pharmacologic profile of Dec that avoids cytotoxicity while simultaneously increasing non-cytotoxic DNMT1-depletion.

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 (THU) (published data).

THU dose	THU Route	Combination drug	Number of patients	Toxicity attributed to THU?
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
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
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 hepatoxicity, diarrhea. No attribution to direct THU effect (attributed to Ara-C co-administered with THU)
2 - 4 mg/kg (approx 75-150 mg/m^2) for 3	oral	Oral 5-azacytidine 0.2mg/kg for 3	2	None reported

consecutive days every week		consecutive days every week		
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
200 mg/m^2 x 1	IV, SQ and oral	none	5	None reported

Side-Effects of THU: The single dose toxicology of THU was evaluated in dogs and Rhesus monkeys at intravenous doses of up to 1000mg/kg[104]: with the exception of local inflammation at the injection site, no notable toxic effects were attributed to the administration of THU. 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, including with oral administration for more than 1 year[92-98] (summarized in **Table 2**). 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.[92] 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[98]. A similar experience is described when THU was administered at 700 mg/m^2 daily for 4 consecutive days together with cytosine arabinoside.[96] 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).

Side-Effects of THU and decitabine in combination: In the Phase 1 clinical trial of oral THU and decitabine in combination administered 2X/week on consecutive days for 8 weeks at the doses used in this clinical trial, the side-effects of the combination treatment were the side-effects expected with non-cytotoxic DNMT1-depletion by decitabine, that is, an increase in the platelet counts and total hemoglobin concurrent with a decrease in the absolute neutrophil counts.

Astex Pharmaceuticals has evaluated the combination of an orally ingested THU analog (a fluorinated THU) in combination with oral decitabine 15-40 mg per day for 5 consecutive days repeated every 28 days to treat patients with myeloid malignancies. The dose of decitabine evaluated in combination with the THU analog was thus ~2-3X higher than the doses of decitabine used in this clinical trial. There were no significant non-hematologic adverse events attributed to the combination, with the main risks being related to myelosuppression as seen with similar doses of decitabine alone.

Side-Effects of Nivolumab: See section 1.4.2 and Nivolumab Investigator Brochure for information on the Adverse events of nivolumab, the standard of care drug in this protocol.

8.2 Definitions

8.2.1 Adverse Event

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

NONSERIOUS ADVERSE EVENT

- Nonserious Adverse Events are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g. IND US trial] as part of an annual reporting requirement.
- Nonserious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

A ***nonserious adverse event*** is an AE not classified as serious.

Nonserious Adverse Event Collection and Reporting

The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30days following the last dose of study treatment, unless they start other treatments for the cancer within that time, transition to hospice or expire within 30 days after treatment. AE evaluation can be done by chart review and/or telephone encounter.

Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption

or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

8.2.2 Serious Adverse Events

SERIOUS ADVERSE EVENTS

A S***erious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Potential drug induced liver injury (DILI) is also considered an important medical event.

- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs Potential drug induced liver injury is defined as:

1) ALT or AST elevation > 3 times upper limit of normal (ULN)

AND

2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

3) No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product

exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety).

The investigator must immediately notify Worldwide Safety at BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form which is provided upon request from BMS.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

8.2.3 Adverse Event Evaluation

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.

The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE)
- Description of the event
- Date of onset and resolution
- Expectedness of the toxicity
- Grade of toxicity
- Attribution of relatedness to the investigational agent- (this must be assigned by an investigator, sub-investigator, or treating physician)
- Action taken as a result of the event, including but not limited to; no changes,

dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received conmed or other intervention, etc.
- Outcome of event

Descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

Protocol must specify if attribution is required for individual components of the treatment regimen or the treatment regimen as a whole.

8.3 SAE Report Form

SAEs will be recorded on the FDA Form 3500A (MedWatch) but should only be reported as instructed below. The electronic FDA SAE reporting forms should not be used.

8.4 Reporting Procedures for Serious Adverse Events

Since there is an early stopping rule for this clinical trial (Section 6.4), all AEs related to toxicity that cause patients to withdraw from the study in the first 5 patients should be reported to the DSTC in "real-time".

For the purposes of safety reporting, all adverse events will be reported that occur on or after Cycle 1 Day 1, through 30 days after the final dose of study drug. Adverse events, both serious and non-serious, and deaths that occur during this period will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es). Related AEs will be followed until resolution to baseline or grade 1 or stabilization.

8.4.1 SAE Reporting Requirements

Participating investigators (all sites) must report all serious adverse events to the Sponsorwithin **24 hours** of discovery or notification of the event. The participating investigator must also provide follow-up information on the SAE until final resolution. Sponsor contact:

- Yogen Saunthararajah, saunthy@ccf.org and research coordinator, Sherry Fada, FADAS@ccf.org

The Sponsor will review the SAE and report the event to the FDA, external collaborator(s), and IRB as applicable.

It is the Sponsor-Investigator's responsibility (e.g. lead site PI) to ensure that ALL serious adverse events that occur on the study (e.g. ALL SAEs that occur at each enrolling institution) are reported to all participating sites.

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

- An SAE report should be completed for any event where doubt exists regarding its seriousness.
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection in the protocol.
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)
- If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization. All SAEs should be followed to resolution or stabilization.

Institutional Review Board Reporting Requirements:
Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.5 SAEs and OnCore

All SAEs will be entered into OnCore.

A copy of the SAE form(s) submitted to the sponsor-investigator is also uploaded into Oncore.

8.6 Data Safety and Toxicity Committee

It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the Case Comprehensive Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

The sponsor-investigator is responsible for submitting an annual report to the DSTC as per CCCC Data and Safety Monitoring Plan.

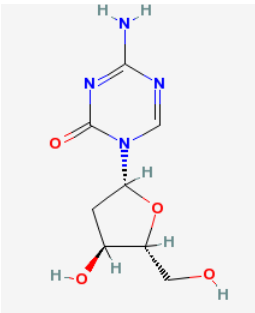
8.7 Data and Safety Monitoring Plan (DSMP)

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 8.

9.1 Decitabine (Dec)



Decitabine (Dec), NSC127716
5-aza-2'-dexocytidine

9.1.1 Product Description

Dec is manufactured by ScinoPharm (Tainan, Taiwan) and formulated by KP Pharmaceutical Technologies (Bloomington, IN, USA) as per current Federal Good Manufacturing Practice guidelines. Dec is supplied as capsules containing 5 mg of Dec per capsule, in bottles containing 8 capsules per bottle, together with a drying agent. Bottles should be tightly closed and stored in the refrigerator at 2-8ºC. 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.

9.1.2 Therapeutic Classification:
DNA methyl-transferase enzymes (DNMTs) recognize and methylate target deoxycytidines that precede deoxyguanines (CpG), and are components of larger multi-protein complexes that mediate gene repression (silencing). Decitabine (Dec) is a deoxycytidine analogue with an unmodified deoxyribose and a single-base modification in the pyrimidine ring. This chemistry produces DNMT1-depletion without termination of DNA chain elongation or cytotoxicity within certain concentration ranges. The mechanism of action of Dec begins with uptake into cells, which involves membrane nucleoside transporters such as the human equilibrative nucleoside transporter (hENT1), followed by Dec phosphorylation, which is initiated by deoxycytidine kinase (DCK), then Dec nucleotide incorporation into the replicating genome. DNMT1 is covalently trapped, modified and depleted as it tries to methylate the 5-N position of the triazine ring of DNA-incorporated Dec. Replacement of 0.3% of cytidine in genomic DNA with Dec is sufficient to deplete 95% of DNMT1[66]. Unlike cytidine analogues such as cytarabine or gemcitabine, the sugar back-bone of Dec is physiologic. Therefore, at low concentrations, Dec does not terminate DNA chain synthesis[67,68], and can deplete DNMT1 without causing significant DNA damage or cytotoxicity, both *in vitro* and *in vivo*[67-72]. Low doses of Dec sufficient to deplete DNMT1 and produce hypomethylation do not overwhelm the cellular DNA-repair machinery, and although temporary growth arrest (cytostatic effect) may occur, cell division ultimately continues but with depleted DNMT1, DNA hypomethylation and consequently altered gene expression[66,70,73,74]. At high Dec concentrations, hydrolytic cleavage at DNMT-CpG complexes overwhelms the cellular DNA-repair machinery, damaging the replicating genome to an extent that results in cell-death[73]. Within cells, and at mucosal barriers and in the plasma, cytidine deaminase (CDA) is the enzyme most important in the break-down of Dec.

9.1.3 Method of Administration

- **Starting dose of oral THU and Dec is by weight (all subsequent doses are based on the Cycle 1 Day 1 dose with dose modifications based on toxicities and tumor response as described in the protocol)**:
Weight 40-60kg = 2 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 61-80kg = 3 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 81-100kg or higher = 4 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.

Subsequent dose changes should be based on hematologic paramters, non-hematologic toxicities attributed to study drug or as specified in the protocol.

- **Timing between THU and Dec**: Oral THU capsules followed ~60 minutes later by oral Dec capsules (oral Dec can be taken up to 6 hours after oral THU).

- **Frequency of THU and Dec ingestion**:

Oral THU ~10 mg/kg is administered 60 minutes before oral decitabine ~0.2 mg/kg 2X/week on consecutive days if there is no ≥ grade 3 treatment-related non-hematologic toxicity and the ANC is >$0.5x10^9$/L (if ANC is <$0.5x10^9$/L, treatment is held until recovery above this threshold then resumed with a dose reduction as per Table 1).

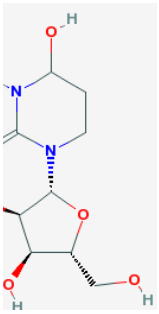
- Patients will be required to have the treatment on the same consecutive days every week. If patients miss a dose they need they can make up the dose the same week (±3 days) and the study team needs to be notified.

9.1.4 Storage requirements: Capsules should be stored in the original bottles in which they are supplied in the refrigerator at 2-8ºC.

9.1.5 Drug Accountability: The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

9.1.6 Drug Destruction: At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return of unused study drug. Unused drug is to be shipped back to source at completion of the study. Please contact Dr Yogen Saunthararajah, 9500 Euclid Avenue, R40, Cleveland, OH 44195, tel: 216 444 8170, email: saunthy@ccf.org regarding shipping arrangements.

9.2 Tetrahydrouridine (THU)



3,4,5,6-Tetrahydrouridine, NSC112907
2(1H)-pyrimidinone, tetrahydro-4-hydroxy-1-beta-D-ribofuranosyl

9.2.1 Product description: The THU is manufactured by ASH Stevens (Detroit, MI, USA) and formulated by KP Pharmaceutical Technologies (Bloomington, IN, USA) as per Federal Good Manufacturing Practice guidelines. THU is supplied as capsules containing 250 mg of THU per capsule, in bottles containing 8 capsules per bottle, together with a drying agent. Bottles should be tightly closed and stored in the refrigerator at 2-8ºC. 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.

9.2.2 Therapeutic Classification

Tetrahydrouridine (THU) is a competitive inhibitor of the pyrimidine metabolism enzyme cytidine deaminase (CDA) with K_i values of 3-5x10^{-8}M[75,76]. 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[76]. CDA binds THU approximately 4-orders of magnitude more tightly than uridine, the substrate for the reverse reaction catalyzed by CDA[76]. However, slow onset of inhibition suggests that structural reorganization precedes the formation of a stable enzyme-inhibitor complex[76].

9.2.3 Dosing Information:

- **Starting dose of oral THU and Dec is by weight (all subsequent doses are based on the Cycle 1 Day 1 dose with dose modifications based on toxicities and tumor response as described in the protocol)**:

Weight 40-60kg = 2 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 61-80kg = 3 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.
Weight 81-100kg or higher = 4 capsules of each drug. Dec capsules are ingested ~60 minutes after THU capsules.

Subsequent dose changes should be based on hematologic parameters, non-hematologic toxicities attributed to study drug or as specified in the protocol.

- **Timing between THU and Dec**: Oral THU capsules followed ~60 minutes later by oral Dec capsules (oral Dec can be taken up to 6 hours after oral THU).

- **Frequency of THU and Dec ingestion**:

Oral THU ~10 mg/kg is administered 60 minutes before oral decitabine ~0.2 mg/kg 2X/week on consecutive days if there is no ≥ grade 3 treatment-related non-hematologic toxicity and the ANC is >0.5x10^9/L (if ANC is <0.5x10^9/L, treatment is held until recovery above this threshold then resumed with a dose reduction as per Table 1).

- Patients will be required to have the treatment on the same consecutive days every week. If patients miss a dose they need they can make up the dose the same week (±3 days) and the study team needs to be notified.

9.2.4 Storage requirements: Capsules should be stored in the original bottles in which they are supplied in the refrigerator at 2-8ºC.

9.2.5 Drug Accountability: The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no

circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

9.2.6 Drug Destruction: At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return of unused study drug. Unused drug is to be shipped back to source at completion of the study. Please contact Dr Yogen Saunthararajah, 9500 Euclid Avenue, R40, Cleveland, OH 44195, tel: 216 444 8170, email: saunthy@ccf.org regarding shipping arrangements.

9.3 Nivolumab

Nivolumab (Opdivo™) is manufactured by Bristol Myers Squibb (New Jersey, USA) as per Federal Good Manufacturing Practice guidelines. It is FDA approved for use in metastatic NSCLC. The drug for the study will be provided by Bristol Myers. See section 1.4 and 6.1 for details on the drug and dosing.

PRODUCT INFORMATION TABLE: Please also see Drug Information in Investigators brochure

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

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

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

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for "Recommended Storage and Use Conditions"

Handling and Dispensing

The investigator should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as per product information and the Investigator Brochure and per local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact BMS immediately.

Please refer to the current version of the Investigator Brochure and/or shipment reference sheets for additional information on storage, handling, dispensing, and infusion information for nivolumab.

Destruction

Sponsor/Investigator drug destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the Sponsor SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, ie, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for BMS to review throughout the clinical trial period as per the study agreement.

If conditions for destruction cannot be met, please contact BMS.

It is the Sponsor Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

Dose calculations and administration

Describe timing of first dose from registration/randomization. For preparation and administration details please refer to the current Investigator Brochure.

Nivolumab will be given every two weeks at a dose of 240mg IV flat dose to be administered as a 60 minute IV infusion.

Subjects may be dosed no less than 12 days from the previous dose of drug. There are no premedications recommended for nivolumab on the first cycle.

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

Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to Protocol Section 6.1. Doses of nivolumab may be interrupted, delayed, or discontinued depending on how well the subject tolerates the treatment.

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) and 40 mg/mL (10 mg/mL) Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 1 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

Dose Modifications

Dose reductions or dose escalations are not permitted.

Management Algorithms

Guidelines for the management of immune related events can be found in the current Investigator Brochure AND in the approved USPI in the US. Investigators should decide the appropriate source of AE management for each protocol.

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

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

For subjects expected to require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider recommendations provided in the algorithms. These algorithms are found in the Nivolumab IB [and in Appendix] of this protocol. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

- Grade 4 lymphopenia or leucopenia
- Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Investigator

- Any dosing interruption lasting > 6 weeks with the following exceptions:
  - Dosing delays or interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted or delayed
  - Dosing interruptions or delays lasting > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing
- Treatment beyond radiographic progression is allowed if it is felt by the investigators that there is clinical benefit. If new or pre-existing asymptomatic brain lesions are noted on surveillance MRI. These patients need to be followed closely with follow up brain imaging on treatment and can receive gamma knife therapy while actively receiving therapy on study – study drugs do not require disruption while receiving gamma knife therapy unless clinically indicated to do so. Use of steroids after Gamma knife therapy should be minimized but can be used per clinical discretion of the treating radiation oncologist.

Treatment of Nivolumab Related Infusion Reactions

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE Version 4.0 guidelines.

Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab will be administered at that visit. Administer diphenhydramine

50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

10.0 STUDY CALENDAR

Trial Period:	**Screening Phase**		**Treatment Cycles[a]**																**End of Treatment**	**Post-Treatment**	
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1		2		3		4		To be repeated beyond 8 cycles: 5		6		7		8		Discon	Safety Follow-up	Survival Follow Up[b]
Scheduling Window (Days):		-28 to -1	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	D1 ±4 days	D15 ±4 days	At time of Discon	30 days post discon	Every 12 weeks post discon
Administrative Procedures																					
Informed Consent	X																				
Inclusion/Exclusion Criteria	X																				
Demographics and Medical History	X																				
Prior and Concomitant Medication Review	X																				
Randomization		X																			
Nivolumab			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
THU-Dec			2X/week																		
G-CSF neutropenia prophylaxis			G-CSF (e.g., Neupogen, Granix) Recommended 1X/week if ANC is <1.5 x 10^9/L																		
Post-study anticancer therapy status																				X	X
Survival Status																				X	X
Clinical Procedures/Assessments																					
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Full Physical Examination		X																			
Directed Physical Examination		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory																					
Pregnancy Test – Urine or Serum β-HCG		X																			
CBC with Differential		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Comprehensive Serum Chemistry Panel		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Urinalysis		X																			
T3, FT4 and TSH		X	X				X				X				X						
Efficacy Measurements																					
Tumor Imaging		X[c]					X				X				X						
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood																					
Archival or Newly Obtained Tissue Collection		X[d]			X[d]																
Correlative Studies Blood Collection		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

a) Patients will receive Nivolumab every 2 weeks (± 4 days per protocol) (4 weeks i.e 2 treatments=1 cycle) until progression (by RECIST v1.1) or unacceptable toxicity. A treatment break for upto 6 weeks for will be allowed upon discussion with study PI
b) Patients will be monitored post study every 12 weeks (± 3 weeks) for post-study treatment status, clinical safety updates and survival; these could be done by review of patient's medical records or contacting patient's medical provider or the subject.
c) Patients disease will be monitored with CT scans of the chest, abdomen and pelvis after every 2 cycles (i.e prior to every odd cycle +/- 7 days) or sooner if clinically indicated;
d) Patients who have recent (<6 weeks from start of study treatment) tissue biopsy or patients who have had recurrence after surgery within 6 months of surgical resection may be eligible without a fresh biopsy; Rest of the patients will require a biopsy prior to study enrollment. Patients will require a repeat biopsy in 6-10 weeks post treatment prior to cycle 3 of nivolumab. If the biopsy is felt to be high risk by the treating physician or interventional radiologist, an exception may be made upon discussing with the PI.
e) For Cycle 1 Day 1 (C1D1) labs, screening labs can be used if ≤1 week; pregnancy tests need to be done within 24hrs of first dose.

11.0 CORRELATIVE STUDIES

Tumor Biopsies:
Percutaneous US or CT-guided core-needle biopsy: Patients are ineligible if they refuse percutaneous biopsy or if in the judgement of interventional radiology or pulmonologist the risk of significant complications exceeds 2%[105]. If percutaneous biopsy is feasible, it is planned at protocol screening, between week 6-10, and at disease progression (biopsy at progression is mandatory if a subject wishes to cross-over). Where possible, FFPE tissue from the time of original diagnosis should also be obtained.

Percutaneous, bronchoscopy or surgical biopsies could be considered; The objective is to obtain 0.5-1.5 cm cores. The tissue samples are processed according to specifications in the correlatives/lab manual;

Peripheral blood collections for PK and correlative studies: Patients will have baseline and follow up on treatment pre-treatment blood sample for correlatives to evaluate markers described in **section 1.6**; Detailed blood collection procedures, processing, handling and shipping information are provided in the accompanying **Laboratory Manual**.

12.0 MEASUREMENT OF EFFECT

12.1 Primary End Point
Objective response: Objective response (OR) is the primary endpoint of this trial, subjects with measurable disease will be assessed by standard criteria. For the purposes of this study, subjects should be re-evaluated with CT scan with contrast (if clinically indicated

non-contrast CT or PET/CT could be used in lieu of CT with contrast) every 8 of weeks i.e after every 2 cycles (4 treatments) of Nivolumab.

Evaluation of response: Response and progression will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[106]. For the purpose of this study treatment will be discontinued in patients who experience disease progression by RECIST v1.1 (see Appendix IVa) with the following caveat: since it is known that there can be initial progression by RECIST v1.1 criteria with immune checkpoint blockade followed by subsequent regression and clinical benefit, if the treating clinical team and the patient judge that they have benefitted from therapy, treatment continuation is allowed even if there is disease progression by RECIST v1.1 criteria. However, patients should have confirmed stable disease or better at the time of the following scheduled imaging time-point.**ORR is defined as** the proportion of all randomized subjects whose best overall response (BOR) from baseline is either a CR or PR per RECIST v1.1 criteria. BOR is determined by the best response designation recorded between the date of randomization and the date of objectively documented progression or the date of subsequent anti-cancer therapy, whichever occurs first. For subjects without documented progression or subsequent anticancer therapy, all available response designations will contribute to the BOR determination. For subjects who continue treatment beyond progression, the BOR should be determined based on response designations recorded at the time of the initial RECIST v1.1.

12.2 Secondary End Point

a) *Progression free survival:* It is defined as the time from randomization to the date of the first documented tumor progression (per RECIST v1.1) or death due to anycause. Subjects who die without a reported prior progression will be considered to have progressed on the date of their death. Subjects who did not progress or die will be censored on the date of their last evaluable tumor assessment. Subjects who did not have any on study tumor assessments and did not die will be censored on the date they were randomized. Subjects who started any subsequent anti-cancer therapy without a prior reported progression will be censored at the last evaluable tumor assessment prior to initiation of the subsequent anti-cancer therapy. Progression will be assessed every 6 weeks (from the first on-study radiographic assessment) until disease progression is noted.

b) *Overall survival:* It is defined as the time from randomization to the date of death. A subject who has not died will be censored at last known date alive. OS will be followed continuously while subjects are on the study drug and every 3 months via in-person or phone contact after subjects discontinue the study drug.

c) *Safety and tolerability:* Safety and tolerability objective will be measured by the incidence of adverse events, serious adverse events, deaths, and laboratory abnormalities. Adverse event assessments and laboratory tests are performed at baseline, and continuously throughout the study at the beginning of each subsequent cycle. The safety analysis will be performed in all treated subjects. Descriptive statistics of safety will be presented using

National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 by treatment arm. All treatment emergent AEs, drug-related AEs, SAEs and drug-related SAEs will be tabulated using worst grade per NCI CTCAE v 4.0 criteria by system organ class and preferred term. On-study lab parameters including hematology, coagulation, chemistry, liver function and renal function will be summarized using worst grade per NCI CTCAE v 4.0 criteria.

d) *Exploratory analysis:* Other exploratory endpoints for include mainly anti-tumor immune response evaluation (tumor adaptive and innate cell immune responses), tumor tissue biomarker analysis, imaging based biomarkers and molecular pharmacodynamics analysis (DNMT1-protein in peripheral blood buffy coat cells and by measurement of plasma CDA enzyme activity by HPLC enzyme assay). These are discussed in detail in section 1.6.

All subjects who signed an informed consent form, randomized and registered for the study will be included in the primary dataset for analyses of baseline characteristics, efficacy and biomarker analysis. All treated subjects, i.e all randomized subjects who received at least one dose of nivolumab or 1 dose of THU-Dec combination will be evaluated for safety and tolerability.

13.0 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

The Overture and OnCore™ Databases will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for both accrual entry and trial data management. Overture and OnCore™ are Clinical Trials Management Systems housed on secure servers maintained at Case Western Reserve University. Access to data through Overture and OnCore™ is restricted by user accounts and assigned roles. Once logged into the Overture or OnCore™ system with a user ID and password, Overture and OnCore™ define roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu.

Overture and OnCore™ are designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Form completion in the Overture database. A calendar of events and required forms are available in Overture and OnCore™.

13.2 **Regulatory Considerations**

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

13.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided. The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject. Additionally, documentation of the consenting process should be located in the research chart.

13.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

13.2.3 Retention of records

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with local, national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

13.2.4 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. For multi-center studies, participating sites must inform the sponsor-investigator of pending audits.

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APPENDIX I

PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity. Full active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	ble 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	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% if waking hours.	60	equires occasional assistance, but is able to care for most of his/her needs.
		50	quires considerable assistance and frequent medical care.
3	n bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	sabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	00% 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

APPENDIX II

SUBJECT CAPSULE DIARY FOR TWICE WEEKLY DOSING

Add or delete columns and rows and revise the information in *red* as appropriate for the trial.

Subject Name _____ **Subject Study ID** _____ **Today's date** ___/___/___

Drug _____ **Cycle #:**_____

INSTRUCTIONS FOR THE SUBJECT:

1. Complete one form every ***4 weeks*** (one treatment cycle).
2. You will take ___*number of capsules* of THU 60 minutes before ___*number of capsules* of Decitabine on _____*day of week* and _____*day of week (*twice a week). ***Take the capsules with or without food, as you wish.***
3. Record the date, the number of capsules of each drug that you took, and what time you took them.
4. If you have any comments or notice any side effects, please record them in the "Comments" column.
5. Please bring this form and your bottles of THU and Decitabine to your physician when you return for each appointment.
6. Please sign your name at the bottom of the diary.

Day	Date	Time of THU dose	# of capsules taken			Time of Decitabine dose	# of capsules taken			Comments
			___ mg	___ mg			___ mg	___ mg		
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
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21										
22										
23										
24										
25										
26										
27										
28										**Add/remove days as needed**

Subject's Signature: _____ Date: _____

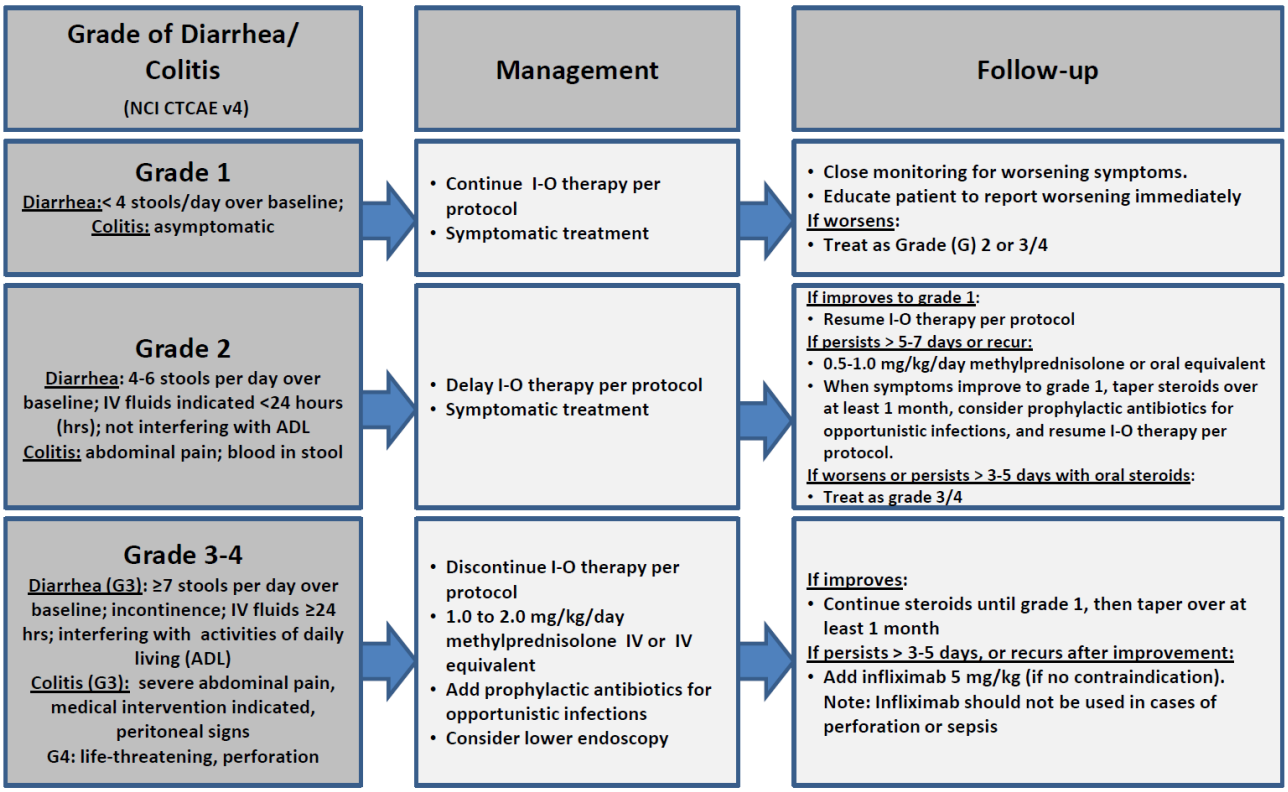
Appendix III

Immune related adverse event management algorithms

These general guidelines constitute guidance to the Investigator. The guidance applies to all immuno-oncology (I-O) agents and regimens. A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated. Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended. The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

GI Adverse Event Management Algorithm

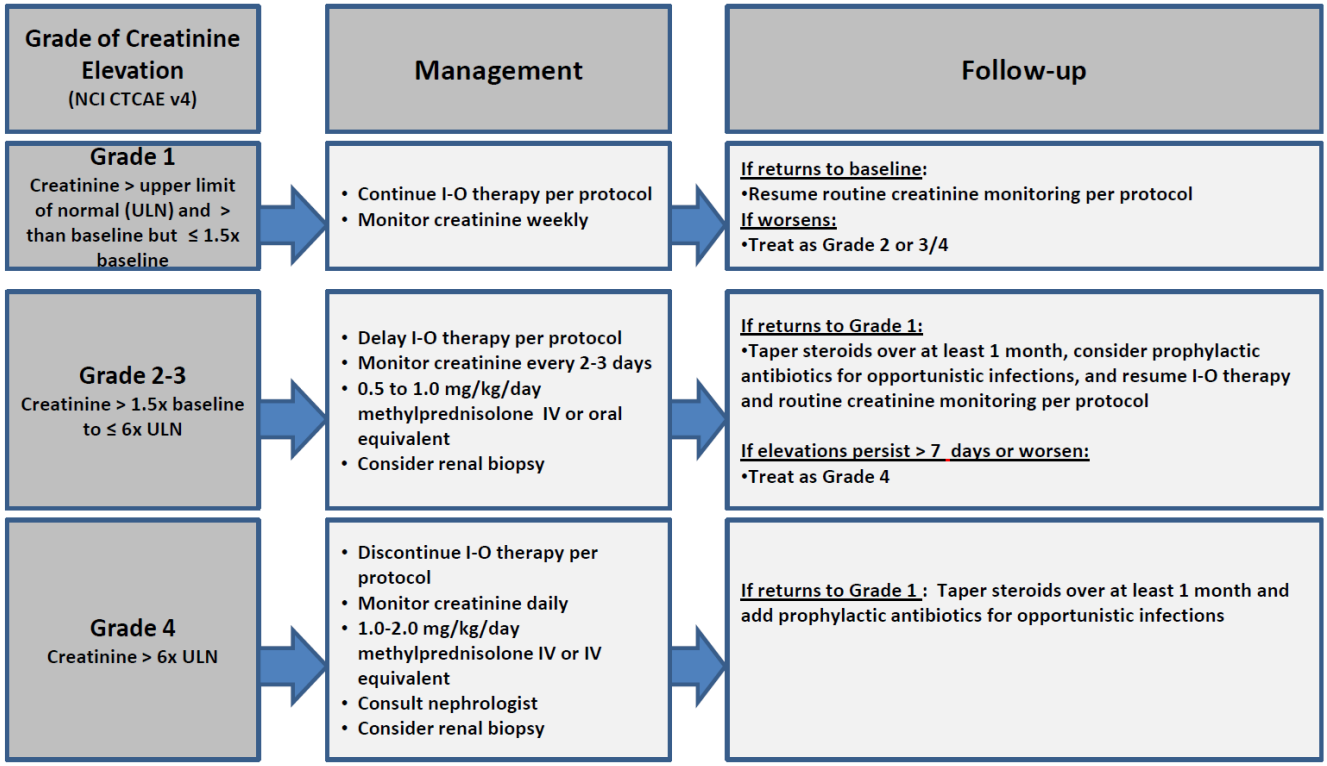
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

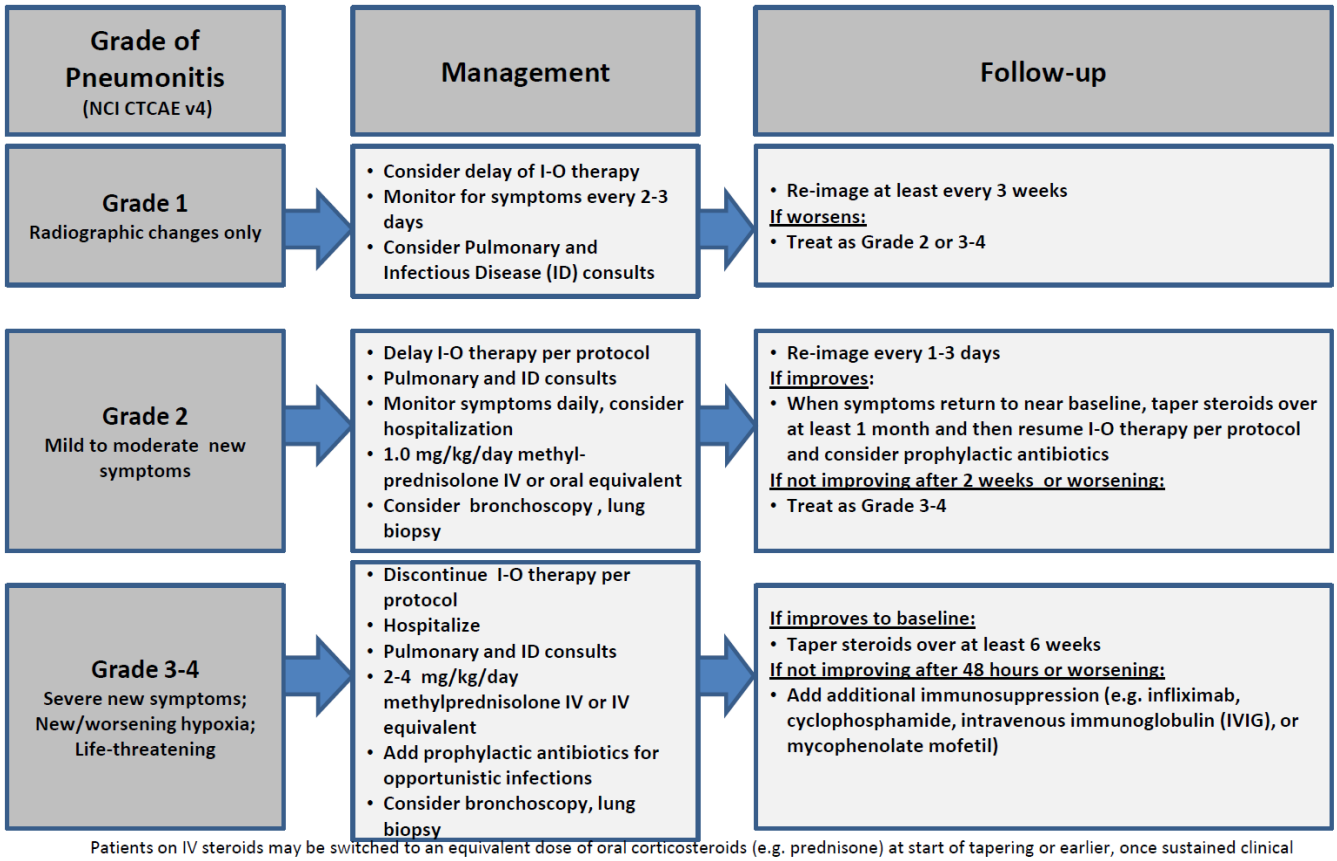
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

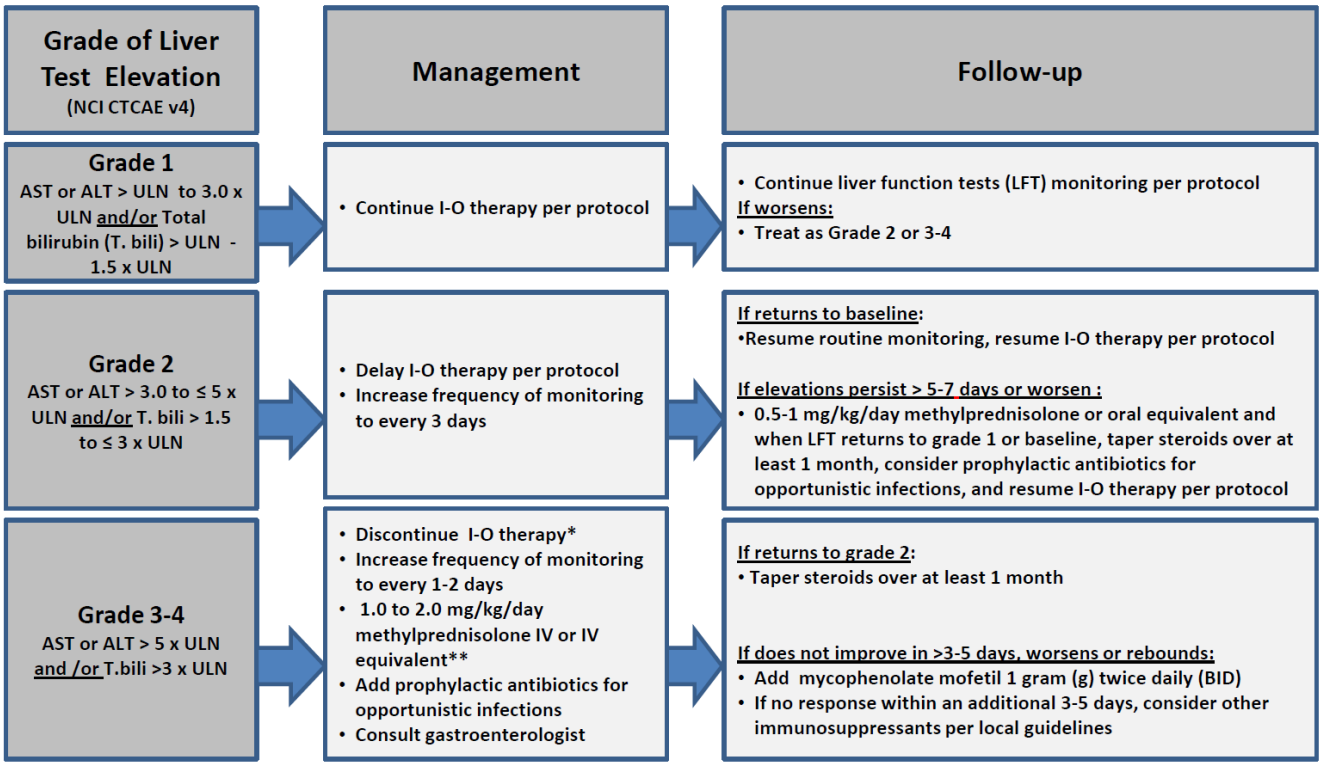
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



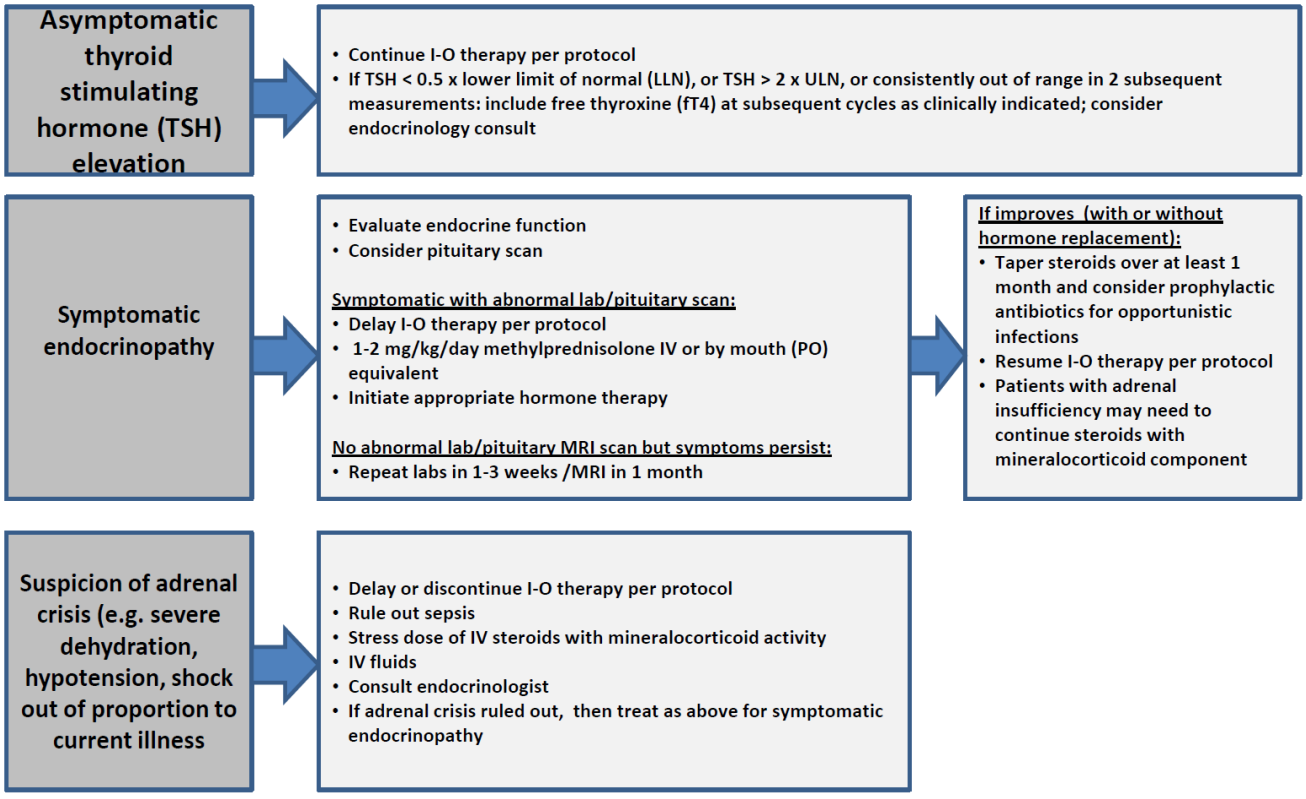
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.**

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Management Algorithm

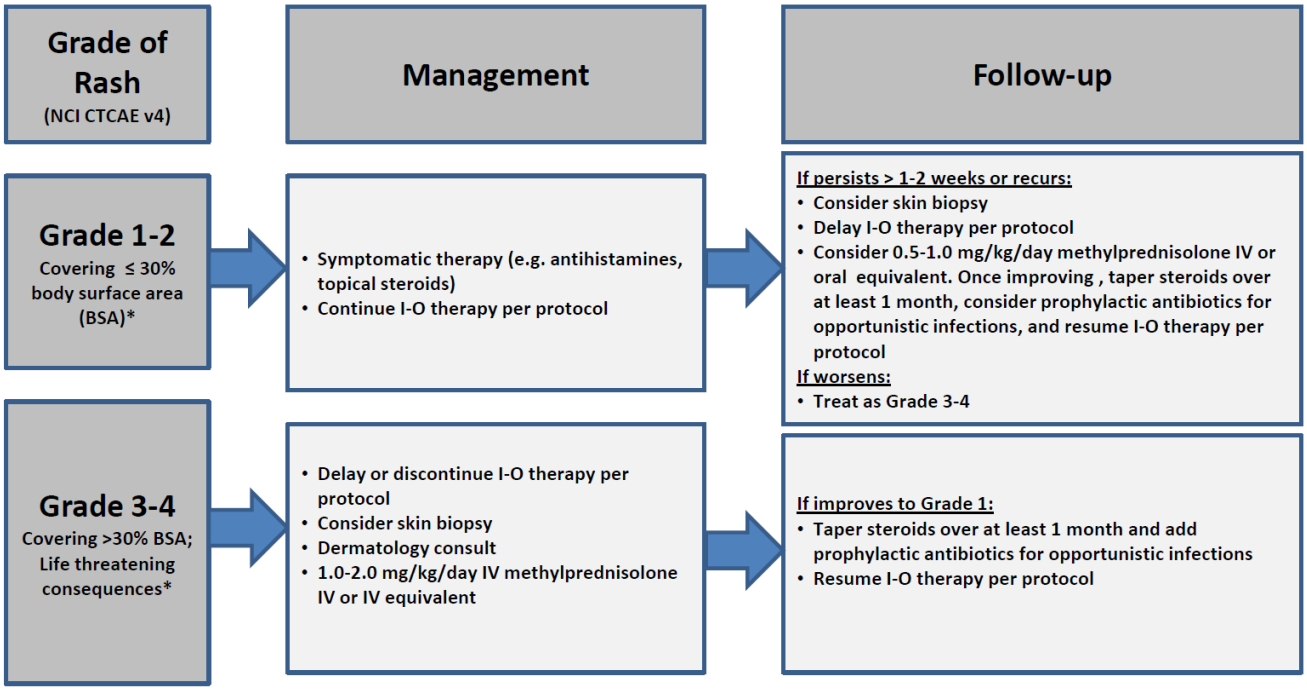
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

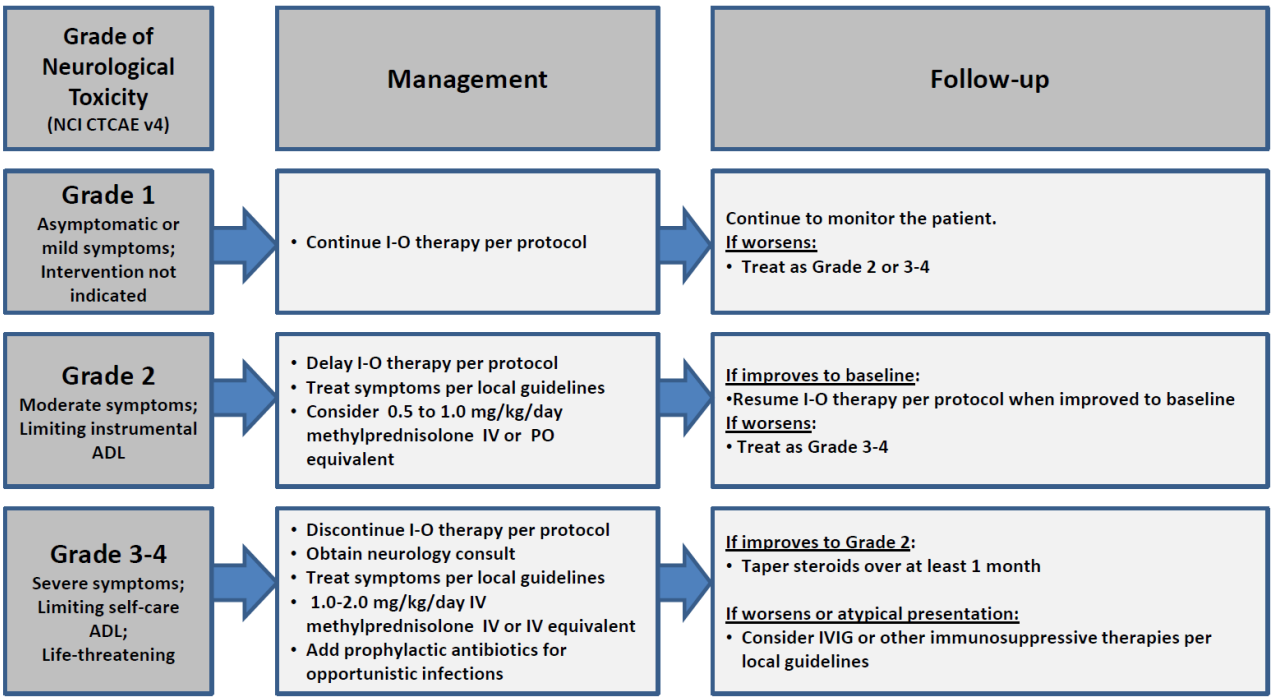


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v4 for term-specific grading criteria.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Appendix IV

A) RECIST Criteria:

Measurable Disease
Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MRI, x-ray) or as ≥10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable Disease
All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, CNS metastases, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target Lesions
All measurable lesions up to a maximum of five lesions per organ and 10 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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target Lesions
All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

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.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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 when both methods have been used to assess the antitumor effect of a treatment.

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

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.

Conventional CT and MRI
These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US)
When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy and Laparoscopy
The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers
n/a

Cytology and Histology
These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain).

Response Criteria

Evaluation of Target Lesions
Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded from the time treatment was started or from the time treatment was held or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment was started or held.

Evaluation of Non-target Lesions
Complete Response (CR): Disappearance of all non-target lesions

Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) or/and 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

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair).
Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

Target Lesions	Non-target Lesions	New Lesions	Overall Response

CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

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

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 lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

Confirmatory Measurement/Duration of Response

Confirmation
n/a

Duration of Overall Response
The duration of overall response is measured from the time measurement criteria are first met for CR or PR (whichever is first recorded) until the first date that progression of disease is objectively documented **while on study drugs** (taking as reference for progressive

disease the smallest measurements recorded since the treatment was started or since treatment was last initiated).

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

Duration of Stable Disease
Stable disease is measured from the start of the treatment until the criteria for progression are met **while on study drugs**, taking as reference the smallest measurements recorded since the treatment started or since treatment was last initiated.

Time to Disease Progression
Time to disease progression will be recorded from the first day of protocol therapy until the criteria for disease progression are met while on sunitinib treatment, patient death from any cause or removal of the patient from study for any reason, whichever comes first.