SUMMARY OF CHANGES – Protocol

For Protocol Amendment # 19

NCI Protocol #: 9706
Local Protocol #: CIRB 15-0185

Protocol Date: 04/25/2023

The following changes were made to protocol Amendment #18:

#	Section #	Description
1.	n/a	Protocol version date updated to 4/25/2023
2.	Cover Page	PI updated from Hongtao Liu to Wendy Stock.
3.	Cover Page	Responsible study coordinator updated to remove J Peterson and add CTSO contact information.
4.	Cover Page	Responsible Research Nurse contact information deleted.

NCI Protocol #: 9706

Local Protocol #: CIRB15-0185

ClinicalTrials.gov Identifier: NCT02275533

TITLE: RANDOMIZED PHASE II STUDY TO ASSESS THE ROLE OF NIVOLUMAB AS SINGLE AGENT TO ELIMINATE MINIMAL RESIDUAL DISEASE AND MAINTAIN REMISSION IN ACUTE MYELOGENOUS LEUKEMIA (AML) PATIENTS AFTER CHEMOTHERAPY (REMAIN TRIAL)

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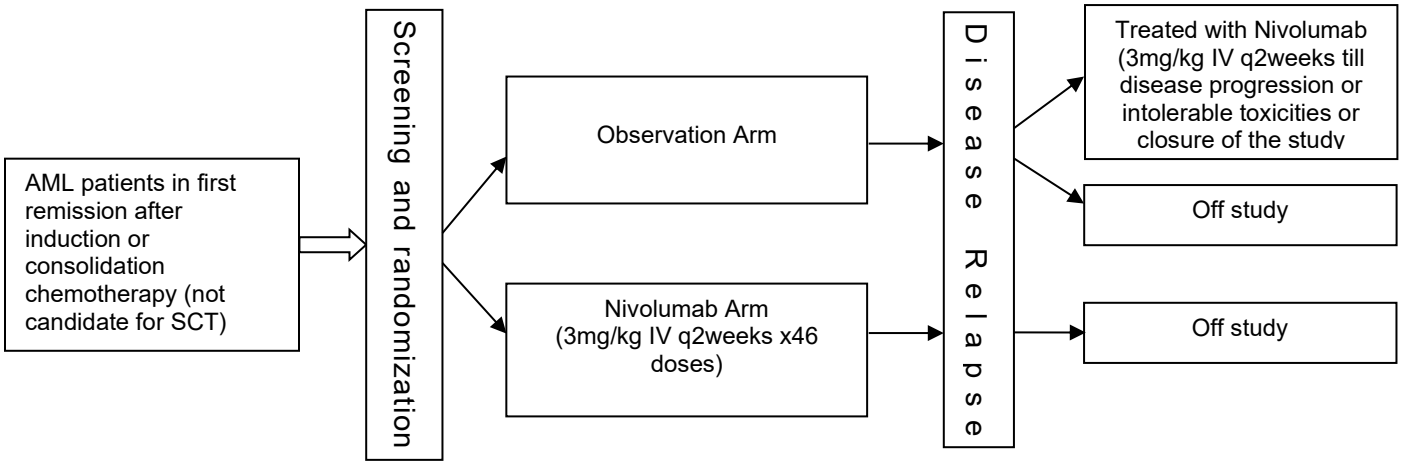
NCI-Supplied Agent): Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC #748726)
Other Agent(s): Not applicable
IND #:
IND Sponsor: DCTD, NCI

PROTOCOL TYPE / VERSION # / VERSION DATE:
Initial Protocol 09/17/2014
Consensus Review 12/04/2014
Follow Up Review 01/07/2015
Follow Up Review 02/16/2015
Follow Up Review 03/06/2015
Follow Up Review 04/21/2015
Initial Review Approval 05/05/2015
Amendment #1 08/13/2015
Amendment #2 10/23/2015
Amendment #3 02/26/2016; 03.11.2016
Amendment #4 07/07/2016
Amendment #5 01/17/2017 (disapproved)

Amendment #6 01/25/2017
Amendment #7 08/30/2017
Amendment #8 02/20/2018
Amendment #9 08/13/2018 (disapproved)
Amendment #10 08/16/2018 (disapproved)
Amendment #11 08/21/2018
Amendment #12 01/29/2019 (disapproved)
Amendment #13 02/21/2019 (disapproved)
Amendment #14 03/22/2019
Amendment#15 07/16/2019 (disapproved)
Amendment#16 08/06/2019
Amendment#17 09/24/2019 (in response to CIRB approved with modifications comments to Amendment #16)
Amendment #18 03/12/2021 (response to RRA to update CAEPR to v2.4)
Amendment #19 04/25/2023 (PI Change)

SCHEMA


AML patients in first remission after induction or consolidation chemotherapy (not candidate for SCT)
Screening and randomization
Observation Arm
Nivolumab Arm (3mg/kg IV q2weeks x46 doses)
Disease Relapse
Treated with Nivolumab (3mg/kg IV q2weeks till disease progression or intolerable toxicities or closure of the study
Off study
Off study

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1. OBJECTIVES

1.1 Primary Objectives

To evaluate and compare the progression free survival rate after randomization in the two treatment arms (Nivolumab vs. observation)

1.2 Secondary Objectives

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1.2.2 To determine and compare the incidence of non-relapse mortality in the two arms
1.2.3 To evaluate the toxicities of Nivolumab as maintenance

1.3 Exploratory Objectives

1.3.1 To analyze PD-LI expression on AML cells from peripheral blood and/or bone marrow samples at diagnosis if available and at the time of study enrollment
1.3.2 To monitor AML MRD by *WT1* PCR at enrollment and at subsequent defined time points in the Nivolumab-treated and control groups
1.3.3 To perform an exploratory analysis on the frequencies, absolute numbers and subsets of T cells (including regulatory T cells) in the Nivolumab-treated and control groups with an emphasis on activation markers
1.3.4 To perform deep sequencing of TCR-α and TCR-β chains on polyclonal T cells at baseline and at subsequent time points in the Nivolumab and control groups

2. BACKGROUND

2.1 AML - Standard treatment

Standard intensive chemotherapy regimens induce complete remission in the majority of adults AML patients under age 60, but maintenance of durable remissions remains a challenge. Post-remission strategies to eradicate minimal residual disease (MRD) after induction chemotherapy include repeated cycles of cytarabine-based consolidation chemotherapy (Mayer, Davis et al. 1994), autologous stem cell transplantation (ASCT) (Farag, Ruppert et al. 2005) and allogeneic stem cell transplantation (Allo-SCT) (Cassileth, Harrington et al. 1998). For AML patients who are not candidates for SCT, the current standard of care is observation after induction/consolidation chemotherapy. Unfortunately, more than 50% of patients eventually will experience a disease relapse (Mayer, Davis et al. 1994). The outcome of older AML patients (>= 60 years) is more dismal with an estimated 2 year progression free survival (PFS) of approximately 20% without SCT (Mrozek, Marcucci et al.). Thus, new post-remission strategies are needed in order to improve the long-term outcomes of AML patients.

2.2 Immunotherapy of cancer

Over the past two decades, evidence has mounted suggesting that the immune system can play an active role in the elimination of malignant cells. However, although spontaneous anti-tumor immune responses are generated in a subset of patients, immune-mediated eradication of

established tumors is rare. These observations suggest the existence of immune evasion mechanisms which are activated in the cancer-bearing host which are capable of potent suppression of the anti-tumor immune response (Gajewski, Meng et al. 2006). A growing number of putative immune evasion mechanisms have been characterized, including extrinsic suppression by regulatory T cells (Tregs), T cell anergy, diminished T cell activation by engagement of negative co-stimulatory T cell molecules, such as PD-1 and CTLA-4 (reviewed in (Gajewski, Meng et al. 2006; Zou 2006; Colombo and Piconese 2007)). Recently, strategies aimed at reversing immune escape pathways have led to impressive clinical results, particularly those which involve blocking so-called "checkpoint" inhibitors of T cell function, such as CTLA-4, and the PD-1/PD-L1 pathway (Hamid, Robert et al. ; Weber, Kudchadkar et al. ; Wolchok, Kluger et al. ; Bashey, Medina et al. 2009; Hodi, O'Day et al. 2010).

2.3 The role of negative co-stimulatory regulators in AML

Up-regulation of negative T cell co-stimulatory receptors, such as CTLA-4 and PD-1, on tumor-specific T cells clearly inhibit their effector function in solid malignancies, such as melanoma. Administration of antibodies which block these receptors, or their ligands in the case of PD-1, leads to clinical benefit in a subset of patients with melanoma and other solid tumors (Hamid, Robert et al. ; Weber, Kudchadkar et al. ; Wolchok, Kluger et al. ; Bashey, Medina et al. 2009; Hodi, O'Day et al. 2010). In pre-clinical AML models, blocking CTLA-4 in combination with a peptide-based vaccine has led to enhanced CTL responses and prolonged survival, providing rationale for targeting this receptor in AML patients.

With regard to the PD-1/PD-L1 pathway, we have generated pre-clinical data to suggest that this pathway is involved in immune evasion in AML. Our group (Dr. Justin Kline working with Dr. Thomas Gajewski) has published that the PD-1/PD-L1 pathway was involved in immune evasion in a murine AML model. When C1498 AML cells were inoculated into animals, they grew progressively and apparently evaded immune destruction (Zhang, Gajewski et al. 2009). Low levels of PD-L1 expression were found on C1498 cells grown *in vitro*. Exposure of AML cells to IFNγ led to significant PD-L1 up-regulation Figure 1A) and (Figure 2A in (Zhang, Gajewski et al. 2009)). In addition, PD-L1 expression was up-regulated on C1498 cells when analyzed directly *ex vivo* (Figure 1B and Figure 2B in (Zhang, Gajewski et al. 2009)). PD-$1^{-/-}$ mice challenged with C1498 cells generated augmented antitumor T-cell responses, showed decreased AML burden in the blood and other organs, and survived significantly longer than wild-type mice did (Figure 1C and Figure 4C in (Zhang, Gajewski et al. 2009)). Similar results were obtained with a PD-L1 blocking antibody (Zhang, Gajewski et al. 2009). To determine whether PD-L1 was expressed on human AML cells, bone marrow samples from AML patients were analyzed for expression patterns of PD-L1. Three staining patterns were observed: 1) no PD-L1 expression on either CD34+ or CD34- cells, 2) expression of PD-L1 on the CD34-, but not on CD34+ cells, and 3) PD-L1 expression on CD34+ AML cells, but not on CD34- cells (Figure 1D). These preliminary data support a hypothesis that PD-L1 expression on a subset of AML cases might serve as a possible baseline biomarker for response to PD-1-blocking antibodies, as has been demonstrated in melanoma patients (Topalian, Hodi et al.). The identification of PD-L1 expression on human AML cells, in addition to pre-clinical data demonstrating a negative impact of the PD-1/PD-L1 pathway on anti-leukemia immunity; provide strong rationale for testing PD-1 blockade in AML patients.

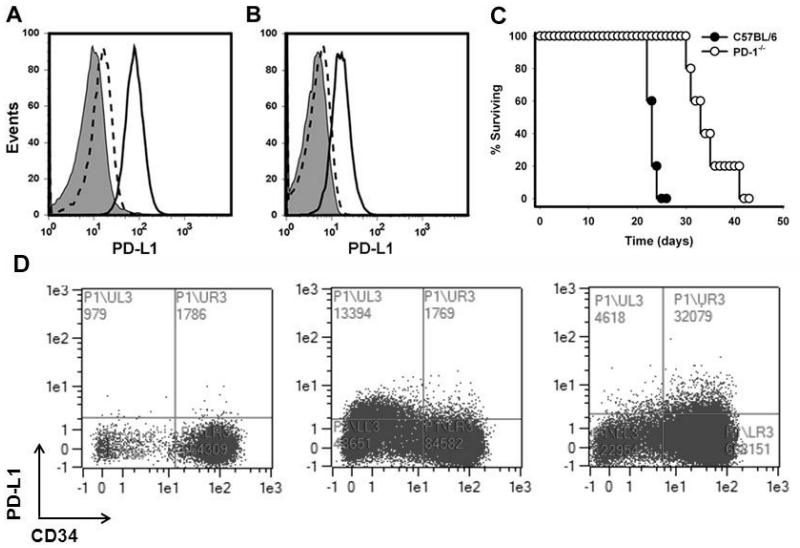

Figure: PD-L1 is expressed on murine and human AML cells and promotes immune evasion in a pre-clinical AML model. (A) PD-L1 expression on C1498 AML cells by flow cytometry either at baseline or after *in vitro* exposure to IFN-□ for 48 hours. The shaded histogram represents staining with an isotope control antibody. The dashed line represents PD-L1 expression at baseline, and the solid line represents PD-L1 expression after exposure to IFN-γ. (B) PD-L1 expression on C1498 AML cells following IV inoculation into C57BL/6 mice. (C) C1498 AML cells were inoculated into syngeneic control or PD-$1^{-/-}$ mice and survival was assessed. (D) Three patterns of PD-L1 expression on bone marrow samples from AML patients. Bone marrow samples were stained with anti-CD34 and anti-PD-L1 antibodies and analyzed by flow cytometry. Representative plots are shown above.

2.4 CTEP IND Agent: Nivolumab

Nivolumab (BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor (Investigator Brochure, 2013). PD-1 is a negative regulatory molecule that is expressed transiently following T-cell activation and on chronically stimulated T cells characterized by an "exhausted" phenotype. Nivolumab binds to cynomolgus monkey PD-1 but not mouse, rat, or rabbit molecules. Clinical activity of nivolumab has been observed in patients with melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC). The combination of nivolumab and ipilimumab (anti-cytotoxic T lymphocyte associated antigen-4 [anti-CTLA-4]) in a phase 1/2 trial showed markedly enhanced clinical activity with an acceptable safety profile in melanoma patients (Wolchok *et al*., 2013).

The clinical use of monoclonal antibodies to T-cell inhibitory receptors has provided transformative information on the nature of the immune system and cancer. An emerging picture suggests that endogenous immune responses can mediate effective tumor regression and/or improved survival even in patients with large volume tumors resistant to other forms of therapy. Some of the unique features of this type of therapy, based largely on experience in advanced melanoma, include: improved overall survival (OS) with or without radiographic responses or improved progression-free survival (PFS); responses that may be delayed or occur after radiographic disease progression; combinations of immune modulators with enhanced or novel

activities (in the example of ipilimumab and nivolumab); and toxicity that is almost exclusively immune or inflammatory in nature. It is not yet clear what factors determine responses and which components of the immune system are needed for this to occur. It seems likely that both memory helper and effector cells would be needed to sustain long-term responses. Increasing emphasis has been placed on understanding the relationships of the tumor, celluar infiltrate, and immunologic milieu surrounding each tumor.

PD-1, a 55-kDa type 1 transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors that include Ig super family member CD28, CTLA-4, inducible co-stimulator (ICOS), and B and T lymphocyte attenuator (BTLA) (Investigator Brochure, 2013). PD-1 is transiently but highly expressed on activated T cells functioning to limit immune effectors at the site of activation. Chronic stimulation may prevent the re-methylation of the PD-1 gene leading to continuous expression and characterizes a state of "exhausted" T cells that lose function and proliferative capacity while enhancing a suppressive tumor microenvironment. PD-1 may act together with other T-cell modulating molecules, including CTLA-4, TIM-3, lymphocyte-activation gene 3 (LAG-3) as well as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta (TGF-beta).

Two ligands specific for PD-1 have been identified: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs], and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). The interaction of PD-1 with PD-L1 and PD-L2 results in negative regulatory stimuli that down-modulate the activated T-cell immune response through SHP-1 phosphastase.

PD-1 knockout mice develop strain-specific lupus-like glomerulonephritis (C57BL/6) and cardiomyopathy (BALB/c). In transplantable tumor models that expressed PD-1 and LAG-3 on tumor-infiltrating $CD4^+$ and $CD8^+$ T cells dual anti-LAG-3/anti-PD-1 antibody treatment cured most mice of established tumors that were largely resistant to single antibody treatment (Woo *et al*., 2012). Despite minimal immunopathologic sequelae in PD-1 and LAG-3 single knockout mice, dual knockout mice abrogated self-tolerance with resultant autoimmune infiltrates in multiple organs, leading to eventual lethality.

PD-L1 expression is found on a number of tumors, and is associated with poor prognoses based on OS in many tumors, including melanoma (Taube *et al*., 2012), renal (Thompson *et al*., 2004; Thompson *et al*., 2005; Thompson *et al*., 2006), esophageal (Ohigashi, *et al.* 2005), gastric (Wu *et al*., 2006), ovarian (Dong *et al*., 2003), pancreatic (Nomi, *et al*., 2007), lung (Zitvogel, *et al*., 2006), and other cancers (Investigator Brochure, 2014).

The PD-1/PD-L1 axis plays a role in human infections, particularly in hepatitis C virus (HCV) and human immunodeficiency virus (HIV). In these cases, high expression levels of PD-1 were found in viral-specific $CD8^+$ T cells that also display a non-responsive or exhausted phenotype. Non-responsive PD-1-high T cells were observed in simian immunodeficiency virus (SIV) infection in rhesus macaques. Treatment of SIV-infected macaques with an anti-PD-1 mAb (3 mg/kg x4) resulted in decreased viral loads and increased survival along with expanded T cells with increased T-cell functionality.

2.4.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated (Investigator Brochure, 2013). Combination studies have highlighted the potential for toxicity when combined with ipilimumab, MDX-1408, and BMS-986016. Nivolumab bound specifically to PD-1 (and not to related members of the CD28 family such as CD28, ICOS, CTLA-4, and BTLA) with a K_d = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

2.4.2 Clinical Development of Nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC, hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications (Investigator Brochure, 2013). In addition, two investigator-sponsored trials (ISTs) of nivolumab in combination with a peptide vaccine in melanoma are being conducted in the adjuvant setting and advanced disease.

Seven nivolumab studies were conducted in Japan, including six studies in advanced solid tumors and recurrent or unresectable stage III/IV melanoma sponsored by Ono Pharmaceuticals Co. Ltd., and one IST in recurrent or advanced platinum-refractory ovarian cancer.

2.4.3 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (C_{max}) and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), with low to moderate inter-subject variability observed at each dose level (Investigator Brochure, 2013). Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

2.4.4 Efficacy

In a phase 1 (1, 3, and 10 mg/kg nivolumab doses) dose-escalation study the 3 mg/kg dose was chosen for expanded cohorts. Among 236 patients, objective responses (ORs) (complete or partial responses [CR or PR]) were seen in NSCLC, melanoma, and RCC. ORs were observed at all doses (Sznol *et al*., 2013). Median OS was 16.8 months across doses and 20.3 months at the 3 mg/kg dose. Median OS across all dose cohorts was 9.2

months and 9.6 months for squamous and non-squamous NSCLC, respectively (Brahmer *et al*., 2013). In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year (Drake *et al*., 2013).

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered IV every 3 weeks for 4 doses followed by nivolumab alone every 3 weeks for 4 doses (concurrent regimen) (Wolchok *et al*., 2013). The combined treatment was subsequently administered every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent regimen (53 patients), 53% of patients had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced-regimen (33 patients), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC patients, 43 patients were treated with nivolumab + PT-doublet (Rizvi *et al*., 2013). No dose-limiting toxicities (DLTs) were reported and total/confirmed ORRs were 43/33%, 40/33%, and 31/31% in nivolumab/gemcitabine/cisplatin, nivolumab/pemetrexed/cisplatin, and nivolumab/carboplatin/paclitaxel arms, respectively.

2.4.5 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined (Topalian *et al*., 2012). Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, and vasculitis have also been reported.). In combination with ipilimumab in the concurrent-regimen group (Wolchok *et al*., 2013), grade 3 or 4 treatment-related events were noted in 53% of patients. Skin rash represents the majority of these events.

The possible side effects of Nivolumab are summarized below (included in the Investigator Brochure).

The following are the key safety findings for the subjects with advanced malignancies mainly solid tumors.

- Drug-related AEs (any grade) were reported in 75.2%, and drug-related Grade 3-4 AEs were reported in 17.0% of subjects.
  - The most frequently reported drug-related AE was fatigue (28.1%). Other drug-related AEs Grade 3-4 reported in more than 2 subjects were pneumonitis (1.3%), lymphopenia (1.3%), diarrhea (1.0%), abdominal pain (1.0%), CD4 lymphocytes decreased (1.0%), and hypophosphatemia (1.0%).

- The most frequently reported drug-related SAE was pneumonitis (7 subjects, 2.3%).

Drug-related Grade 3-4 pneumonitis was reported in 4 (1.3%).

- The most frequently reported drug-related select AE categories (any grade) were skin (24.5%), GI (14.1%), and endocrine (9.5%).
  - AEs belonging to the pulmonary and renal select AE categories were unexpected, drug-related toxicities associated with the use of nivolumab.
  - AEs belonging to select AE categories were generally manageable and reversible with the use of immunosuppressants.

Below is the safety experience from the single agent nivolumab Phase 1 trial in patients with relapsed or refractory hematologic malignancies (included in the Investigator Brochure).

- The most frequently reported drug-related AEs (> 10% of subjects) were fatigue (12.6%) and rash (10.7%). The majority were Grade 1-2 in severity.
- The most frequently reported drug-related SAE was pneumonitis (4.9%).
- AEs leading to discontinuation were reported for 15.5% of subjects. The most frequently reported AE leading to discontinuation were pneumonitis and nodule (1.9%, each).
- Thirteen subjects have died, with 1 death reported due to study drug toxicity (1 subject in the 3 mg/kg treatment group died due to Grade 5 pneumonitis with onset 10 days after the subject received the only dose of nivolumab).

It is very important to monitor AEs for the patients on the Nivolumab treatment, since getting medical treatment right away may keep these problems from becoming more serious. The local investigators or treating physicians need to be aware the main AEs, and should not hesitate to contact the study chair for any question and management. The management of AEs will be further discussed in Section 5 (Treatment Plan, especially section 5.6: Criteria to resume treatment) and Section 6: Dose delay/Dose modification.

2.4.6 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count (Wolchok *et al*., 2013). With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell $CD8^+$ are of current interest. Until more reliable data based on standardized procedures for tissue collection

and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

The combination of ipilimumab with nivolumab has been reported to result in improved responses in advanced melanoma marked by time to response, number of responses, depth and duration of responses, PFS, and OS compared to single agent ipilimumab (Wolchok *et al*., 2013).

For RCC results have been reported (Hammers *et al*., 2014).

The combination is being evaluated in other disease settings typically with 3mg/kg nivolumab and 1mg/kg ipilimumab q 3 weeks x 4 induction doses.

See Section 8.1.2 for drug information.

2.5 Rationale to utilize the PD-1-blocking antibody, Nivolumab, to eliminate MRD and maintain remission in AML patients after induction/consolidation chemotherapy

Based on our exciting pre-clinical results of PD-1/PD-L1 blockade (Zhang, Gajewski et al. 2009), along with our data demonstrating that PD-L1 is expressed on AML cells in a subset of patients, a logical strategy to eliminate MRD in AML is to enhance the host anti-leukemia immune response by targeting PD-1 (Nivolumab). Thus, we propose a multi-center randomized phase II study to assess the efficacy of Nivolumab as maintenance therapy for AML patients who are not candidates for stem cell transplantation. Nivolumab will be administered IV at 3mg/kg every 2 weeks for forty-six doses. The duration of Nivolumab treatment will be 2 years following randomization based on the experience in melanoma patients (Wolchok, Kluger et al.). Patients will be followed for disease relapse, adverse events, non-relapse mortality and survival. The primary objective will be progression free survival at 2 years. Secondary objectives will be OS, and evaluation of adverse events following Nivolumab administration. Exploratory objectives will include: determination of PD-LI expression on $CD34^+$ and $CD34^-$ cells within the bone marrow as predictive biomarker; eradication of MRD assessed by quantitative RT-PCR of *WT-1* during the Nivolumab treatment; immune responses including WT1 specific CTLs, Treg changes; and TCR repertoire sequencing to determine the TCR clonal evolution during Nivolumab treatment.

2.6 Correlative Studies Background

2.6.1 WT1 as a marker of minimal residual disease in AML

Wilm's tumor 1 (WT1) is a zinc finger transcription factor that is normally expressed in tissues of mesodermal origin during embryogenesis. WT1 is over-expressed in various hematological malignancies, including AML (80-100% of cases). WT1 mRNA levels in the peripheral blood and bone marrow correlate with disease status during and after treatment, and elevation of WT1 after chemotherapy and stem cell transplant predicts clinical relapse. Thus, *WT1* RT-PCR is now being used to monitor minimal residual disease (MRD) during treatment of AML (Reviewed in (Grimwade, Vyas et al. 2010; Dominietto 2011)).

Dr. Wendy Stock's lab assessed MRD for a GVAX vaccine study in patients with AML using quantitative RT-PCR detection of *WT1* expression. Although > 90% of patients who achieved clinical complete remission (CR) had significant declines in *WT1* in both blood and bone marrow, 95% of patients had persistently detectable *WT1* levels in bone marrow demonstrating clear MRD in AML patients in CR (Figure 3 in (Borrello, Levitsky et al. 2009)). Importantly, the achievement of a significant decrease or elimination of *WT1* transcripts during the course of vaccination correlated with significant superior relapse-free survival (table 3 in (Borrello, Levitsky et al. 2009)). Furthermore, patients who relapsed during or following vaccination had a significant increase in *WT1* expression, while patients in continuous CR maintained very low or undetectable *WT1* levels (Figure 4 in (Borrello, Levitsky et al. 2009)).

WT1 MRD monitoring appears to correlate well with remission status in conventionally-treated AML patients as well. Among AML patients in clinical CR after induction and consolidation therapy, 80-90% and 30-40% of patients continue to have detectable *WT1* transcript in the marrow or blood, respectively (Miyawaki, Hatsumi et al.). *WT1* levels in the peripheral blood can predict relapse after CR, and its levels after consolidation therapy are closely correlated with disease free survival (DFS), OS, and early relapse. The DFS rate at 3 years was 20% and 50% for *WT1* MRD positive and negative patients, respectively (Miyawaki, Hatsumi et al.). Thus, *WT1* monitoring is a useful tool not only for predicting relapse in AML patients in clinical CR, but also for monitoring response to post-remission immunotherapeutic strategies, such as vaccination and possibly after post-remission treatment with PD-1 blocking antibodies.

2.6.2 **TCR sequencing during immunotherapy**

Recent advances in deep sequencing technology make it possible to characterize the T cell receptor (TCR) repertoire generated following immunotherapy, such as WT1 peptide vaccination (i.e. the "clonality" of the response). Preliminary studies have demonstrated biased usage of TCR-Vβ gene families in WT1 peptide vaccinated patients (Ochsenreither, Fusi et al. ; Ochsenreither, Fusi et al. ; Tanaka-Harada, Kawakami et al.), which was confirmed by our own TCR sequencing data in a patient who received WT1 peptide vaccination post SCT (Figure 4 in preliminary result section). Dr. Yusuke Nakamura at our institution is one of the pioneers in the assessment of genetic variations (VNTR and SNP markers) and whole-genome analysis in cancer cells. Using a novel sequencing approach, we have been working together to understand the TCR "landscape" during immune-based therapies for AML. TCR sequencing of peripheral blood and/or bone marrow samples during immunotherapy, such as PD-1 blockade, will allow us to assess for an emerging T cell repertoire in patients who respond versus those who do not. This type of analysis may also be useful to identify high-affinity TCRs for use in down-stream adaptive T cell therapy approaches for AML, as well as to enable the identification of new AML associated antigens**.**

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 AML patients in first CR (CR1) or first CRi after induction and/or consolidation chemotherapy; except young (<60 years) AML patients in European LeukemiaNet favorable group. (Since young AML patients in the European LeukemiaNet favorable group have excellent 2 year PFS at around 64%, further maintenance therapy might not provide additional benefit; thus the current trial will exclude young favorable group AML patients (Mrozek, Marcucci et al.). Patients could receive any cycle consolidation or no consolidation per the discretion by the treating physician.

3.1.2 Within 60 days after bone marrow biopsy confirmed remission after the patients recover from their last course of chemotherapy, the goal to consent the eligible patient prior to the remission confirmation bone marrow biopsy at the end of the planned chemotherapy. Ideally, the research samples will be collected during the bone marrow biopsy, and the patient will be enrolled to the study within 2 weeks of the bone marrow biopsy.

If there is delay to enroll the patient after the bone marrow biopsy and research sample collection, it is ok not to repeat bone marrow biopsy within 4 weeks, after the last bone marrow biopsy, if there is no sign of disease relapse.

A repeat bone marrow biopsy should be done if the delay of enrollment is more than 4 weeks after the last bone marrow biopsy.

Patients with confirmed remission within 60 days after the last bone marrow biopsy, without research samples collection, should have a repeat bone marrow biopsy conducted within two weeks prior to enrolling on the study.

3.1.3 Patient is not a candidate for stem cell transplant due to advanced age or co-morbidities; or the enrollee does not have donor available; or the enrollee declines stem cell transplant due to personal belief; or stem cell transplant is not standard of care based on the risk category of disease

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of nivolumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.5 ECOG/Karnofsky performance status of 0 or 1 (Karnofsky ≥70%, see Appendix A).

3.1.6 Life expectancy of greater than 6 months

3.1.7 Patients must have acceptable organ and marrow function as defined below:

- leukocytes ≥1,500/mcL
- absolute neutrophil count ≥1,000/mcL
- platelets ≥50,000/mcL or recovery to the baseline count
- total bilirubin ≤1.5× institutional upper limit of normal (ULN) (except patients with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)

- AST(SGOT)/ALT(SGPT) ≤2.5× ULN
- Amylase and lipase ≤1.5× ULN without any symptoms of pancreatitis
-
- Serum creatinine ≤1.5× ULN
  OR
- creatinine clearance (CrCl) ≥50 mL/min (if using the Cockcroft-Gault formula below):

Female CrCl = $\frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{serum creatinine in mg/dL}}$

Male CrCl = $\frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$

3.1.8 The effects of nivolumab on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. WOCBP should use an adequate method to avoid pregnancy for 23 weeks) after the last dose of investigational drug Nivolumab. Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab. Women must not be breastfeeding. Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. 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. Women who are not of childbearing potential (*i.e.*, who are postmenopausal or surgically sterile as well as azoospermic men) do not require contraception.

Women of childbearing potential (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 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL.

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.

Should a woman become pregnant or suspect she is pregnant while she or her partner is

participating in this study, she (or the participating partner) should inform the treating physician immediately.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events (AEs) due to agents administered more than 4 weeks earlier.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients should be excluded if they have had prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways

3.2.4 Patients with known CNS involvement may be excluded because of poor prognosis and concerns regarding progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. However, if CNS disease is cleared before the treatment with Nivolumab, patients could be allowed if no permanent CNS damage.

3.2.5 History of severe hypersensitivity reaction to any monoclonal antibody.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Pregnant women are excluded from this study because Nivolumab is an agent with 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 *Nivolumab,* breastfeeding should be discontinued if the mother is treated with *Nivolumab*.

3.2.8 Patients with known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS) might be enrolled if the viral load by PCR is undetectable with/without active treatment and absolute lymphocyte count >= 350/ul.

Patients with a positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection might be enrolled if the viral load by PCR is undetectable with/without active treatment.

3.2.9 Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids, should be excluded. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease. Patients with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjögren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

3.2.10 Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger (precipitating event).

3.2.11 Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses ≤10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if ≤10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (*e.g.*, contrast dye allergy) or for treatment of non-autoimmune conditions (*e.g.*, delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

3.2.12 Patients who have had evidence of active or acute diverticulitis, intra-abdominal abscess, GI obstruction and abdominal carcinomatosis which are known risk factors for bowel perforation should be evaluated for the potential need for additional treatment before coming on study.

3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other

circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

We will try to enroll equal number of patients from different genders (the final ratio of male / female should be very close since there is significant difference of AML in different gender). We will encourage the enrollment of patients from different race and ethnicity without any discrimination, but more Caucasians still might be enrolled to the study. Please see the detail in Planned Enrollment Report under Section 13.2.

4. REGISTRATION PROCEDURES (ROSTERED PROTOCOL MODEL)

4.1 Investigator and Research Associate Registration with CTEP

4.1.1 CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at http://ctep.cancer.gov/investigatorResources/investigator_registration.htm

4.1.2 CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account is needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, and is critical to the conduct of this study, including document access, patient enrollment, and clinical data submission.

Additional information can be found on the CTEP website at http://ctep.cancer.gov/branches/pmb/associate_registration.htm.

4.1.3 For Questions and Support

For questions about Investigator Registration, please contact the CTEP Investigator Registration Help Desk: pmbregpend@ctep.nci.nih.gov.

For questions about Associate Registration or CTEP-IAM Account Creation, please contact the CTEP Registration Help Desk: ctepreghelp@ctep.nci.nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain Institutional Review Board (IRB) approval for this protocol and submit all required regulatory documents (including any protocol specific documents) to the CTSU Regulatory Office before they can be approved to enroll patients.

The CTSU Regulatory Office tracks receipt of these documents in the CTSU Regulatory Support System (RSS), reviews for compliance, and transmits site approval data to CTEP.

Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing, or amendment review. However, sites must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB (via IRBManager) to indicate their intention to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office for compliance in the RSS. The Signatory Institution must inform the CTSU which CIRB approved institutions aligned with the Signatory Institution are participating in the study so that the study approval can be applied to those institutions. Other site registration requirements (e.g., laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the 9706 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol

organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, then select EDDOP-IL-057, and protocol #9706
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will automatically load to RSS.)

4.2.2 Requirements For 9706 Site Registration:

- CTSU IRB Certification (for sites not participating via the NCI CIRB)
- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)
- Local informed consent document

Submit required forms and documents to the CTSU Regulatory Office, where it will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-866-651-2878
Fax: 215-569-0206
E-mail: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

4.2.3 Checking **Site** Registration Status

Sites can check the status of their registration packets by querying the Site Registration subtab of the members' section of the CTSU Web site. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in using your CTEP IAM username and password.
- Click on the Regulatory tab at the top of your screen.
- Click on the Site Registration subtab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: If possible, please allow three working days for site registration approval before attempting to enroll your first patient.

4.3 Patient Registration*

***Please notify the University of Chicago general e-mail at phaseIIcra@medicine.bsd.uchicago.edu when you have a potential patient that you would like to screen for the study and to obtain a pre-registration ID for screening BMBX**

samples.

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

For this protocol, sites must first contact the lead institution (University of Chicago) prior to registering the patient in OPEN/IWRS so that the patient can be randomized. They should send an e-mail to the UC Data Manager at phaseIIcra@medicine.bsd.uchicago.edu containing the following information:

Clinical site name
Patient's first and last initials
Date of birth
Age group: <60 or >=60 years
AML risk category: Adverse, Intermediate-1, Intermediate-2, or Favorable

The UC Data Manager will obtain the randomized treatment assignment from REDCap. The UC Data Manager will then login to OPEN/IWRS, enter the assigned treatment, and reserve a slot for the patient. He/she will then notify the clinical site that a slot has been reserved, and the site will access OPEN/IWRS and complete registration of the patient.

The OPEN system will provide the site with a printable confirmation of registration and the treatment assigned to the patient (Nivolumab or Observation). Please print this confirmation for your records.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar.
- To approve slot reservations or access cohort management: Be identified to Theradex as the "Client Admin" for the study.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the CTSU web site as a tool to verify eligibility.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://theradex.com/CTMS/Downloads.aspx. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk: 609-619-7802 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within *14* days. Issues that would cause treatment delays should be discussed with the lead site Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The UC Study Coordinator should be notified of cancellations as soon as possible at: phaseIIcra@medicine.bsd.uchicago.edu

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1.1 Nivolumab

Nivolumab will be given every two weeks (±2 days) (two weeks is equivalent to one cycle) at a dose of 3 mg/kg. Patients may be dosed no less than 12 days from the previous dose of drug.

The dosing calculations should be based on the actual body weight. If the patient'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 if dose rounding is allowed as per institutional guidelines. There will be no

dose modifications allowed.

Nivolumab is to be administered as a 30-minute (+/- 10 minutes) IV infusion, using a volumetric pump with a 0.2-1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be below 1 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

5.2 General Concomitant Medication and Supportive Care Guidelines

There is no clear evidence to demonstrate a potential for interaction of Nivolumab with other concomitantly administered drugs through the cytochrome P450 system.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 46 cycles (92 weeks) or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) which include the following (see also section 6 and specific algorithms in Appendix H):
    - Any grade 4 events.
    - Grade 3 drug-related autoimmune or inflammatory events including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation except as noted below:

:

    - Any other grade 3 non-skin, drug-related AE lasting >7 days including fatigue.
    - Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, <u>not associated with underlying organ pathology</u>, that does not require treatment except for electrolyte replacements **does not** require treatment discontinuation.
    - Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation clinical manifestations of pancreatitis and which decrease to <u>≤</u> Grade 2 within 1 week of onset **may** resume study treatment when resolved.
    - 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

- Patients requiring > two dose delays for the same type of event should go off protocol therapy.

- 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 study drug dosing

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

  Patients being tapered after high dose corticosteroids over one month followed by a two-week observation period will be allowed an additional two weeks to restart treatment (a maximum eight week interruption). Dosing interruptions >6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Principal Investigator must be consulted.

- Tumor assessments should continue as per protocol even if dosing is interrupted.

- Any patients who require additional immune suppressive treatment beyond steroids should go off study treatment

- Patient decides to withdraw from the study, or

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

5.4 Duration of Follow Up

Patients will be followed for up to two years from the enrollment after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Patients who have come off treatment/observation after two years on study without relapse will continue to be followed every 6 months for the first year and yearly until disease relapse or death (whichever occurs first).

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the applicable criteria, including relapse of disease, adverse events, patient withdrawal or inability to follow study protocol as listed in Section 5.4. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.6 Criteria to Resume Treatment

Restarting applies only to grade 2 events and some grade 3 events (skin rash and thyroiditis). The section should emphasize stopping treatment and starting steroids earlier to obtain resolution with the possibility for restarting rather than waiting for higher grade events.

For non-autoimmune or non-inflammatory events patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of Grade 2 fatigue
- Evaluation to exclude any additional immune mediated events endocrine, GI, and liver / pancreas function as clinically indicated must be made prior to restarting.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol the treatment should resume at the earliest convenient point that is within the six week delay period.

If treatment is delayed for >6 weeks, (>8 weeks for patients on a steroid taper), the patient must be permanently discontinued from study therapy, except as specified in Section 5.4 (Duration of Therapy).

For patients treated with corticosteroids for autoimmune side effects:

Grade 2 events must resolve to ≤ Grade 1 before considering retreatment.

All patients treated with steroids for grade ≥2 events should have nivolumab held until resolution to ≤ Grade 1 for at least 2 weeks following complete removal from steroid treatment except for maintenance replacement doses for adrenal insufficiency (preferably no greater than 10mg prednisone equivalent daily).

All patients treated with steroids for grade ≥3 events should have nivolumab discontinued. Patients with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Patients with hepatitis, pancreatitis, pneumonitis, and colitis are at risk for exacerbation with retreatment if there is residual inflammation and should resolve to Grade 0 or baseline before retreatment. Baseline can mean the initial grade *i.e.* grade <1 where permitted on study.

Patients with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses only of corticosteroids. Please note that grading and for hypophysitis with symptoms of headache, visual or neurologic

changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.

New immune related events or exacerbation of existing events during steroid treatment or taper suggest the presence of ongoing immune activation and should require permanent discontinuation of nivolumab.

A patient who is treated with steroids, evaluated, and found to not have an autoimmune or inflammatory event requiring steroid treatment, may be restarted if asymptomatic off steroids for 2 weeks and other restarting criteria are met.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be drawn if clinically feasible to document baseline function and distinguish the pituitary from peripheral organ dysfunction and later from steroid (or thyroid) treatment associated ACTH (or TSH) suppression. Steroids should be started prior to obtaining results based on clinical indications.

5.7 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, urticaria, angioedema, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

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 5.0 guidelines.

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

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. Infusion rate may be slowed. 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 patient closely.

The following prophylactic premedications 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, slowing infusion rate as above.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [*e.g.*, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; close observation for

recurrence and treatment medications may need to be continued for 24-48 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 patient until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient 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 patient 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 (acetaminophen) (or paracetamol) 325 to 1000 mg should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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

Grade 4 symptoms: (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. Patient 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 patient until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (*e.g.*, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (*e.g.*, oral antihistamine, or corticosteroids).

Please note that late occurring events including isolated fever and fatigue may represent the presentation of systemic inflammation. Please evaluate accordingly.

5.8 Treatment with Nivolumab after Relapse in the Control Arm

If the patients in the observation arm choose to get Nivolumab at the time of relapse; they will be followed for toxicities, mortality not related to AML progression and survival, but analyzed separately. Correlative study samples will be collected following the schedules for the patients randomized on the Nivolumab arm.

For the patients in the control arm who cross over to receive Nivolumab treatment, the primary endpoint will be overall response rate (ORR) (including CR2, CR2i, partial response, and stable disease) after eight q2weeks treatment. Patients who progress during the treatment will be taken

off the study. Patients can receive a maximum of 46 doses (92 weeks).

The International Working Group (IWG) response criteria were developed to assess the activity of drugs in AML. According to the criteria, relapse following complete remission is defined as reappearance of leukemic blasts in the peripheral blood or the finding of more than 5% blasts in the bone marrow, not attributable to another cause, or extramedullary relapse.

Patients may be permitted to be treated with Nivolumab at the time to disease relapse in the control arm as long as they meet the inclusion criteria, except that the blood count requirement will be lower as listed below, and the following criteria:

- Patients must be clinically stable with no significant change in performance status due to disease progression
- No indication for immediate alternative treatment
- Patient assessed by the treating physician is going to have clinical benefit and tolerates study drug. The assessment of clinical benefit should take into account whether the subject is clinically stable or deteriorating and likely or unlikely to receive further benefit from continued treatment. In general, the relapsed AML will not require urgent chemotherapy.. Hydroxyurea could be used to control AML prior to initiating Nivolumab treatment. The dose and duration of hydroxyurea would be based on the current practice for controlling AML prior to treatment
- The cell counts will meet the following criteria at the time of first dose of Nivolumab:
  - leukocytes ≥1,000/mcL
  - absolute neutrophil count ≥500/mcL
  - platelets ≥20,000/mcL

6. DOSING DELAYS/DOSE MODIFICATIONS

Below are DOSE DELAY tables for Nivolumab for the following adverse events

Please refer to the Nivolumab Investigator Brochure or Appendix H to the protocol for toxicity management algorithms which include specific treatment guidelines. These algorithms should be followed unless there are specific clinical circumstances which the treating physician indicates variations or alternative treatment is needed.

Generally we strongly encourage early evaluation, withholding drug, and appropriate treatment as indicated in the management tables and following event specific guidelines.

ALL OTHER EVENTS*	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1 continue at investigator discretion
Grade 4	Off protocol therapy
* **Not agent related**, or agent related non-immunologically mediated	

ALL OTHER EVENTS*	**Management/Next Dose for Nivolumab**
Recommended management: As clinically indicated	
ALL OTHER EVENTS**	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline* When resolved < or following steroids resume at same dose level .
Grade 3	Off protocol therapy (exceptions noted in 5.4)
Grade 4	Off protocol therapy
** immunologically mediated	
Recommended management: As clinically indicated	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose
Grade 2	Hold until 1≤ Grade resolved. Consider steroid treatment > 7 days Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same level at investigator discretion
Grade 4	Off protocol therapy
*Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: AE management guidelines	

Liver Function AST	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold until UNL or baseline. Resume at same dose level.
Grade 2	Hold until UNL or baseline. Resume at same dose level. Consider steroid treatment > 7 days
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Continued treatment of active immune mediated heapatitis may exacerbates ongoing inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.	
Recommended management: see Hepatitic AE management algorithm	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold until Grade 0 or baseline. No change in dose
Grade 2	Hold until Grade 0 or baseline. Consider steroid treatment > 7 days
Grade 3	Off protocol therapy.

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
Grade 4	Off protocol therapy
See GI AE Algorithm for management of symptomatic colitis. Patients with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Patients who require steroids should be taken off study treatment. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: see GI AE management Algorithm	

Pancreatitis Amylapse/Lipase	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose until grade 0
Grade 2	Hold until Grade 0. Resume at same dose level if asymptomatic
Grade 3	Hold* until Grade 0. Resume at same dose level if asymptomatic. *Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Grade 4	Off protocol therapy; Asymptomatic grade 4 amylase/lipase may be re-treated as in Grade 3.
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm	

Pneumonitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and resolution to ≤ Grade 0 or baseline including baseline pO2. Resume no change in dose after pulmonary and/or ID consultation
Grade 2	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded. Off study if steroids are required. ^
Grade 3	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation only if lymphocytic pneumonitis is excluded. Off protocol therapy
Grade 4	Off protocol therapy
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza killed vaccine for all patients.	
Recommended management: See Pulmonary Adverse Event Management Algorithm	

Other GI N-V	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	Hold pending evaluation for gastritis duodenitis and other immune adverse events or other causes. Resume at same dose level after resolution to ≤ Grade 1.
Grade 3	Hold pending evaluation until ≤ Grade 1. Resume at same dose level. If symptoms do not resolve within 7 days with symptomatic treatment patients should go off protocol therapy
Grade 4	Off protocol therapy
Patients with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.	

Fatigue	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 2. Resume at same dose level
Grade 4	Off protocol therapy
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Neurologic events	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and observation. ^ Resume with no change in dose. *
Grade 2	Hold dose pending evaluation and observation. ^ Hold until ≤ Grade 1.*Off protocol therapy if treatment with steroids is required. ^ Resume at same dose level for peripheral isolated n. VII (Bell's palsy)^
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
*Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, myasthenia gravis should be off study.	
Recommended management: See Neurologic Adverse Event Management Algorithm	

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
≤ Grade 1	Asymptomatic TSH elevation * Hold pending evaluation, endocrine consult
Grade 2	Hold until patients are on a stable replacement hormone regimen. If

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
	treated with steroids patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	Off study treatment.
Grade 4	Off protocol therapy
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm	

Fever	**Management/Next Dose for Nivolumab**
≤ Grade 1	Continue nivolumab at same dose. If no improvement after 5-7 days or worsening, then treat as Grade 2.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	
See section 5. infusion reactions	

If treatment is delayed >6 weeks, >8 weeks for patients on high dose steroids with recommended 4 weeks taper and 2 week observation, the patient must be permanently discontinued from study therapy, except as specified in Section 5.7 (Criteria to Resume Treatment.)

Patients requiring a delay of >6 weeks, >8 weeks for patients on high dose steroids with required 4 weeks minimal taper and 2 week observation, should go off protocol therapy.
Patients requiring > two dose delays for the same event should go off protocol therapy.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be obtained to document baseline.

Patients may be dose-delayed for evaluation and restarted depending on results.

Any patient started on corticosteroids initially who is determined to not require steroids treatment for an autoimmune adverse event may resume therapy after a 2 week observation period without further symptoms at the discretion of the PI or investigator.

Please refer to the Drug modification table for cardiomyopathy myocarditis:

Cardiac *	Management/Next Dose for BMS-936558 (Nivolumab) + Ipilimumab Cardiac Toxicities
≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation
Grade ≥2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Consult algorithm for more details. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
Grade ≥2 with confirmed myocarditis	Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone Add ATG or tacrolimus if no improvement. Off treatment.
**Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin* ***Patients with evidence of myositis without myocarditis may be treated according as "other event"* Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.	

- Drug will be held for grade 2 cardiac dysfunction pending evaluation
- Drug will be permanently discontinued for grade 3 or 4 cardiac dysfunction and grade 2 events that do not recover to baseline or that reoccur
- Missed doses of study drug are not made up. If drug was held for an adverse event (or other non-medical reason), site must indicate that no drug was given for that cycle.
- Treatment with steroids as clinically indicated
- Add the table as follows in the treatment modification and AE management section

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR) for BMS-936556 (Nivolumab, MDX-1106, NSC 748726)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or

potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2069 patients.* Below is the CAEPR for BMS-936558 (Nivolumab, MDX-1106).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.4, December 2, 2020[1]

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
		Pericarditis	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[3]		
	Hyperthyroidism[3]		
	Hypophysitis[3]		
	Hypothyroidism[3]		
EYE DISORDERS			
		Blurred vision	
		Dry eye	
		Eye disorders - Other (diplopia)[3]	
		Eye disorders - Other (Graves ophthalmopathy)[3]	
		Eye disorders - Other (optic neuritis retrobulbar)[3]	
		Eye disorders - Other (Vogt-Koyanagi-Harada)	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Enterocolitis	
		Gastritis	
		Mucositis oral	

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		***Injection site reaction (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatobiliary disorders - Other (immune-mediated hepatitis)	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Autoimmune disorder[3]	
		Cytokine release syndrome[5]	
		Immune system disorders - Other (GVHD in the setting of allotransplant)[3,6]	
		Immune system disorders - Other (sarcoidosis)[3]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		
INVESTIGATIONS			
	Alanine aminotransferase increased[3]		***Alanine aminotransferase increased[3] (Gr 3)***
	Aspartate aminotransferase increased[3]		***Aspartate aminotransferase increased[3] (Gr 3)***
	Blood bilirubin increased[3]		***Blood bilirubin increased[3] (Gr 2)***
	CD4 lymphocytes decreased		***CD4 lymphocyte decreased (Gr 4)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)[3]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Musculoskeletal and connective tissue disorder - Other (polymyositis)	
		Myositis	
		Rhabdomyolysis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy[3]	
		Facial nerve disorder[3]	
		Guillain-Barre syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)[3]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
		Reversible posterior leukoencephalopathy syndrome[3]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury[3]	
		Renal and urinary disorders - Other (immune-mediated nephritis)	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)[3]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme[3]	
	Pruritus[3]		***Pruritus[3] (Gr 2)***
	Rash maculo-papular[3]		***Rash maculo-papular[3] (Gr 2)***
		Skin and subcutaneous tissue disorders - Other (bullous pemphigoid)	

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)[3]		
	Skin hypopigmentation[3]		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Pericardial tamponade may be related to possible inflammatory reaction at tumor site.

[3]Nivolumab being a member of class of agents involved in the inhibition of "immune checkpoints", may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[4]Pancreatitis may result in increased serum amylase and/or more frequently lipase.

[5]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopenia.

[6]Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving Nivolumab. These complications may occur despite intervening therapy between receiving Nivolumab and allo-SCT.

[7]Infusion reactions, including high-grade hypersensitivity reactions which have been observed following administration of nivolumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of nivolumab.

Adverse events reported on Nivolumab trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Nivolumab caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Heart failure; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Vestibular disorder
EYE DISORDERS - Eye disorders - Other (iridocyclitis); Optic nerve disorder; Periorbital edema
GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Flatulence; Gastrointestinal

disorders - Other (mouth sores); Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Pain
HEPATOBILIARY DISORDERS - Bile duct stenosis
IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (autoimmune thrombotic microangiopathy); Immune system disorders - Other (limbic encephalitis)
INFECTIONS AND INFESTATIONS - Bronchial infection; Lung infection; Sepsis; Upper respiratory infection
INVESTIGATIONS - Blood lactate dehydrogenase increased; GGT increased; Investigations - Other (protein total decreased); Lymphocyte count increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Musculoskeletal and connective tissue disorder - Other (musculoskeletal pain); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Histiocytic necrotizing lymphadenitis)
NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (tubulointerstitial nephritis)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pain of skin; Photosensitivity; Rash acneiform; Skin and subcutaneous tissue disorders - Other (rosacea)
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Vasculitis

Note: Nivolumab in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

- CTCAE v5.0 documents, including a mapping document, are available on the CTEP website: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm .

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.

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

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients. Please also copy all adverse events to the following email address: phaseIIcra@medicine.bsd.uchicago.edu

The Coordinating Center of the Corresponding Organization is responsible for submitting to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be

submitted.

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported**

expeditiously through CTEP-AERS must <u>also</u> be reported in routine study data submissions.

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent: Nivolumab (NSC 748726)

Amino Acid Sequence: 4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

Other Names: BMS-936558, MDX1106

Classification: Anti-PD-1MAb

M.W.: 146,221 daltons

Mode of Action: Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate dihydrate, sodium chloride, mannitol, diethylenetriaminepentacetic acid (pentetic acid) polysorbate 80 (Tween® 80), and water for injection. Dilute solutions of hydrochloric acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5)

How Supplied: Nivolumab is supplied by Bristol-Myers Squibb and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as 100 mg vials (10 mg/mL) with a 0.7 mL overfill. It is supplied in 10 mL type I flint glass vials, with fluoropolymer film-laminated rubber stoppers and aluminum seals.

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose. When the dose is based on patient weight (i.e., mg/kg), nivolumab injection can be infused undiluted or diluted to protein concentrations as low as 0.35 mg/mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

Storage: Vials of Nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

If a storage temperature excursion is identified, promptly return Nivolumab to 2°C-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf-life surveillance of the intact vials is ongoing.

The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.

Route of Administration: Intravenous infusion. Do not administer as an IV push or bolus injection.

Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter.

Potential Drug Interactions: The indirect drug-drug interaction potential of nivolumab was assessed using systemic cytokine modulation data for cytokines known to modulate CYP enzymes. There were no meaningful changes in cytokines known to have indirect effects on CYP enzymes across all dose levels of nivolumab. This lack of cytokine modulation suggests that nivolumab has no or low potential for modulating CYP enzymes, thereby indicating a low risk of therapeutic protein-drug interaction.

Availability

Nivolumab is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Nivolumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.1 Agent Ordering and Agent Accountability

8.1.1.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.1.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMC using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.1.1.3 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Integral Laboratory

N/A

9.2 Integrated Correlative Studies (Mandatory studies)

9.2.1 **Analysis of PD-LI expression on AML cells from peripheral blood and/or bone marrow samples at diagnosis if available and at the time of study enrollment**.

Background: As discussed in the background section 2.3, to determine whether PD-L1 was expressed on human AML cells, bone marrow samples from AML patients were analyzed for expression patterns of PD-L1. Three staining patterns were observed: 1) no PD-L1 expression on either CD34+ or CD34- cells, 2) expression of PD-L1 on the CD34-, but not on CD34+ cells, and 3) PD-L1 expression on CD34+ AML cells, but not on CD34- cells. These preliminary data support a hypothesis that PD-L1 expression on a subset of AML cases might serve as a possible baseline biomarker for response to PD-1-blocking antibodies, as has been demonstrated in melanoma patients (Topalian, Hodi et al.). We intend to test the expression of PD-LI inside the bone marrow environments and in the blood to determine if PD-LI expression will predict the response to the Nivolumab treatment.

Method: Bone marrow core biopsy samples at the time of diagnosis (when available), study enrollment, 3, 6, 12 months on the study and at the end of the study either due to relapse or at 24 months on the study will be collected from patients on each study arm. Immunohistochemistry (IHC) staining using anti-PD-L1 and CD34 antibody will be conducted by BMS at the central lab if the test is ready at the time of the initiation of the study. Otherwise, PD-LI expression will be done by Flow cytometry using bone marrow aspiration and peripheral blood at Dr. Kline's laboratory at University of Chicago as demonstrated in the Background section 2.3. These results will be used to determine if expression level of PD-L1 on bone marrow cells will predict patient's response to Nivolumab treatment using logistic regression modeling.

Analysis: The expression of PD-LI will quantified using the staining intensity or the mean fluorescence intensity from 0 to 1+ to 4+. To determine association between PD-LI expression and clinical response, patients will be divided based on response to Nivolumab maintenance therapy: responders (continuing CR) versus non-responders (disease relapse on Nivolumab). The differences will be assessed using a conventional T-test, followed by a modified T-test employed using the eBayes function in limma (R version 9.2).

9.2.1.1 Collection of Specimen(s):

5-10* unstained bone marrow core biopsy slides (x3) (at time of diagnosis, at the time of enrollment, and at the time of relapse) and 5ml bone marrow aspiration in green top tube (x3) (at the time of enrollment and at the time of disease relapse) will be collected for PD-LI expression detection.
* More slides (up to 20 total) may be requested if samples are not adequate for testing purposes.

For bone marrow core biopsy unstained slides: The bone marrow core biopsy will processed

locally by the department of pathology at the participating institution as part of the standard of care. 5-10 unstained bone marrow core biopsy slides will be shipped to University of Chicago medical center and stored for future PD-LI IHC staining ..

For bone marrow aspiration samples: 5-10ml bone marrow aspiration will be collected using Green top tube. The fresh samples will be shipped on ice pack same day or overnight to University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, mononuclear cells (MNC) could be isolated using Ficoll method and then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen before shipment in batches. Fresh samples should be sent with ice pack, and frozen samples should be sent on dry ice. All specimens must be accompanied by a specimen transmission (Appendix C-F) form that includes:

Patient name
Hospital record number
NCI protocol number
Sample type (aspirate, core biopsy, peripheral blood etc)
Date when biopsy was obtained

9.2.1.2 Handling of Specimens (See also Appendix F)

The shipment of all human tissue samples must comply with appropriate regulations as specified by the carrier. Fresh samples should be sent with ice pack, and frozen samples should be sent on dry ice. At a minimum, all samples must be packaged within two containers with absorbent material between containers to control any spill or leakage. The outer container must be puncture resistant (e.g., cardboard mail tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers.

9.2.1.3 Shipping of Specimen(s)

All bone marrow core biopsy blocks and bone marrow aspiration must be accompanied by a pathology report and a sample transmission form (appendix C-F) and shipped the following address:

SHIPPING METHODS

1. The fresh samples will be shipped on ice pack same day or overnight to University of Chicago Dr. Wendy Stock laboratory for sample processing.

2. For centers with ability to process the samples, MNC could be isolated using Ficoll method and then be frozen in 90% fetal bovine serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches to Dr. Wendy Stock laboratory

a. Lab Address:
W. Stock Lab
900 East 57th Street
KCBD Room 9160 LB49
Chicago, IL 60637

b. Contact Number:
773-795-3727

3. Archival tissue slides or blocks should be sent to the University of Chicago Phase II

Multi-site Coordinator for storage.

a. Address:
5841 S. Maryland Ave., MC 2115
Chicago, IL 60637

b. Contact number:
773-834-1746

Please send an email along with the shipment tracking information to Bartholomew Eisfelder at beisfelder@medicine.bsd.uchicago.edu Noreen Fulton at nfulton1@bsd.uchicago.edu (fresh samples) and copy the Phase 2 general e-mail **phaseIIcra@medicine.bsd.uchicago.edu** for tissue and slides. Please do NOT ship samples on Fridays as there will not be anyone here to receive the package or process the sample.

9.2.1.4 Site Performing Correlative Study:
For Flow cytometry, it will be done by the lab of Dr. Kline at the University of Chicago.
For IHC, it will be done by the by the lab of Dr. Kline or the hematopathology department at the University of Chicago.

9.3 Exploratory Correlative Studies

All the exploratory correlative studies are mandatory.

9.3.1.1 Minimal residual disease (MRD) monitor

Background: WT1 as a marker of minimal residual disease in AML
Wilm's tumor 1 (WT1) is a zinc finger transcription factor that is normally expressed in tissues of mesodermal origin during embryogenesis. WT1 is over-expressed in various hematological malignancies, including AML (80-100% of cases). WT1 mRNA levels in the peripheral blood and bone marrow correlate with disease status during and after treatment, and elevation of WT1 after chemotherapy and stem cell transplant predicts clinical relapse. Thus, *WT1* RT-PCR is now being used to monitor minimal residual disease (MRD) during treatment of AML (Reviewed in (Grimwade, Vyas et al. 2010; Dominietto 2011)).

Dr. Wendy Stock's lab assessed MRD for a GVAX vaccine study in patients with AML using quantitative RT-PCR detection of *WT1* expression. Although > 90% of patients who achieved clinical complete remission (CR) had significant declines in *WT1* in both blood and bone marrow, 95% of patients had persistently detectable *WT1* levels in bone marrow demonstrating clear MRD in AML patients in CR (Figure 3 in (Borrello, Levitsky et al. 2009)). Importantly, the achievement of a significant decrease or elimination of *WT1* transcripts during the course of vaccination correlated with significant superior relapse-free survival (table 3 in (Borrello, Levitsky et al. 2009)). Furthermore, patients who relapsed during or following vaccination had a significant increase in *WT1* expression, while patients in continuous CR maintained very low or undetectable *WT1* levels (Figure 4 in (Borrello, Levitsky et al. 2009)).

WT1 MRD monitoring appears to correlate well with remission status in conventionally-treated

AML patients as well. Among AML patients in clinical CR after induction and consolidation therapy, 80-90% and 30-40% of patients continue to have detectable *WT1* transcript in the marrow or blood, respectively (Miyawaki, Hatsumi et al.). *WT1* levels in the peripheral blood can predict relapse after CR, and its levels after consolidation therapy are closely correlated with disease free survival (DFS), OS, and early relapse. The DFS rate at 3 years was 20% and 50% for *WT1* MRD positive and negative patients, respectively (Miyawaki, Hatsumi et al.). Thus, *WT1* monitoring is a useful tool not only for predicting relapse in AML patients in clinical CR, but also for monitoring response to post-remission immunotherapeutic strategies, such as vaccination and possibly after post-remission treatment with PD-1 blocking antibodies.

Method: Assessment of MRD: MRD, measured by quantitative RT-PCR (qPCR) for *WT1* transcripts will be monitored in peripheral blood and bone marrow samples during the course of the study. Briefly, total RNA will be extracted from blood and bone marrow MNC, and cDNA synthesized using standard techniques. Amplifications of patient samples, K562 cell line cDNA (WT1-expressing), and no-template controls will be performed in triplicate. *WT1* expression levels will be detected using a transcript-specific primer and probe set. To compensate for differences in RNA integrity and cDNA synthesis efficiency, the absolute *WT1* transcript copy number will be normalized to the endogenous control gene, ABL. The limit of normalized WT1 transcript quantification is 10^{-3} WT1/ABL. Disappearance of detectable *WT1* transcript will be considered a complete molecular response (clearance of MRD). A one log decline in *WT1* transcript will define a partial molecular response. Molecular stability will be defined by no reduction or increase in *WT1* transcript level by more than one log (10 fold). Molecular progression will be defined by greater than one log increase in *WT1* transcript. Molecular response and progression will be tabulated by treatment arm and analyzed using ordinal logistic regression.

Analysis: To determine association between MRD and clinical response, patients will be divided based on response to Nivolumab maintenance therapy: responders (MRD-) versus non-responders (MRD+). The differences will be assessed using a conventional T-test, followed by a modified T-test employed using the eBayes function in limma (R version 9.2).

9.3.1.2 Collection of Specimen(s)

10-20ml bone marrow aspiration and peripheral blood will be collected using Green top tube. The bone marrow samples will be collected 5 times at the time of scheduled bone marrow biopsies on the study (At enrollment, week 13, 25, 53 on the study, and at the time clinically indicated bone marrow to evaluate relapse/off study). (See the study calendar for the detail).

The peripheral blood samples will be collected on the same day of the scheduled bone marrow biopsy, an additional peripheral blood will be collected after 2 months on the study (week 9).

For bone marrow aspiration samples: 10-20ml bone marrow aspiration will be collected. The fresh samples will be shipped on ice pack same day or overnight to University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, MNC could be isolated using Ficoll method and then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches.

For peripheral blood samples: The samples will be shipped on ice pack same day or overnight to

University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, MNC will be isolated per the standard procedure. Briefly, blood will be diluted 1:1 with PBS. Diluted blood will be layered on top of 5mls ficoll. Specimens will be spun at 350g for 20 minutes at room temperature. The buffy coat layer containing MNC will be collected and washed two times with PBS. MNC will then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches..

9.3.1.3 Handling of Specimens(s)
Same as 9.2.1.2.

9.3.1.4 Shipping of Specimen(s)
Same as 9.2.1.3

9.3.1.5 Site(s) Performing Correlative Study
It will be done by the lab of Dr. Stock at the University of Chicago. Dr. Christopher Hourigan (Myeloid Malignancies, NHLBI) will be a collaborator for the MRD correlate section.

9.3.2 **T cell subset analysis including Tregs**

Background: A recent study demonstrated that human AML cells constitutively express the immuno-suppression enzyme indoleamine 2, 3-dioxygenase (Curti, Aluigi et al. 2007) and co-culture with IDO positive AML leukemia cells with lymphocytes could transform $CD3^+CD25^-$ T cells to $CD3^+CD25^+$ T cells *in vitro*, which function as Treg cells because they do not proliferate, do not produce interleukin IL–2, and inhibit *naive* T-cell proliferation. These data suggest that AML leukemic cell-induced regulatory T cells might play a role in the pathogenesis of refractory or relapsed AML (Curti, Pandolfi et al. 2007). It has been demonstrated that the frequency of $CD4^+CD25^+$ Treg in peripheral blood in AML patients is significantly higher when compared with healthy individuals (Wang, Zheng et al. 2005). A recent study demonstrated that Treg cell numbers prior to chemotherapy correlated with the response to chemotherapy. Specifically, patients who achieved a CR after induction chemotherapy had a significantly lower Treg frequency at diagnosis compared with patients who did not respond. Even though it needs to be proven that the observed high suppressor activity levels of Treg in patients with CR are related to higher relapse rates, it suggests that immunotherapy approaches to deplete or down-regulation of functional activity of Tregs are attractive to consider for patients with AML(Szczepanski, Szajnik et al. 2009).

There is clear data to demonstrated that immune negative regulators, such as CTLA4 (reviewed in (Bour-Jordan, Esensten et al.), and PD1-PDL1 axis (Amarnath, Mangus et al.); could regulate the function of T regulatory cells. We intent to monitor the T cell subset changes during immunotherapy with PD1 antibody in the AML patients. Since Nivolumab will target PD1 on the T cells, we will also monitor the expression of PD1 on the T cells prior to the treatment, during the treatment and at the time of disease relapse.

Method: Assessment of effect of Nivolumab on T cell subsets and T cell activation status,

and PD1 expression on the T cells: T cell frequencies and will be enumerated following staining of PBMC samples with anti-CD4, anti-CD8 and anti-FoxP3 staining and flow cytometry. Absolute numbers will be enumerated by multiplying the frequency of CD8+, CD4+ and CD4+FoxP3+ (Tregs) cells by the total WBC from each sample. T cell activation status will be performed in parallel, and include the following markers: HLA-DR, CD69, ICOS, and CD25. PD1 expression on T cells especially CD8 positive T cells will be assessed by flow cytometry. Others may be included depending on preliminary results.

Analysis: Differences in pre- and post-treatment levels, as well as comparisons between blood samples, will be compared using a paired t-test. Changes in levels of responders versus non-responders will be analyzed using a non-parametric Wilcoxon rank-sum test.

9.3.2.1 Collection of Specimen(s)
5-10ml peripheral blood will be collected using Green top tube.
The peripheral blood samples will be collected 6 times on the study. (At enrollment, week 9, 13, 25, 53 on the study, and at the time of clinically indicated bone marrow to evaluate relapse/off study).

For PD1 expression on the T cells, the samples will only be collected 3 times (at enrollment, week 9 after treatment and at the time of relapse).

The fresh samples will be shipped on ice pack same day or overnight to University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, MNC will be isolated per the standard procedure. Briefly, blood will be diluted 1:1 with PBS. Diluted blood will be layered on top of 5mls ficoll. Specimens will be spun at 350g for 20 minutes at room temperature. The buffy coat layer containing MNC will be collected and washed two times with PBS. MNC will then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches.

9.3.2.2 Handling of Specimens(s)
Same as 9.2.1.2

9.3.2.3 Shipping of Specimen(s)
Same as 9.2.1.3

9.3.2.4 Site(s) Performing Correlative Study :
The tests will be conducted at the Human Immune Monitoring facility directed by Dr. Thomas Gajewski at University of Chicago.

9.3.3 **Deep sequencing of TCR-α and TCR-β chains on polyclonal T cells**

Background: Recent advances in deep sequencing technology make it possible to characterize the T cell receptor (TCR) repertoire generated following immunotherapy, such as WT1 peptide vaccination (i.e. the "clonality" of the response). Preliminary studies have demonstrated biased usage of TCR-Vβ gene families in WT1 peptide vaccinated patients (Ochsenreither, Fusi et al. ; Ochsenreither, Fusi et al. ; Tanaka-Harada, Kawakami et al.), which was confirmed by our own TCR sequencing data in a patient who received WT1 peptide vaccination post SCT (Figure 4 in

preliminary result section). Dr. Yusuke Nakamura at our institution is one of the pioneers in the assessment of genetic variations (VNTR and SNP markers) and whole-genome analysis in cancer cells. Using a novel sequencing approach, we have been working together to understand the TCR "landscape" during immune-based therapies for AML. TCR sequencing of peripheral blood and/or bone marrow samples during immunotherapy, such as PD-1 blockade, will allow us to assess for an emerging T cell repertoire in patients who respond versus those who do not. This type of analysis may also be useful to identify high-affinity TCRs for use in down-stream adaptive T cell therapy approaches for AML, as well as to enable the identification of new AML associated antigens**.**

Method: TCR Sequencing: To determine whether Nivolumab treatment leads to changes in the "clonality" of the polyclonal T cell repertoire, cDNA libraries for *TCRA* and *TCRB* genes will be constructed by polymerase chain reaction (PCR) using a primer set designed for adaptor sequence and C segment region in the *TCR* genes. Following that, emulsion-PCR will be conducted on the Ion Torrent system (Life Technologies, Carlsbad, CA) that templates clonal copies of DNA libraries onto the Ion Sphere Particles. The complex of DNA and particle will be deposited onto semiconductor chips for sequencing by the Ion Torrent PGM sequencer (Life Technologies, Carlsbad, CA). This analysis will be performed on selected patient peripheral blood and marrow samples.

Analysis: Differences in pre- and post-treatment levels, as well as comparisons between blood samples will be compared using a paired t-test. Changes in levels of responders versus non-responders will be analyzed using a non-parametric Wilcoxon rank-sum test.

9.3.3.1 Collection of Specimen(s)

5-10ml bone marrow aspiration and peripheral blood will be collected using Green top tube. The bone marrow samples will be collected 5 times at the time of scheduled bone marrow biopsies on the study (At enrollment, week 13, 25, 54 on the study and at the time clinically indicated bone marrow to evaluate relapse/off study). (See the study calendar for the detail).

The peripheral blood samples will be collected on the same day of the scheduled bone marrow biopsy, an additional peripheral blood will be collected after 2 months (week 9) on the study.

For bone marrow aspiration samples: 5-10ml bone marrow aspiration will be collected. The fresh samples will be shipped on ice pack same day or overnight to University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, MNC could be isolated using Ficoll method and then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches.
For peripheral blood samples: The fresh samples will be shipped on ice pack same day or overnight to University of Chicago central lab (Dr. Stock's lab) for sample processing. For centers with ability to process the samples, MNC will be isolated per the standard procedure. Briefly, blood will be diluted 1:1 with PBS. Diluted blood will be layered on top of 5mls ficoll. Specimens will be spun at 350g for 20 minutes at room temperature. The buffy coat layer containing MNC will be collected and washed two times with PBS. MNC will then be frozen in 90% fetal calf serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches.

9.3.3.2 Handling of Specimens(s)
Same as 9.2.1.2

9.3.3.3 Shipping of Specimen(s)
Same as 9.2.1.3

9.3.3.4 Site(s) Performing Correlative Study
The study will be done at the laboratory of Dr. Yusuke Nakamura at University of Chicago.

9.4 Special Studies

n/a

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 week prior to start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 72 hours prior to initiation of the next cycle of therapy.

Study Calendar for Nivolumab and Observation Arms							
	Pre-Study (Day -14 to 0)	Wk 1& (±1 Wk)	Wk 9@ (±1 Wk)	Wk 13 (±1 Wk)	Wk 25 (±1 Wk)	Wk 53 (±1 Wk)	Off Study or at the time of clinically suspected relapse [H β]
Nivolumab[A]		B	B	B	B	B	
Informed consent	X						
Demographics	X						
Medical history	X						
Concurrent meds	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Height	X						
Weight	X	X	X	X	X	X	X
Performance status	X	X	X	X	X	X	X
CBC w/diff, plts [C*]	X	X	X	X	X	X	X
Serum chemistry [D*]	X	X	X	X	X	X	X
EKG (as indicated)[Ω]	X						
HIV and hepatitis test [E]	X						
Adverse event evaluation	X	X	X	X	X	X	X
B-HCG	X[F]						X[F]
Bone marrow biopsy [F] (mandatory)	X#			X	X	X	X
Correlative studies: (Mandatory)							
PD-L1 staining/mutation load (Bone marrow core biopsy slides or Bone marrow aspiration)	X						X
WT1 MRD detection/digital PCR panel (5-10cc peripheral blood at each time point and 5-10cc bone marrow aspiration on the day of scheduled bone marrow biopsy)	X		X	X	X	X	X
T cell subsets analysis /CyTOF analysis. (5-10cc peripheral blood at each time point and 5-10cc bone marrow aspiration on the day of scheduled bone marrow biopsy	X		X	X	X	X	X
T cell PD-1 expression (5-10cc peripheral blood in green top tube)	X		X				X
TCR sequencing (5-10cc peripheral blood and 5-10cc bone marrow aspiration if available)	X		X	X	X	X	X

A: Nivolumab only given to patients on treatment arm, same calendar of events should be followed for all patients on study aside from the biweekly Nivolumab treatments. If a patient on observation relapses, patient may elect to cross over to the Nivolumab arm and follow the same calendar as patients whom originally randomized to the Nivolumab arm, with exception to the Bone Marrow biopsy at 9 weeks, which is ONLY required for patients on observation who have crossed over to Nivolumab.
B: Nivolumab: 3mg/kg IV every 2 weeks for 46 doses.
C: Patients on both arms will have CBCs done every 4 weeks for the first year and every 8 weeks in the second year. An unexplained drop in the counts especially platelet should will mandatorily trigger an unscheduled bone marrow exam to assess for relapse.
D: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium , amylase, lipase, TSH (with reflexive Free T4 and Free T3)
E: If patient has history of HIV or Hepatitis B or C on or off treatment, or patient has positive antibody test, viral load by quantitative PCR should be ordered, and viral loading negativity is required to be eligible on the study.
F: Complete bone marrow exams (with flow, cytogenetics, and molecular MRD) should be done prior to Nivolumab, at 3, 6, and 12 months, and then only as clinically indicated (e.g., appearance of peripheral blasts or dropping platelets), and at the time off the study on both arms of the trial.
G: Serum pregnancy test (women of childbearing potential). A serum or urine pregnancy testing is required within 72 hrs of study enrollment or randomization. and one week after discontinuation from nivolumab
H: Off-study evaluation or at the time of clinically suspected relapse.
* Laboratory testing prior to Nivolumab treatment (will be done before every 2 cycles of Nivolumab treatment; every 4 weeks): Within 72 hrs prior to re-dosing to include CBC w/ differential, LFTs, BUN or serum urea level, creatinine, Ca, Mg, Na, K, Cl, Glucose, Phosphate. (amylase, lipase, TSH only if clinically indicated)
# This bone marrow biopsy could be done anytime within 60 days after the last chemotherapy; as long as the correlative studies samples are obtained and there is no evidence of disease relapse from the time of bone marrow biopsy to the start of treatment on the trial. Screening bone marrow should be repeated within two weeks of starting on protocol treatment.
&: Any labs within 2 weeks to the start of treatment could be used.
@: for patients who crossed over to Nivolumab Arm, will have response re-assessment at Week 9, to determine if the patient will continue Nivolumab or not.
Ω:EKG and ECHO cardiogram for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs as clinically indicated. For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, ECHO cardiogram".
β: Patients who have come off treatment/observation after two years on study without relapse will continue to be followed every 6 months for the first year and yearly for disease relapse and survival. If patients come off treatment prior to two years, patients should be followed according to the protocol: CBCs every month for the first year and every two months for the second year. Patient will then be followed every 6 months for the first year and then yearly for disease relapse and survival.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – AML

Since all the patients initially enrolled on the study will be in morphologic complete remission (CR) or CRi, defined by the International Working Group Criteria (Dohner, Estey et al. ; Cheson, Bennett et al. 2003)

Morphologic complete remission (CR): Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0 x 10^9/L (1000/uL); platelet count >100 x 10^9/L (100 000/uL); independence of red cell transfusions.

Morphologic complete remission with incomplete blood count recovery (CRi): Same as CR but ANC may be <1,000/μL and/or platelet count <100,000/μL in a cellular marrow (greater than ≥ 20%).

The patients on the study will be monitor for disease relapse and PFS and OS defined as below.

Morphologic Relapse following complete response: is defined as bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease.

Consensus to determine disease relapse or progression:

In general, the AML relapse on the study will be defined by morphologic relapse.

In addition, cytogenetic relapse defined by the reappearance of a cytogenetic abnormality, in the absence of morphologic relapse, could be used to determine disease relapse on the study based on all the evaluable clinic data. The determination should be discussed with the study PI.

Molecular relapse defined by reappearance of a molecular abnormality; and appearance of new dysplastic changes might be used to define disease relapse after the review of the clinical data and the discussion with the study PI.

Progression-free survival (PFS): Defined as the time from randomization to disease relapse or death from any cause. Patients not known to have relapsed or died at last follow-up are censored on the date they were last examined.

Overall Survival (OS) is defined for all patients of a trial; measured from the date of entry into a study to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive.

Cumulative incidence of relapse (CIR): Defined for all patients achieving CR or CRi measured from the date of achievement of a remission until the date of relapse; patients not known to have relapsed are censored on the date they were last examined; patients who died without relapse are counted as a competing cause of failure.

For the patient in the control arm that has disease relapse during the follow-up and cross over to be treated with Nivolumab. The response to Nivolumab treatment will be also evaluated by the International Working Group Criteria.

Morphologic complete remission (CR): Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0 x 10^9/L (1000/uL); platelet count >100 x 10^9/L (100 000/uL); independence of red cell transfusions.

Morphologic complete remission with incomplete blood count recovery (CRi): Same as CR but ANC may be <1,000/μL and/or platelet count <100,000/μL in a cellular marrow (greater than ≥ 20%).

Partial Remission (PR): Requires all hematologic values for a CR (i.e. ANC ≥1,000/μL, platelet count ≥100,000/μL), but with a decrease of at least 50% in the percentage of marrow blasts to 5% to 25% in the bone marrow aspirate.

Treatment Failure (TF): Any one of the following:

Failure to achieve a CR, CRi or PR

Failure due to resistant disease (patient survives ≥7 days post-chemotherapy with persistent disease in blood or bone marrow); Persistent disease is defined as an increase of at least 25% in the absolute number of leukemic cells in peripheral blood or bone marrow/aspirate, the development of extramedullary disease, or other

evidence of increased tumor burden.

Failure due to aplasia [patient survives ≥7 days post-chemotherapy; death while cytopenic and aplastic (<20% marrow cellularity) bone marrow with no leukemic blasts]

Failure due to indeterminate cause (patients who die <7 days post-chemotherapy; patients who die >7 days post-chemotherapy with no peripheral blood blasts, but no bone marrow examination; patients who do not complete the first course of Induction therapy)

11.2 Other Response Parameters:

Cytogenetic CR (CRc): Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow.

Molecular CR (CRm): No standard definition; but in general, it will be defined as disappearance of detectable molecule marker at the diagnosis, such as NPM1 mutation, FLT3-ITD mutation and CEBPα mutation et al at the time of morphologic CR (or CRi).

Molecular and/or cytogenetic relapse is characterized by reappearance of a cytogenetic or molecular abnormality.

Minimal residual disease (MRD) monitoring: In this protocol, MRD will be monitored by quantitative RT-PCR (qRT-PCR) of *WT1* gene which will be normalized by control gene *ABL*. The limit of normalized WT1 transcript quantification is 10^{-3} WT1/ABL.

MRD negative (MRD-): Disappearance of detectable *WT1* transcript will be considered a complete molecular response (clearance of MRD).

Partial MRD response: A one log decline in *WT1* transcript will define a partial molecular response.

Stable MRD: Molecular stability will be defined by no reduction or increase in *WT1* transcript level by more than one log (10 fold).

MRD progression: Molecular progression will be defined by greater than one log increase in *WT1* transcript.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

During the phase 2 portion of the study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance. Decisions to proceed to the second stage of a Phase 2 trial will require sign-off by the Protocol Principal Investigator and the Protocol Statistician through IWRS and Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.2 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (https://eapps-ctep.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read-Only, or Site Investigator) on either the Corresponding Organization or Participating Organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at http://www.theradex.com/CTMS. On-site audits will be conducted three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at ctms@theradex.com for additional support with Rave and completion of CRFs.

12.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI. CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

12.3 CTEP Multicenter Guidelines

N/A.

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12.4 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

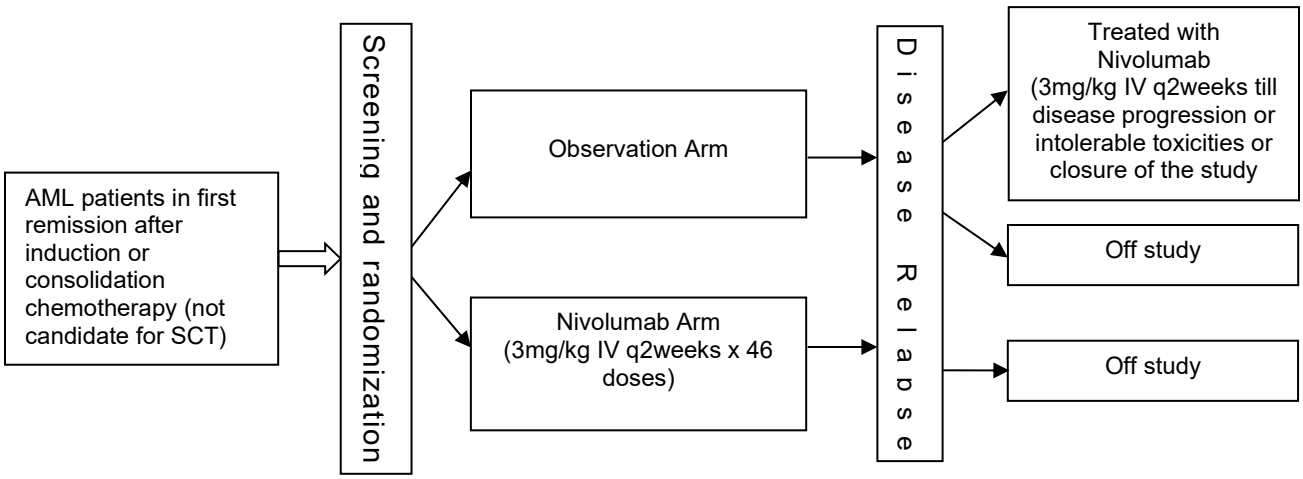
The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open-label, randomized Phase II study to evaluate the efficacy of single agent of Nivolumab as maintenance treatment to prevent relapse in AML patients in first CR after induction

chemotherapy or consolidation chemotherapy. Eighty patients with AML in first CR or CRi after induction and/or consolidation chemotherapy will be randomized into an observation arm (standard of care) or to Nivolumab treatment. Patients assigned to the Nivolumab arm will receive Nivolumab at 3mg/kg IV every 2 weeks for 2 years (46 doses). Patients on both arms will be monitored for relapse, adverse events and survival for a minimum of 2 years following randomization. During and after treatment, patients will be evaluated for toxicities, particularly immunologic adverse events including pneumonitis, autoimmune colitis, dermatitis etc. Peripheral blood and bone marrow samples will be collected before and during the treatment course for the correlative studies outlined above. Complete blood counts and differential counts will be obtained every 4 weeks for the first year, and every 8 weeks for the second year on the study, for patients on both arms. Bone marrow examination for disease assessment and correlative studies will be performed at the following time points: at the time of study enrollment; 3, 6 and 12 months following enrollment and/or at the time of study discontinuation due to toxicity or disease relapse.



Eligible patients will be randomized to observation arm vs Nivolumab arm with stratification by AML risk category (Adverse, Intermediate-1, Intermediate-2 and favorable) and age (<60 years, >=60 years). Patients randomized to the Nivolumab arm will receive IV Nivolumab at 3mg/kg every 2 weeks for forty-six doses (2 years). After that, patients will be followed up for adverse events, relapse and survival until at least 2 years after the last patient on the study is randomized. Patients who have come off treatment/observation after two years on study without relapse will continue to be followed every 6 months for the first year and yearly for disease relapse and survival.

When the patients on the observation arm are confirmed to have disease relapse (defined as below), at the treating physicians' discretion, the patients have the option to cross over to receive 3mg/kg Nivolumab every 2 weeks in the same manner as the patients on the Nivolumab arm till disease progression or intolerable toxicities or closure of the trial. Upon disease relapse for patients on the observation arm, there will not be a randomization to be treated with Nivolumab or to be off the study. The decision to cross-over from the observation arm to be treated with Nivolumab at disease relapse will be made by the treating physician after discussion with the patient.
The total duration of the treatment with Nivolumab for these patients will be no more than 2 years after the accrual to the study is completed. Before treatment with Nivolumab, these patients should still meet the inclusion criteria and exclusion criteria other than the disease status. Alternatively, these patients could be taken off study to pursue further salvage chemotherapy at any time at the treating physician's discretion. However, all of these patients will be followed for survival. Many of the patients on the observation arm at the time of relapse might elect to receive salvage chemotherapy followed in some cases by stem cell transplantation. However, treatment with Nivolumab for early relapse in AML patients, especially elderly AML patients who are not candidates for intensive chemotherapy and SCT, might provide valuable clinical and scientific insight without affecting the primary objective of PFS of the study.

If the patients in the observation arm choose to get Nivolumab at the time of relapse; they will be followed for toxicities, mortality not related to AML progression and survival, but analyzed separately. Correlative study samples will be collected following the schedules for the patients

randomized on the Nivolumab arm.

For the patients in the control arm who cross over to receive Nivolumab treatment, the primary endpoint will be overall response rate (ORR) (including CR2, CR2i, partial response, and stable disease) after eight q2weeks treatment. Patients who progress during the treatment will be taken off the study.

Patients will have a baseline eligibility screen within 1-2 weeks prior to randomization, including medical history, physical examination, assessment of performance status, and assessment of hematology and chemistry parameters. Baseline assessment of status of AML will depend on bone marrow aspirate and biopsy by morphology and flow cytometry. Residual disease from cytogenetic and molecular analyses is allowed. Patient will have bone marrow biopsy prior to the randomization to confirm the status of AML, and obtain pre-treatment bone marrow samples along with peripheral blood samples for the correlative studies. The minimal residual disease (MRD) status will be determined by cytogenetics/FISH study; molecular studies if patients have identifiable disease markers, such as the *NPM1* mutation. All the patients will also have quantitative RT-PCR to check for *WT1* expression. The pre-treatment MRD status is not a requirement to be enrolled in the clinical trial.

Bone marrow samples will be collected after four cycles of receiving every 2 weeks doses of Nivolumab to determine disease status, MRD status and to be used for correlative studies. Complete blood counts and differentials will be obtained monthly, and any appearance of peripheral blasts or unexpected drop in blood counts will trigger a bone marrow exam to evaluate for relapse. Peripheral blood samples will be collected after four and eight every 2 weeks doses, at 1 year on the treatment and at the end of the treatment for correlative studies. All the patients will be followed up for clinical relapse or death. The relapse rate and progression free survival will be calculated for each arm; and in subset analysis for MRD+ or MRD- patients separately. In order to have comparison, patients on the observation arm will have all the scheduled sample collection.

Analysis of primary endpoint:
The primary endpoint is progression free survival (PFS). PFS is defined as the time from randomization to disease relapse or death from any cause. Patients alive and progression free will be censored at the date of the last negative bone marrow examination. Patients will be accrued over a two-year period and follow-up will continue for 2 years after the last patient is enrolled, providing a minimum of 2 years and maximum 4 years follow-up of each patient. Kaplan-Meier plots (Kaplan and Meier 1958) will be used to estimate PFS in each arm and a stratified log rank test (Peto, Pike et al. 1976) will be performed to compare the two groups. In addition, Cox proportional hazards models (Cox 1972) will be fit to provide estimates of the hazard ratio (HR) and associated 95% confidence interval (CIs), both unadjusted and adjusted for baseline covariates.

This is a randomized Phase II study to compare relapse (progression) free survival between observation and Nivolumab maintenance treatment. According to the historical data, the 2 year PFS survival of AML after induction chemotherapy and 3-4 cycles high dose cytarabine consolidation is around 40% in young patients (Mayer, Davis et al. 1994) and about 35%

including older patients extrapolated from the data of patients with primary AML enrolled onto CALGB first-line treatment trials who did not undergo allogeneic SCT in first complete remission per protocol (Figure 4A and 4C in (Mrozek, Marcucci et al.)). Since young AML patients in the European LeukemiaNet favorable group have excellent 2 year PFS at around 64%, further maintenance therapy might not provide additional benefit; thus the current trial will exclude young favorable group AML patients. The estimated 2 year PFS for all the AML patients excluding young favorable group is around 25% (Mrozek, Marcucci et al.). We expect that Nivolumab maintenance after induction and consolidation will improve the 2 year PFS rate from 25% to 45%. Assuming exponential distributions, this corresponds to a HR of 0.58.

Using a one-sided alpha of 0.10, to have 80% power to detect a HR of 0.58 would require 80 patients (40 patients per arm) (Schoenfeld 1983). This assumes 2 years of accrual and 2 years follow-up of the last patient. An interim futility analysis will be conducted after half the number of expected events (i.e., 31 of 62 expected) occur. If, at this point, the observed HR does not favor the experimental arm, the study will be stopped for futility. This futility rule is associated with a minimal (<2%) reduction in power (Wieand, Schroeder et al. 1994).

After reviewing the interim analysis results, the Data Monitoring Committee recommended that the trial be continued. Accrual will proceed until the randomization of 80 eligible patients.

Due to the heterogeneous population of AML patients, during the randomization, the patients will be stratified according to AML European LeukemiaNet risk categories (Adverse, intermediate-1, intermediate-2 or favorable) and age (<60 years vs. >= 60 years) (Mrozek, Marcucci et al.). Randomization sequences will be prepared by the statistician at University of Chicago and REDCap will be used for web-based treatment allocation.

13.2 Sample Size/Accrual Rate

A total 82 (The first two patients enrolled on the trial were mistakenly not randomized and were administered nivolumab) will be enrolled into this randomized Phase II study. The two patients mistakenly not randomized will not be included in primary efficacy outcome, however, they will be included overall safety analysis.

We have agreement from all the cancer centers within our Phase II consortium to participate in this clinical trial. Our consortium consists of the following institutions: University of Chicago, University of Maryland, University of Michigan, and Northwestern University, NorthShore University Health System, Indiana University and all the community cancer centers in our Phase II consortium. We have strong record of collaboration with these institutions. We also have agreement from University of Wisconsin; City of Hope and Penn State Hershey Cancer Institute through University of California Davis Comprehensive Cancer Center P2C; Vanderbilt University through H Lee Moffitt Cancer Center P2C; Princess Margaret Cancer Center in Canada through University Health Network Princess Margaret Cancer Center P2C to participate in the study. Thus, there will be at least 7-8 major institutions to assure timely accrual.

Furthermore, all P2C will have the opportunity to participate in this trial via the ETCTN

mechanism.

We anticipate enrollment of 3-4 patients/per month from all the participating institutions to accomplish the total accrual goal of 82 patients within 2 years.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	1	1			**2**
Asian	2	2			**4**
Native Hawaiian or Other Pacific Islander	1	1			**2**
Black or African American	10	10			**20**
White	20	20	5	5	**50**
More Than One Race	1	1			**2**
Total	**35**	**35**	**5**	**5**	**80**

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13.3 Stratification Factors

Due to the heterogeneous population of AML patients, during the randomization, the patients will be stratified according to AML European LeukemiaNet risk categories (Adverse, intermediate-1, intermediate-2 or favorable) and age (<60 years vs. >= 60 years) (Mrozek, Marcucci et al.). Randomization sequences will be prepared by the statistician at University of Chicago and REDCap will be used for web-based treatment allocation.

13.4 Analysis of Secondary Endpoints

The secondary endpoints include: Overall Survival (OS), non-relapse mortality (NRM) and adverse effects of Nivolumab.

OS is defined as the time from randomization to the date of death from any cause. NRM is

defined as the duration between the date of randomization and the date of patient death due to reasons other than relapse.

For overall survival, Kaplan-Meier plots will be generated for each treatment arm and the curves will be compared using a log rank test. Kaplan-Meier estimates with 95% CI will be summarized every 6 month using Greenwood's formula for the standard error of the Kaplan-Meier estimate. In addition to the Kaplan-Meier estimates, Cox proportional hazards models will be fit to estimate HR's and to assess and adjust for the effects of covariates. NRM will be analyzed using competing risks models (Gooley, Leisenring et al. 1999) with deaths due to relapse as a competing risk. Cumulative incidence curves will be generated and treatment effects summarized using the sub-distribution hazard ratio (Fine and Gray 1999) with and without adjustment for covariates.

Descriptive, subset analysis of PFS and OS will be performed for MRD+ and MRD- patients, respectively.

Adverse Events Monitoring:
Summary tables for adverse events (AEs) will include only AEs that started or worsened after randomization. The incidence of treatment-related adverse events (new or worsening from baseline after randomization) will be summarized by system organ class and/ or preferred term, severity (based on CTCAE grades), type of adverse event, and relation to study treatment by treatment group. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by type of adverse event and treatment group. All laboratory values will be converted into SI units and the severity grade calculated using Common Toxicity Criteria for Adverse Events (CTCAE). All laboratory values will be listed by laboratory parameter and patient. Separate listings will display notable laboratory abnormalities (i.e. newly occurring CTCAE grade 3 or 4 laboratory toxicities).

Toxicity rates will be compared between the two treatment arms via chi-square or Fisher's exact test. Formal comparisons will be conducted at the time of the interim analysis (i.e., after 31 events) and at completion of the trial. However, we will also undertake more frequent safety monitoring to ensure that serious AEs (deaths or hospitalizations) or selected AEs are not occurring at an unacceptably high rate in the Nivolumab arm. The selected AEs will be those listed in Section 7.1 as having a possible relationship to Nivolumab based on previous trials, with the stipulation that they are of grade 4 or higher. First, all serious AEs are reviewed at our weekly Phase II monitoring conferences. Second, safety reports containing comparative results will be generated quarterly and presented at the phase II conference. If the rate of SAEs exceeds 20% in the Nivolumab arm or the difference in rates between the Nivolumab and control arms is statistically significant at the $p<0.01$ level (to adjust for multiplicity), consideration will be given to stopping the trial. Accrual will be suspended pending consultation between the principal and co-investigators to determine whether the trial should be terminated.

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity

All patients including participants randomized to the control arm will be evaluable for toxicity from the time of their first treatment with Nivolumab.

13.5.2 Evaluation of Response

All eligible participants who were randomized will be accounted for, during the assessment for response to treatment, since the primary analysis will be an intent-to-treat analysis. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

14. STUDY STATUS UPDATES AND STUDY CLOSURE

14.1 Definitions of Study Status Changes

14.1.1 Temporarily Closed to Accrual

The study status is Temporarily Closed to Accrual when no patient slots are currently available, but there is the possibility that the trial will re-open for accrual (patient slots become available). Sites are not permitted to accrue additional patients until CTEP is notified of Re-Activation.

Study status will need to be changed to Temporarily Closed to Accrual when any of the following criteria are met:

- Sites are notified by CTEP (via Request for Rapid Amendment [RRA]) of changes in the risk/benefit ratio that necessitate changes to the patient Informed Consent document. Requested changes will be specified in the RRA and must be reviewed by the study's IRB.

- CTEP and the lead investigator agree that unacceptable toxicities necessitate a discussion to change the dosing/regimen.
- A protocol-defined benchmark has been achieved (such as an interim analysis before proceeding to the next stage).
- Investigators encounter any of the stopping criteria described in Section 5.5.

14.1.2 Closed to Accrual

The study status is (permanently) Closed to Accrual when no more patient enrollment slots are available, and at least one patient is still actively receiving the study treatment. Sites are no longer permitted to enroll additional patients.

Patient slots are no longer available when the following criteria are met:

- The pre-specified number of evaluable patients has been successfully enrolled, treated, and evaluated.
- The study treatment has failed to meet the pre-specified efficacy goal at the stage 1 interim analysis.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment.
- Investigators encounter any of the stopping criteria described in Section 5.5.

14.1.3 Closed to Accrual and Treatment

The study status is Closed to Accrual and Treatment when no more patient enrollment slots are available and no patients are currently receiving the study treatment. Patients may still be enrolled on the protocol only for the purposes of follow-up.

Patient accrual and treatment will be permanently halted when any of the following criteria are met:

- Enrollment was previously closed (study status of "Closed to Accrual"), and no patients are receiving the study treatment.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment. In this case, CTEP and the investigators must collaborate to alter the regimen or to halt the study treatment altogether as soon as it can be safely done for patients currently receiving treatment.

CTEP and Theradex **must be notified** when patients are no longer receiving treatment [*i.e.*, when the last patient(s) to be receiving treatment is/are no longer receiving the study regimen for any reason].

14.1.4 Closed to Follow-Up

The study is considered Closed to Follow-Up when all protocol-defined follow-up procedures have been completed for all patients who have not been removed from the

study for other reasons. That is, there are no outstanding follow-up procedures to be performed as mandated by the protocol.

CTEP does **not** need to be notified of a status change to "Closed to Follow Up."

14.1.5 Complete

Study is considered Complete if it has been at least thirty (30) days since the last patient follow-up evaluation.

A citation to a final study report (manuscript, meeting abstract, etc.) is required with the submission of the Protocol Status Update Form to CTEP PIO.

14.1.6 Future plan:

If this Phase II study demonstrates that Nivolumab can prolong 2 year PFS as maintenance therapy compared to observation alone, we will then plan to conduct a randomized Phase III trial to compare Nivolumab vs observation aiming for FDA approval of Nivolumab for the indication of maintenance treatment in AML patients.

14.2 Responsibility for Filing Protocol Status Update Forms

CTEP must be notified of all study status changes in Section 14.1 (except for Closed to Follow-Up) by the Corresponding Organization via Protocol Status Update Form, available from the CTEP website at http://ctep.cancer.gov/protocolDevelopment/default.htm#amendments.

Theradex must be notified as soon as all patients are off treatment (*i.e.*, when study status changes to Closed to Accrual and Treatment). Theradex will produce a report within 90 days of this notification.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B BLOOD SAMPLE COLLECTION FORM
The University of Chicago Protocol# CIRB15-0185
Blood Sample Collection Form

Clinician/Research Nurse: Please Fill Out
Blood Samples

Patient Name: _____ Week#_____

Patient Protocol ID #:_____ Date Blood Obtained: _____

Date of Birth: _____ Attending Physician: _____

***Institution:* _____**

Cross-over samples (COS)? (circle): yes / no
if YES Cross-over samples: label COS-CXDX on tubes

Date consent was signed: _____ Day Started on the clinical protocol: _____

Visit	Collection Tubes to use:	Date Drawn	Fresh Samples (shipped on ICE pack) Date Sent	Processed MNC (shipped on Dry Ice) Date Sent	Please check all sent samples
Pre-study Screening (Four green top tubes (5-10cc each))	**1. WT1 MRD** **2. T cell subsets** **3. TCR sequencing** **4. T cell PD-1 Expression**				**□ WT1 MRD** **□ T cell subsets** **□ TCR sequencing** **□ T cell PD-1 Expression**
Week 9 (Four green top tubes (5-10cc each))	**1. WT1 MRD** **2. T cell subsets** **3. TCR sequencing** **4. T cell PD-1 Expression**				**□ WT1 MRD** **□ T cell subsets** **□ TCR sequencing** **□ T cell PD-1 Expression**
Week 13 (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. T cell subsets** **3. TCR sequencing**				**□ WT1 MRD** **□ T cell subsets** **□ TCR sequencing**
Week 25 (Three green top tubes	**1. WT1 MRD** **2. T cell subsets**				**□ WT1 MRD** **□ T cell subsets**

(5-10cc each))	**3. TCR sequencing**				**□ TCR sequencing**
Week 53 (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. T cell subsets** **3. TCR sequencing**				**□ WT1 MRD** **□ T cell subsets** **□ TCR sequencing** **□ T cell PD-1 Expression**
At the time of off study (Four green top tubes (5-10cc each))	**1. WT1 MRD** **2. T cell subsets** **3. TCR sequencing** **4. T cell PD-1 Expression**				**□ WT1 MRD** **□ T cell subsets** **□ TCR sequencing** **□ T cell PD-1 Expression**

APPENDIX C BONE MARROW ASPIRATION COLLECTION FORM
The University of Chicago Protocol# CIRB15-0185
Bone Marrow Aspiration Collection Form

Clinician/Research Nurse: Please Fill Out
Bone Marrow Aspiration Sample

Patient Name: _____ Week#_____

Patient Protocol ID #:_____ Date Obtained: _____

Date of Birth: _____ Attending Physician: _____

Institution: **_____**

Cross-over samples (COS)? (circle): yes / no
if YES Cross-over samples: label COS-CXDX on tubes

Date consent was signed: _____ Day Started on the clinical protocol: _____

Visit	Collection Tubes to use:	Date Drawn	Fresh samples (shipped on Ice pack)	Processed MNC (Shipped on Dry Ice)	Please check all sent samples
Pre-study Screening (Four green top tubes (5-10cc each))	**1. PD-L1 flow cytometry** **2. WT1 MRD** **3. T cell subsets/CyTOF** **4. TCR sequencing**				
Week 13 (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. TCR sequencing** **3. T cell subsets/CyTOF**				
Week 25 (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. TCR sequencing** **3. T cell subsets/CyTOF**				
Week 53 (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. TCR sequencing** **3. T cell subsets/CyTOF**				

At the time of off study (Three green top tubes (5-10cc each))	**1. WT1 MRD** **2. TCR sequencing** **3. T cell subsets/CyTOF**				

APPENDIX D ARCHIVED BONE MARROW BIOPSY ACQUISITION FORM

The University of Chicago **Protocol#**
Archived Bone Marrow Core Biopsy Acquisition Form **_____**

Clinician/Research Nurse: Please Fill Out
Bone Marrow Core Biopsy Tissue (Blocks/Unstained slides)

Patient Name: _____ **Week#_____**

Patient Protocol ID #:_____ **Date Obtained: _____**

Date of Birth: _____ **Attending Physician: _____**

Origin of Tissue: _____ **Institution: _____**

Cross-over samples (COS)? (circle): yes / no
if YES Cross-over samples: label COS-CXDX on tubes

Date consent was signed: _____ **Day Started on the clinical protocol: _____**

Researcher: Please Fill Out

Date Samples received: _____ **Data entered into Database: Yes No**

Name of Data Manager informed: _____ Date Informed:_____

Visit	Collection	Date of biopsy
At the diagnosis of initial AML	**1. block ____** **2. Unstained Slides_____**	
At the time of study screening	**1. block ____** **2. Unstained Slides_____**	
After AML relapse, at the time of cross over to get Nivolumab	**1. block ____** **2. Unstained Slides_____**	

APPENDIX E ARCHIVED BONE MARROW ACQUISITION FORM
The University of Chicago **Protocol#**
Archived Bone Marrow Aspiration Acquisition Form **_____**

Clinician/Research Nurse: Please Fill Out
Bone Marrow Aspiration (frozen bone marrow cells)

Patient Name: _____ **Week#_____**

Patient Protocol ID #:_____ **Date Obtained: _____**

Date of Birth: _____ **Attending Physician: _____**

Origin of Tissue: _____ **Institution: _____**

Cross-over samples (COS)? (circle): yes / no
if YES Cross-over samples: label COS-CXDX on tubes

Date consent was signed: _____ **Day Started on the clinical protocol: _____**

Researcher: Please Fill Out

Date Samples received: _____ **Data entered into Database: Yes No**

Name of Data Manager informed: _____ Date Informed:_____

Visit	**Collection**	**Date of biopsy**	**Date samples received**	**Date enter to database**
At the diagnosis of initial AML	**Frozen cell vial _____**			
Note:				

APPENDIX F SHIPPING INSTRUCTIONS

SHIPPING INSTRUCTIONS
All shipments must contain a completed Sample Identification form and Tracking form.

******Each site will be required to pay for shipping using their per- patient amounts, and really should only batch ship once per patient.**

*** Green top tubes should be collected and processed as per Section 9.**

Paraffin sections can be sent at room temperature.

The blood samples should be labeled with the patient protocol ID#, Week identifiers:

SHIPPING METHODS

1. The fresh (unprocessed) samples will be shipped on ice packs, NOT dry ice, same day or overnight to University of Chicago Wendy Stock laboratory for sample processing.

2. For centers with ability to process the samples, MNC could be isolated using Ficoll method and then be frozen in 90% fetal bovine serum/10% DMSO and stored in liquid nitrogen or -80ºC freezer before shipment in batches to Wendy Stock laboratory
    a. Lab Address:
    W. Stock Lab
    900 East 57th Street
    KCBD Room 9160 LB49
    Chicago, IL 60637
    b. Contact Number:
    773-795-3727

3. Archival tissue slides or blocks should be sent to the University of Chicago Phase II Consortium for storage. Please include a copy of patient pathology report with submission of slides, or send in email
    a. Address:

    Phase II Consortium
    5841 S. Maryland Ave., MC 2115
    Chicago, IL 60637
    b. Contact number:
    773-834-1746
    Email: phaseIIcra@medicine.bsd.uchicago.edu

****Prior to shipment** (cells, and tissue) please EMAIL Bartholomew Eisfelder at beisfelder@medicine.bsd.uchicago.edu, Noreen Fulton at nfulton1@bsd.uchicago.edu (fresh

samples) and phaseIIcra@medicine.bsd.uchicago.edu (tissue and slides) to notify that samples are being sent. **This is a requirement.**

*** Please ship preferentially on Monday, Tuesday or Wednesday, to allow for arrival prior to weekends.

Instructions for Shipping Specimens
The Federal Aviation Administration (FAA) is an arm of the Department of Transportation (DOT). The DOT requires that anyone involved in the transport of hazardous materials (such as infectious substances, diagnostic specimens, genetically modified organisms, biological products, and dry ice) needs to comply with the Federal transportation law (49 CFR 172.700). These regulations are applicable to anyone who handles, offers for transport, and transports dangerous goods or causes dangerous goods to be transported. Please comply with federal regulations 49 CFR and IATA 1.5.

Helpful Hints for Shipping
Dry Ice: Packing Instruction
Processed MNC of blood and bone marrow cell pellet.

1. *Please fill cooler at least half full with dry ice and cover specimen bag with dry ice to prevent thawing*.
2. Enclose embedded tissue or serum/plasma vial in a sealed biohazard bag.
3. Include an absorbent pad within primary sealed biohazard bag.
4. Place the primary bag in a secondary bag.
5. Box (cooler) must be able to release gas build-up.
6. Indicate the dry ice weight in kg on both the waybill and on the box.
7. Include the marking "Dry Ice, UN1845" on the box.
8. Place a Class 9 label on the box.

Training courses and additional information about shipping requirements can be also found at:
http://www.saftpak.com/
http://hazmat.dot.gov

Whole Blood MNC and cells from bone marrow aspiration
-Please write the study number, patient initials, cycle number, and date on tubes.
-Store in liquid nitrogen**.**

Please note, the only thing shipped on dry ice should by already isolated/ frozen mononuclear cells (MNC) or RNA lysates. Unprocessed whole blood and bone marrow aspirates should be shipped on ice packs, NOT dry ice.

Bone Marrow Core Biopsy Tissue
Preferably, whole Tissue blocks sent to University of Chicago and processed. Blocks will be returned. Alternatively, please process paraffin embedded tissue as follows:
1. For IHC- Unstained formalin fixed paraffin embedded tissue sections should be cut at **4-5 micron** thickness. Cut **20** serial sections and store at room temperature.

APPENDIX G FICOLL ISOLATION OF LYMPHOCYTES FROM BONE MARROW ASPIRATES AND WHOLE BLOOD SAMPLES

CONSUMABLES:

- 25, 10, and 1 mL pipettes
- Gauze
- 1.5 mL tubes
- 50 mL conical tubes
- 15 mL conical tubes
- 15 & 50 mL conical tube racks
- Transfer pipette
- RPMI media or PBS (stored at 4ºC)
- Ficoll-Paque Plus (GE Healthcare) (stored at 4ºC)
- Isopropanol
- 70% ethanol
- RNA STAT-60 or TRIzol
- Freezing Media (90% FBS, 10% DMSO; stored at -20 ºC)
- Liquid waste container

EQUIPMENT:

- Biological Safety Cabinet
- Centrifuge
- Pipette aid
- Automated cell counter
- Controlled rate freezing container (e.g., Mr. Frosty, CoolCell, etc.)

PROCEDURE

1. Once samples have been received, information on patient sheet including study name, MRN, patient ID and/or initials, date of sample, sample timepoint (pretreatment, etc.) and tissue type (BM/PB) should be cross-checked with the labels on the samples.

2. Prepare centrifuge: 400 g, room temperature with brakes ***OFF***.

3. Biological safety cabinet should be UV-sterilized for at least 15 minutes and wiped down with 70% ethanol prior to beginning.

4. Label all 50, 15, and 1.5 mL conical tubes with sample ID and tissue type.

5. Aliquot 20 mL cold Ficoll-Paque Plus to a 50 mL tube for each sample.

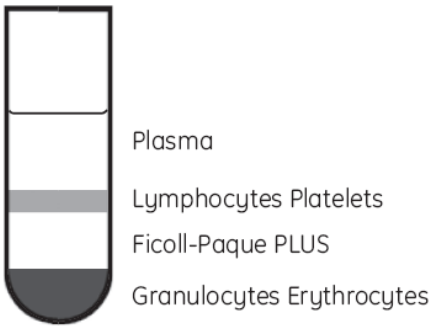
6. Using gauze, carefully open PB/BM samples. Immediately discard cap and gauze in biohazardous waste.

7. Pour up to 20 mL of PB/BM sample into empty 50 mL conical tubes. Record volume on sample sheet.
   - **If sample volume is greater than 20 mL**, split sample equally into two 50 mL conical tubes.

8. Add cold RPMI or PBS to sample to bring volume up to 30 mL using a 25 mL pipette, and mix three times.

9. Set Pipet-Aid to **SLOW**. Overlay sample onto Ficoll dropwise. Hold pipette tip at a slanted angle on the inside edge of the conical tube while dispensing at a steady and constant rate without mixing/penetrating Ficoll layer.

10. Centrifuge for 30 minutes at 400 g, room temperature <u>with brakes **OFF**</u>.



11. Following centrifugation, use a transfer pipette to transfer entire lymphocyte layer into an empty 15 mL tube. If samples of the same tissue type were split in Step 8, they may be recombined to one 15 mL tube.

12. Wash by adding RPMI or PBS to the 15 mL conical tube containing lymphocytes to bring volume up to 14 mL.

13. Centrifuge for 15 minutes at 400 g, room temperature with brakes **ON**.

14. Remove supernatant using a transfer pipette. Resuspend cells with RPMI/PBS and count cells using automated cell counter.

15. **Depending on the number of cells, samples may be frozen for viability and lysed for RNA directly. <u>If cell yields are poor, RNA takes priority</u>. Guidelines for this study are:**

Sample	Minimum Cell Number	Reagent Used	Parameters

WT1 MRD*	2 x 10^6	RNA STAT-60 or TRIzol	<5 x 10^6 cells – lyse with 0.5 mL 5-10 x 10^6 cells – lyse with 1 mL
T-Cell Subset	2 x 10^6	Freezing Media (90% FBS, 10% DMSO)	1 mL Freezing Media per cryovial No more than 10 x 10^6 cells per vial
TCR Sequencing*	5 x 10^6	RNA STAT-60 or TRIzol	<5 x 10^6 cells – lyse with 0.5 mL 5-10 x 10^6 cells – lyse with 1 mL

*If cell yield is poor, cells for RNA (WT1 and TCR sequencing) can be combined into one lysate.

16. For cells **frozen for viability (T-Cell Subset)**:

- Centrifuge cells at 400 g, room temperature for 5 minutes.
- Remove supernatant and resuspend with Freezing Media. Aliquot 1 mL into each labeled cryovial.
- Place cryovials in controlled rate freezing container, and store at -80ºC overnight. Transfer to liquid nitrogen storage the following morning.

17. If cells are going to be made into **RNA lysates**:

- Centrifuge cells at 400 g, room temperature for 5 minutes.
- Label 1.5 mL tubes:

  Acc. # and Patient ID
  GITC (Tissue Type (BM or PB))
  Date

- Remove supernatant and lyse cells with RNA STAT-60 or TRIzol according to parameters listed above.
  - <5 x 10^6 cells should be lysed with 0.5 mL RNA STAT-60 or TRIzol
  - 5-10 x 10^6 cells should be lysed with 1 mL RNA STAT-60 or TRIzol
- Store lysates at -80ºC.

APPENDIX H RISKS ASSOCIATED WITH PREGNANCY

Risks Associated with Pregnancy:
The toxicity of Nivolumab was tested in the pregnant cynomolgus monkeys (Investigator Brochure. (2014). While Nivolumab was well tolerated by the pregnant monkeys, in the offspring, maternal nivolumab administration was associated with fetal/neonatal mortality characterized by: 1) dose-dependent increases in third trimester fetal losses and increased neonatal mortality at higher dose. The cause(s) of these fetal losses and infant prematurity could not be determined. The teratogenic effect of Nivolumab in humans cannot be ruled out. Therefore, a risk minimization plan to prevent pregnancy must be observed.

Criteria for females of childbearing potential (FCBP)
This protocol defines a female of childbearing potential as a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy or 2) has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Counseling
For a female of childbearing potential, Nivolumab is contraindicated unless all of the following are met (i.e., all females of childbearing potential must be counseled concerning the following risks and requirements prior to the start of Nivolumab):

- She understands the potential teratogenic risk to the unborn child
- She understands the need for effective contraception, without interruption, throughout the entire duration of study treatment, dose interruption and 23 weeks after the end of study treatment
- She should be capable of complying with effective contraceptive measures
- She is informed and understands the potential consequences of pregnancy and the need to notify her study doctor immediately if there is a risk of pregnancy
- She understands the need to commence the study treatment as soon as study drug is dispensed following a negative pregnancy test
-

- She acknowledges that she understands the hazards and necessary precautions associated with the use of Nivolumab

The investigator must ensure that for females of childbearing potential:

- Complies with the conditions for pregnancy risk minimization, including confirmation that she has an adequate level of understanding
- Acknowledge the aforementioned requirements

The effect of Nivolumab on sperm is not clear at this time. Male patients taking Nivolumab must meet the following conditions (i.e., all males must be counseled concerning the following risks and requirements prior to the start of Nivolumab):

- Understand the potential teratogenic risk if engaged in sexual activity with a pregnant female or a female of childbearing potential
- Understand the need for the use of a condom even if he has had a vasectomy, if engaged in sexual activity with a pregnant female or a female of childbearing potential.

Contraception

Females of childbearing potential (FCBP) enrolled in this protocol must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual contact during the following time periods related to this study: 1) for at least 28 days before starting study drug; 2) while participating in the study; 3) during dose interruptions; and 4) for at least 23 weeks after study treatment discontinuation.

The two methods of reliable contraception must include one highly effective method and one additional effective (barrier) method. FCBP must be referred to a qualified provider of contraceptive methods if needed. The following are examples of highly effective and additional effective methods of contraception:

Highly effective methods:

- Intrauterine device (IUD)
- Hormonal (birth control pills, injections, implants)
- Tubal ligation
- Partner's vasectomy

Additional effective methods:

- Male condom
- Diaphragm
- Cervical Cap

Pregnancy testing
Medically supervised pregnancy tests with a minimum sensitivity of 25 mIU/mL must be performed for females of childbearing potential, including females of childbearing potential who commit to complete abstinence, as outlined below.

Before starting study drug

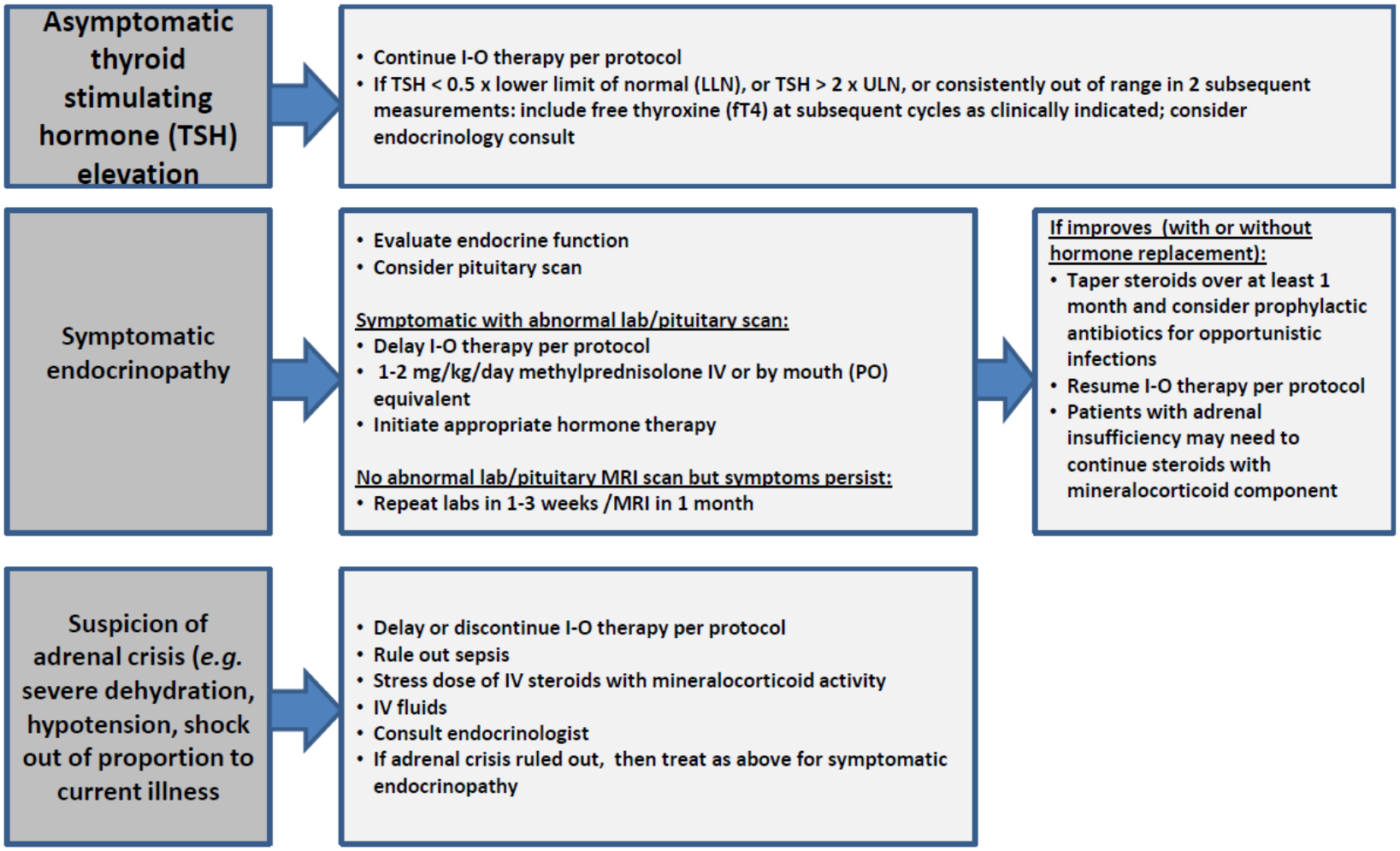
Female Patients:
Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab. The patient may not receive study drug until the study doctor has verified that the results of these pregnancy tests are negative.

Male Patients:
Must practice complete abstinence or agree to use a condom during sexual contact with a pregnant female or a female of childbearing potential while participating in the study, during dose interruptions and for at least 31 weeks (or 7 months) following study drug discontinuation, even if he has undergone a successful vasectomy.

APPENDIX I MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

Endocrinopathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (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.

GI Adverse Event Management Algorithm

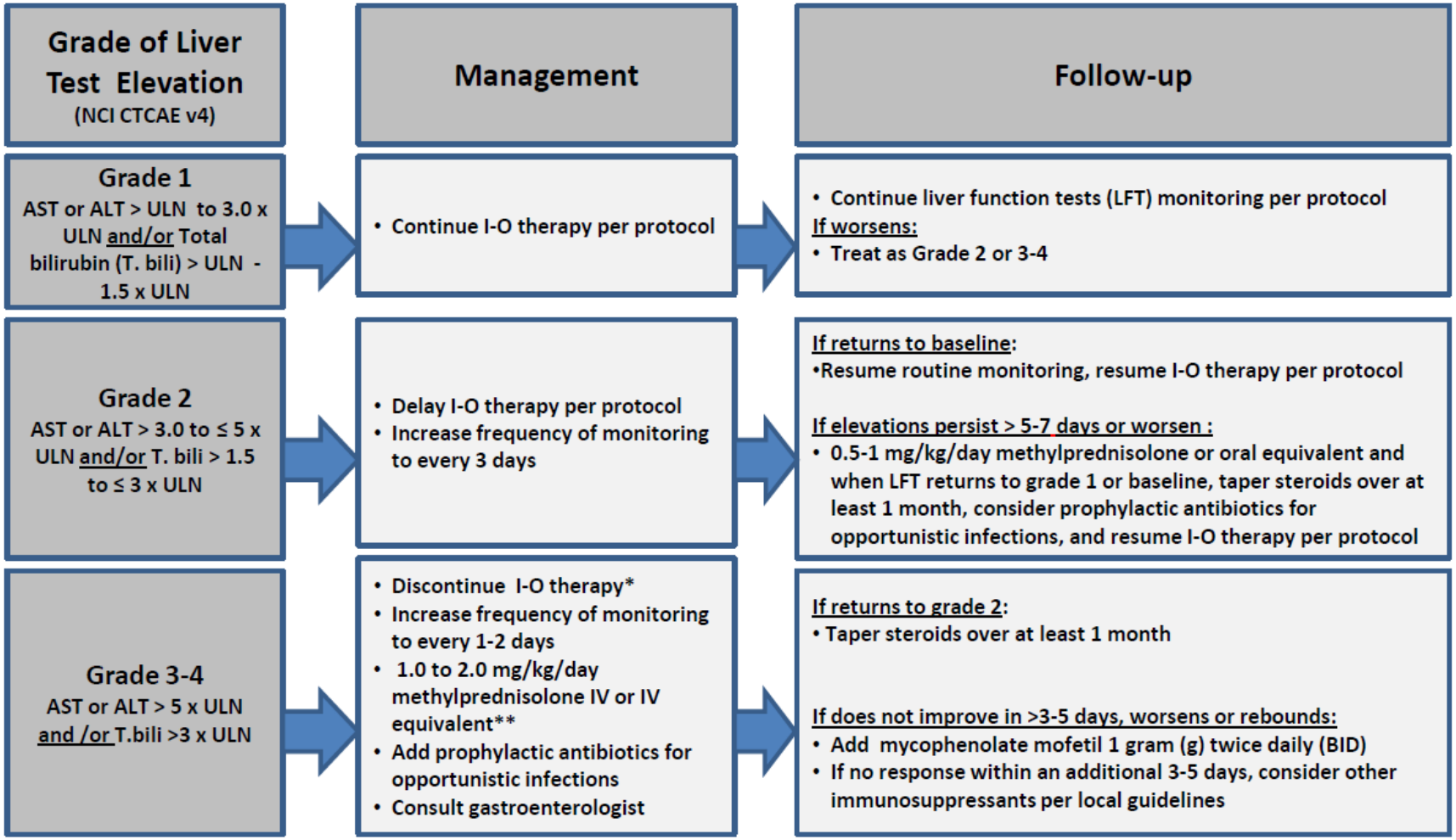
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

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.



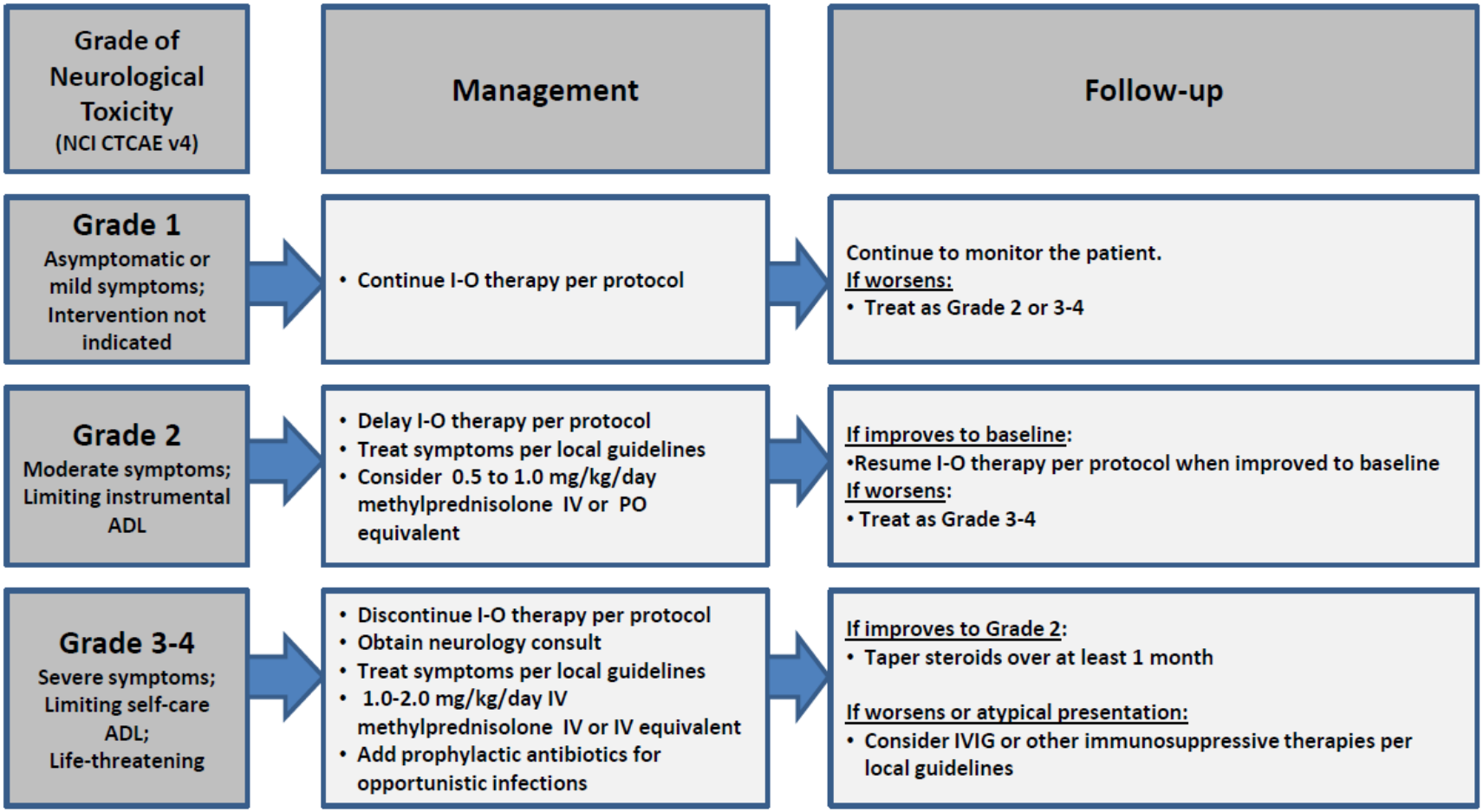
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Neurological Adverse Event Management Algorithm

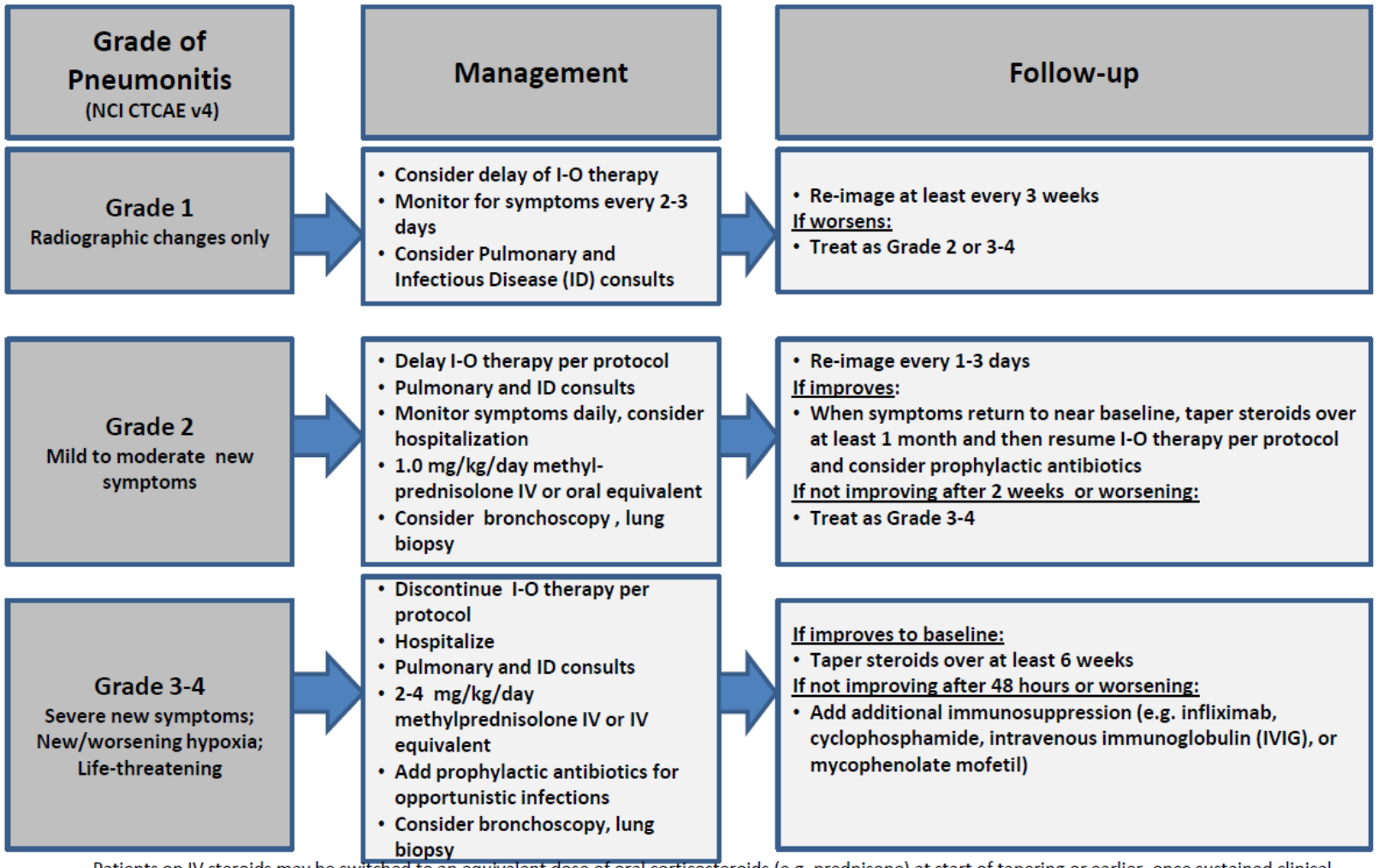
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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.

Pulmonary Adverse Event Management Algorithm

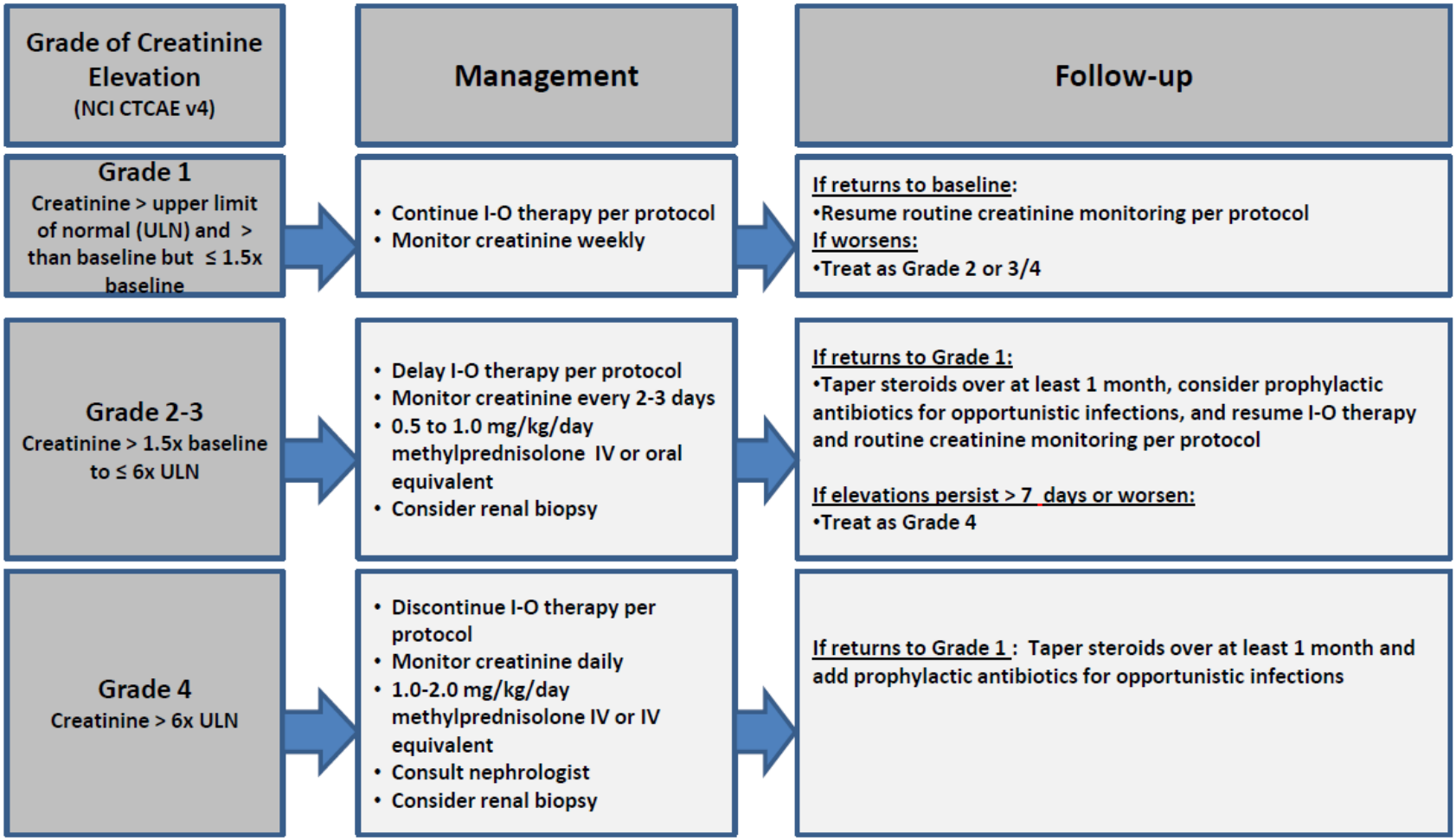
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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.

Renal Adverse Event Management Algorithm

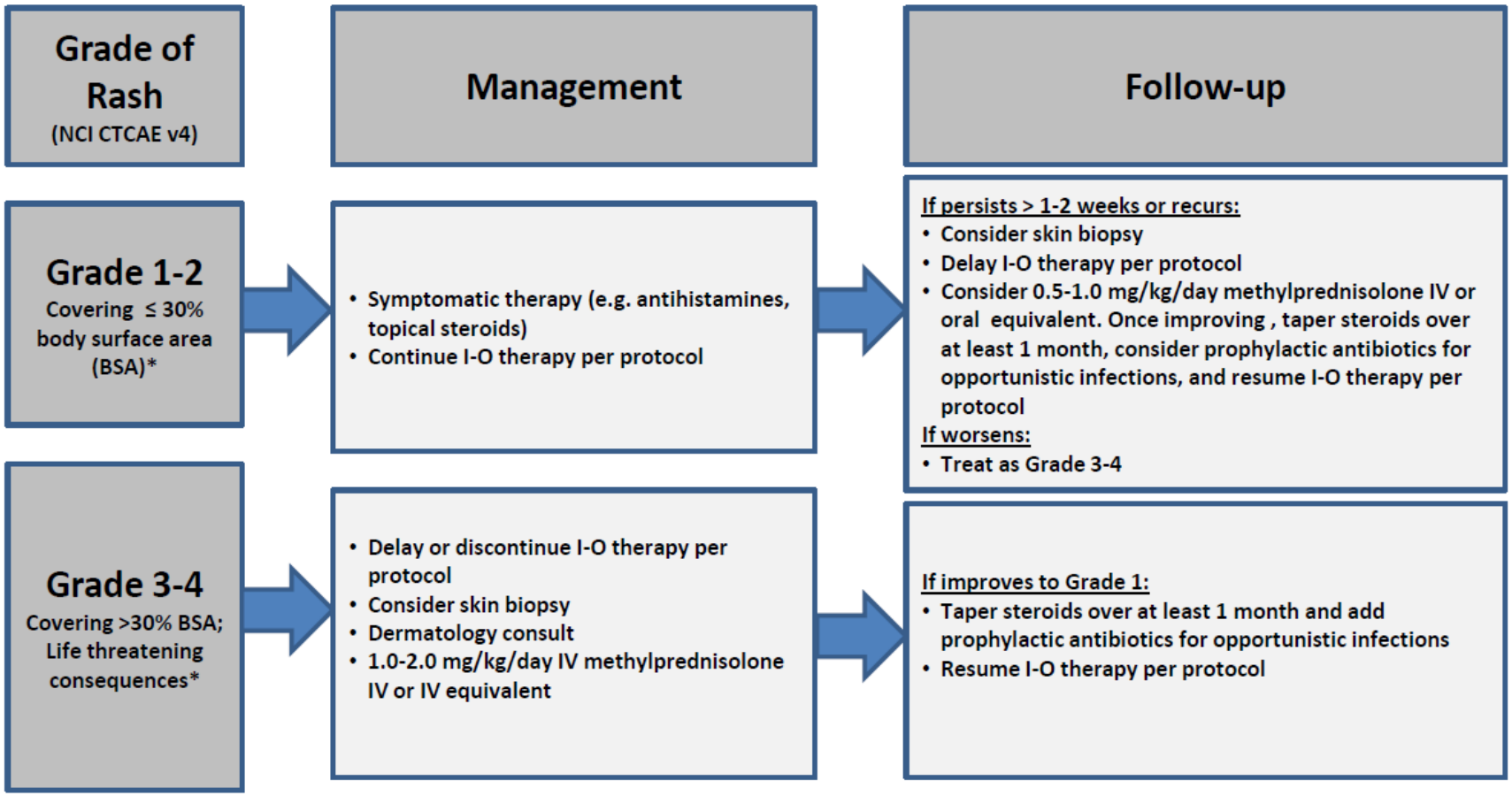
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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.

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.

APPENDIX J AML RISK CATEGORY GUIDELINES

Prognostic Group	Subsets
Favorable	Inv (16) or t(16;16);t(8;21); NK NPM1+FLT3 ITD-;NK and CEBPA+/+
Intermediate -1	NK and NPM-/FLT3 ITD- or CEBPA+/-
Intermediate- 2	Cytogenetic abnormalities not in "favorable" or "adverse" groups;FLT3 ITD positive
Adverse	−5, −7,5q-, abn 3q,17p,11q (other than 9;11), t(6;9) complex cyto; Insufficient metaphases for analysis; mutations in TP53, ASXL1, RUNX1

Estey, Elihu (2016)