DATE: January 3, 2023

TO: CTEP Protocol and Information Office

FROM: Ivana Gojo, MD

SUBJECT: Amendment in response to Notice of FDA Partial Clinical Hold from Dr. Mooney, NCI, dated 12/12/2022 and general formatting throughout the document.

I. SUMMARY OF CHANGES – Protocol

#	Section	Comments
1.	Header, Title Page	Updated version date.
2.	Title Page	Added the ClinicalTrials.gov identifier.
3.	Title Page	Updated LAO Protocol Contact's address and phone number.
4.	4.2.2	Updated who to contact for the SIV.
5.	5.3.1, 8.1.1	Removed reference to 72- and 96-hour infusions as requested in the notice.
6.	Throughout	General formatting and corrected typos throughout the document.

II. SUMMARY OF CHANGES – Consent Form

#	Section	Comments
1.	Header	Updated version date.
2.	Throughout	General formatting.
3.	How the study drugs are given in all groups	Updated to specify the IV bag will be changed every 1-2 days instead of every 1-4 days.

NCI Protocol #: 10030

Local Protocol #: ETCTN10030

ClinicalTrials.gov Identifier: NCT02879695

TITLE: A phase 1 study of blinatumomab in combination with checkpoint inhibitor(s) of PD-1 (nivolumab) or both PD-1 (nivolumab) and CTLA-4 (ipilimumab) in patients with poor-risk, relapsed or refractory $CD19^{+}$ precursor B-lymphoblastic leukemia

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NCI-Supplied Agent(s):
Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC #748726)
Ipilimumab (NSC #732442)
Blinatumomab (AMG-103) (NSC #765986)
IV Solution Stabilizer for Blinatumomab (AMG 103) (NSC #773150)

IND Sponsor: DCTD, NCI

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Amendment/Version 5.0/September 5, 2017
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Amendment / Version 11.0/ January 8, 2019
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CIRB revision to v16.0 was dated April 23, 2020
Amendment / Version 17.0 / January 28, 2021
Amendment / Version 18.0 / January 3, 2023

SCHEMA

Tumor Types: Patients older than 21 years with histologically or cytologically confirmed $CD19^+$ Precursor B-acute lymphoblastic leukemia (Pre-B cell ALL) OR $CD19^+$ mixed phenotype acute leukemia (MPAL) with relapse following or refractory to at least one prior line of therapy, or adults ≥60 years old with a new diagnosis of Pre-B cell ALL $CD19^+$ MPAL who are either not a candidate for or do not wish to receive traditional induction chemotherapy. Patients 16-21 years-old will be eligible following a second relapse or if refractory to at least two prior lines of therapy.

Performance Status: ECOG 0-2

Abnormal Organ Function Permitted:

- Total bilirubin ≤2.0 mg/dL (except patients with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)
- AST(SGOT)/ALT(SGPT) ≤5× ULN
- 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 = $\frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$

Prior Therapy:

- > 90 days after stem cell infusion for allogeneic hematopoietic stem cell transplant (HSCT), have no evidence of acute graft-versus-host disease (GVHD) or active chronic (grade 2-4) GVHD, and are off all transplant-related immunosuppression ≥ 2 weeks
- Prior blinatumomab permitted if leukemia remains $CD19^+$ and no unacceptable toxicity with prior blinatumomab treatment
- If Ph+ ALL, then patient must have failed at least one 2^{nd} or 3^{rd} generation TKIs and been off treatment for > 1week
- > 3 weeks off induction or reinduction chemotherapy
- > 4 weeks off investigational drugs or immunotherapy (e.g. rituximab)

Phase of Study: 1

Treatment Plan: Blinatumomab will be administered by continuous intravenous infusion for 28 days. Nivolumab treatment will start on day 11 of blinatumomab infusion in cycle 1 and continue every 2 weeks. If the combination proves tolerable, then ipilimumab will be added in subsequent cohorts starting on day 11 of cycle 1 and continue every 6 weeks. Treatment will continue for 5 cycles of blinatumomab and up to one year of nivolumab/ipilimumab. An expansion cohort of 6 patients will be treated at MTD for blinatumomab, nivolumab and ipilimumab. Also, an expansion cohort of 6 patients will be treated at MTD for blinatumomab and nivolumab only.

Dose Escalation Schedule[#]			
Dose Level	**Dose**		
	Blinatumomab[&]	**Nivolumab**	**Ipilimumab**
All Dose Levels	Nivolumab and ipilimumab (where indicated) will be initiated on Day 11		
Level A-1[*]	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	80 mg IV every 2 weeks	None
Level A1[*]	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	240 mg IV every 2 weeks	None
Level B-1	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	80 mg IV every 2 weeks	1 mg/kg IV every 6 weeks
Level B1	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	240 mg IV every 2 weeks	1 mg/kg IV every 6 weeks

[*] Safety run-in of blinatumomab and nivolumab: Up to 6 subjects will be treated at dose level A1 and dose level A-1 (if necessary) prior to addition/dose escalation with ipilimumab.
[#] Dose expansion of blinatumomab, nivolumab, and ipilimumab: An expansion cohort of 6 subjects will be treated at the MTD.
Dose expansion of blinatumomab and nivolumab only: An expansion cohort of 6 subjects will be also treated at the MTD for blinatumomab and nivolumab only.
[&] In Cycle 2 and subsequent cycles blinatumomab will be administered at 28 µg/day IV continuous infusion Days 1-28. Blinatumomab cycles are administered every 42 days.

Regimen Description					
Agent	**Premedications ; Precautions**	**Dose**[**]	**Route**	**Schedule**[**]	**Cycle Length**
Blinatumomab[&]	20 mg IV dexamethasone 1 hour prior to initiation in each cycle and at step dose escalation in cycle 1	9 µg/day on Days 1-7 28 µg/day on Days 8-28	Continuous IV infusion	Days 1-28 of each cycle up to 5 cycles	42 days (6 weeks)
Nivolumab	None	80 or 240 mg IV	IV, 30-minute infusion	Day 11, and then every 2 weeks for 1 year	
Ipilimumab	None	1 mg/kg	IV, 90-minute infusion	Day 11, and then every 6 weeks for 1 year	
**Doses and schedule as appropriate for assigned dose level. [&] In Cycle 2 and subsequent cycles blinatumomab will be administered at 28 µg/day IV continuous infusion Days 1-28. Blinatumomab cycles are administered every 42 days.					

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate the safety and tolerability of the blinatumomab given in combination with nivolumab alone, or in combination with both nivolumab and ipilimumab in subjects with poor-risk, relapsed or refractory $CD19^+$ Pre-B cell ALL or $CD19^+$ mixed phenotype acute leukemia (MPAL).

1.1.2 To determine the maximum tolerated dose (MTD) of the combination of blinatumomab plus nivolumab, and blinatumomab plus both nivolumab and ipilimumab and to further confirm the safety of the combination therapy in subjects with poor-risk, relapsed or refractory $CD19^+$ Pre-B cell ALL or $CD19^+$ mixed phenotype acute leukemia (MPAL).

1.2 Secondary Objectives

1.2.1 To observe and record anti-leukemia activity of blinatumomab and nivolumab, and blinatumomab plus both nivolumab and ipilimumab, including the effects on minimal residual disease (MRD). Although the clinical benefit of nivolumab and ipilimumab has not yet been established in ALL, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

1.2.2 To assess preliminary anti-leukemia activity in expansion cohorts of patients with poor-risk, relapsed or refractory $CD19^+$ precursor B-lymphoblastic leukemia, or $CD19^+$ mixed phenotype acute leukemia (MPAL).

1.3 Exploratory Objectives

1.3.1 To examine changes in absolute lymphocyte count and distribution of T cell subsets ($CD4^+$, $CD8^+$, T_{regs}, T_{effs}) and their differentiation status, NK cells, and B cells before and post-blinatumomab, and immune checkpoint inhibitor(s) therapy in both peripheral blood and the bone marrow microenvironment.

1.3.2 To explore changes in T cell co-signaling receptors expression in defined T cell subpopulations and their canonic transcription factor expression in both peripheral blood and bone marrow before and post-blinatumomab, and immune checkpoint inhibitor(s) therapy.

1.3.3 To examine changes in expression of co-signaling molecules on leukemia blasts ($CD10^+/CD19^+/CD34^+$) before and after treatment with blinatumomab and checkpoint inhibitors.

1.3.4 To examine the serum levels of cytokines before and after treatment with blinatumomab and checkpoint inhibitors, including the levels of sCTLA-4.

1.3.5 To perform immune profiling of T cell repertoire and characterize T cell transcriptional signature before and after treatment.

2. BACKGROUND

2.1 Study Diseases

2.1.1 Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (ALL) is highly curable in children with 10-year overall survival (OS) as high as 85% in recent studies.[1] However, cure rates in adult ALL in the United States are much poorer with a 5-year relative survival of 35.5%.[2] The poor survival for adults is likely attributable to the high frequency of relapse and the poor prognosis of patients with relapsed disease. The large Medical Research Council (MRC) UKALL12/ECOG2993 trial that enrolled 1,508 patients showed that 44% of adult ALL patients relapse after first complete remission (CR1) at a median of just 11 months. Median OS for those who relapsed was 24 weeks, and only 22% of relapsed patients were alive at 1 year.[3] A separate study including 357 adults with a first relapse of B-precursor ALL (Pre-B ALL) who were previously treated with chemotherapy alone showed a CR rate of 46% after salvage chemotherapy, but a median survival of just 5.8 months for those who did not achieve CR2. The duration of first remission was an important prognostic factor as those who maintained a longer CR1 (i.e. >18 months) had a higher probability to achieve a second remission.[4] Thus, it is not surprising that the response rate for patients receiving a salvage regimen due to a second relapse or refractory disease is worse with only 18% of patients achieving a CR and a median survival of just 3 months. Overall, 1-year survival for patients who fail to respond to frontline therapy is only 15%.[5,6] Thus there is a significant need for new therapies in adult ALL to treat both relapsed and refractory patients.

In addition to the poor prognosis of patients with relapsed or refractory ALL, older adults often have worse outcomes with standard chemotherapy regimens. While pediatric-inspired regimens have improved outcomes in adolescents and young adults with ALL, this approach remains challenging in older patients with a cumulative incidence of chemotherapy-related death of 23% for those over 45 years old.[7-9] Similarly, patients who are 60 years of age or older and receive the standard induction regimen of hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD) suffer an induction-related mortality rate of 10% with an additional 34% dying in first remission due to chemotherapy-related toxicities.[10] Given these poor outcomes, older patients are often deemed unfit for traditional treatment regimens, so there is a need to develop alternative regimens for this patient population that are both tolerable and effective.

2.1.2 $CD19^+$ Mixed Phenotype Acute Leukemia (MPAL)

Mixed phenotype acute leukemia (MPAL) is rare, representing 0.5-2.4% of acute leukemias based on the 2008 WHO criteria. The disease occurs predominantly in adults (68%), and 60% express CD19 in historical case series with only about 25% of those cases showing evidence of the Philadelphia chromosome.[11,12] Due to the rarity of MPAL there are no prospective randomized trials to point towards the optimal treatment approach, so treatment approaches are varied.[13] In one large case series, first-line treatment was based on ALL therapy in 27 patients, AML therapy in 34 patients, or a combination thereof in 5 patients, while one patient received single agent imatinib.[11] Historically, complete remission rates with ALL-based regimens (64-

85%) have been far superior to remission rates with AML-based regimens (28-41%).[11,14,15] However, despite a reasonable initial response to ALL-based chemotherapy regimens, median survival is just 18 months with the majority of patients dying from relapsed or refractory disease.[11] Thus there is a significant need for new therapies in adult $CD19^+$ MPAL to treat both relapsed and refractory patients.

2.2 CTEP IND Agents

2.2.1 **Blinatumomab**

Blinatumomab (AMG103, MT103, and NSC#-707389) is a fusion protein composed of two single-chain antibodies (scFv), murine anti-CD19 scFv and murine anti-CD3 scFv. It has dual specificity for CD19, a B-cell lineage-specific antigen, and the invariant CD3ε subunit of the T-cell receptor (TCR), which is present on all T lymphocytes.[16] Upon contact with $CD19^+$ target cells, blinatumomab induces polyclonal T cell activation and T cell-mediated tumor cell lysis through both the $CD4^+$ and $CD8^+$ T cell subsets with the $CD8^+$ population (effector memory T cells, T_{EM}) responsible for the most rapid killing.[17] Blinatumomab-activated T cells are capable of serial killing at low effector-to-target (E:T) cell ratios, which is possibly a consequence of the much greater affinity of the BiTE for CD19 (10^{-9} M) as compared to CD3 (10^{-7} M).[18] This differential affinity allows $CD3^+$ T lymphocytes to dissociate from the antibody construct more readily than the target cells and subsequently bind additional tumor cells.[19] Its high therapeutic index is derived from the fact that CD19 is expressed on most B-lineage malignancies including B-precursor ALL and lymphomas but is absent on hematopoietic stem cells (HSCs).[20] Treatment with blinatumomab is associated with a rapid depletion of peripheral B cells, accompanied by T cell activation and a transient increase in cytokines.

2.2.1.1 Nonclinical Development of Blinatumomab

The in vitro efficacy of blinatumomab was first investigated in NALM-6, RAJI, and DoHH2 cell lines using donor T cells from 86 different donors obtained from local blood banks. In contrast to other CD19-specific T-cell-recruiting bispecific antibody formats, blinatumomab demonstrated target cell lysis within a few hours, in the absence of any extra T-cell stimuli, and at low effector:T cell ratios using unstimulated lymphocytes. In these assays, blinatumomab exhibited ED_{50} values of 10^{-12} to 10^{-13} M, which greatly exceeded its binding affinities with K_D ranges of 10^{-7} to 10^{-9}. Notably, the efficacy of blinatumomab as measured by cytotoxicity assays varied significantly depending on the T cell donor.[21] The in vitro success of blinatumomab led to investigation of its in vivo efficacy in NOD/SCID mice. In these experiments, NOD/SCID mice were inoculated with NALM-6 cells leading to either subcutaneous tumors or frank leukemia. In mice with subcutaneous tumors, treatment with blinatumomab and human peripheral blood mononuclear cells (PBMCs) led to increased survival and suppressed tumor outgrowth in the absence of any T cell costimulatory compounds. In the leukemic form, injection of blinatumomab and human PBMCs led to the delayed onset of neurological symptoms including the complete prevention of neurological symptoms in a subset of mice.[22]

2.2.1.2 Clinical Development of Blinatumomab

Blinatumomab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy in completed and ongoing clinical trials in $CD19^+$ B-cell malignancies including Precursor B-lymphoblastic leukemia (Pre-B ALL) and Non-Hodgkin Lymphoma (NHL).

2.2.1.2.1 Pharmacokinetics

Blinatumomab consists of a single chain of 504 amino acids with a molecular weight of approximately 54 kDa. The pharmacokinetics of blinatumomab was assessed over a dose range from 5 to 90 mcg/m^2/day (approximately equivalent to 9-162 mcg/day). Following continuous intravenous infusion, the steady state serum concentration (Css) was achieved within a day and remained stable over time. The estimated mean (SD) volume of distribution based on terminal phase (Vz) was 3.93 (2.32) L in adult ALL patients. The estimated mean (SD) systemic clearance was 1.81 (0.58) L/hour and the estimated mean (SD) half-life was 1.47 (0.53) hours in adult ALL patients. Negligible amounts of blinatumomab were excreted in the urine at the tested clinical doses. Like other protein therapeutics, blinatumomab is expected to be degraded into small peptides and amino acids via catabolic pathways. At the clinical doses of 9 mcg/day and 28 mcg/day for the treatment of adult relapsed/refractory ALL, the mean (SD) Css was 246 (305) pg/mL and 632 (510) pg/mL, respectively.

2.2.1.2.2 Efficacy

Blinatumomab was first studied in patients with relapsed NHL. In the initial phase I study of blinatumomab in patients with indolent NHL, blinatumomab (0.5 to 90 μg/m^2/day) was given over 4-8 weeks and demonstrated dose-dependent efficacy. Responses were observed at ≥15 μg/m^2/day dose level with the MTD being defined at 60 μg/m^2/day. Unfortunately, dose-limiting toxicities including CNS toxicities were first encountered at a dose of 15 μg/m^2/day with 29.6% of patients treated at this and higher dose levels requiring treatment discontinuation due to toxicities, the majority of which were neurologic.[23] A low peripheral blood B-to-T cell ratio was associated with neurologic toxicity, which was reduced with a double stepwise dosing approach. At the MTD, the overall response rate was 69% (24/35) across the indolent lymphoma types.[24] Subsequent long-term follow-up of patients in this trial showed significantly improved overall, progression-free, and treatment-free survival for those patients treated with the target dose of 60 μg/m^2/day compared to those who received lower doses of blinatumomab.[25] In a phase II study of patients with relapsed/refractory diffuse large B cell lymphoma (DLBCL), a stepwise dosing approach of blinatumomab escalating to a dose of 112 μg/day was found to offer the optimal risk-benefit ratio and an overall response rate of 43% was achieved with a median response duration of 11.6 months. In this study, 12% of patients discontinued treatment prematurely due to adverse events.[26]

The success of blinatumomab in ALL was first demonstrated in a study of 21 patients who achieved a hematologic complete response with induction and consolidation but had persistent MRD that could be measured by PCR. Treatment with blinatumomab (15 μg/m^2/day continuously for 4 weeks every 6 weeks up to 4 cycles) led to clearance of MRD in 80% of the evaluable patients with a 61% relapse free survival at a median observation of 33 months, which

compares quite favorably with an expected RFS of 20% in patients with a known molecular relapse.[27,28] A confirmatory single-arm, phase II study using blinatumomab in MRD+ Pre-B ALL enrolled 116 patients with 78% achieving clearance of MRD after one cycle of treatment leading to a median overall survival of 40.4 months as opposed to just 12.0 months for patients who failed to clear their MRD.[29,30]

In an initial trial of thirty-six patients with relapsed/refractory Ph-negative, B-precursor ALL treated with blinatumomab, 69% achieved CR or CR with partial hematologic recovery (CRh), and 88% of responders achieved MRD negative status. During the dose finding stage of this study, 28.5% of patients who were treated with a flat dose of 15 μg/m^2 daily over four weeks were replaced due to adverse events, while none of the patients who were treated with a step-wise dose escalation using 5 μg/m^2 daily for one week followed by 15 μg/m^2 daily for three weeks suffered adverse events requiring replacement.[31] Given the improved tolerability of the step-wise dosing approach, it has become the standard dosing regimen for blinatumomab in subsequent studies in ALL. In a larger study of 189 adults with relapsed/refractory Ph-negative, B-precursor ALL, 44% of patients achieved CR or CRh after 1-2 cycles of blinatumomab with 82% of responders clearing their MRD. An additional 8% of patients achieved a blast free hypoplastic or aplastic bone marrow for a total response rate of 52% after two cycles of treatment.[24] The median relapse-free survival was 5.9 months, with MRD responders having a median RFS of only 6.9 months. The majority of grade 3 or greater adverse events were restricted to hematologic toxicities such as febrile neutropenia (25%), neutropenia (16%), and anemia (14%); except for grade 3 cytokine release syndrome (2%) and grade 3 and/or 4 neurologic events (13%).[32] The success of blinatumomab has not been restricted to Ph-negative ALL, as a recent trial including 45 patients with relapsed or refractory Ph-positive Pre-B ALL who had failed at least one second-generation TKI demonstrated a response rate (CR + CRh) of 36% with 86% of responders achieving clearance of MRD.[33] The TOWER trial is an ongoing, randomized, open-label phase 3 study to evaluate the efficacy of blinatumomab versus investigator choice standard of care (SOC) chemotherapy in adult subjects with relapsed/refractory ALL. Subjects are randomized 2:1 to blinatumomab versus 1 of 4 SOC chemotherapy regimens. Patients receiving blinatumomab in this trial are eligible for up to 9 cycles of treatment with the last 4 cycles designated as maintenance therapy and given every 12 weeks as opposed to the standard 6 week interval.[24]

2.2.1.2.3 Toxicology

Nine hundred thrirty-two subjects (819 adult and 113 pediatric subjects) have received treatment in company-sponsored cIV infusion studies in the blinatumomab clinical development program. Subjects have been treated with blinatumomab at doses ranging from 0.5 μg/m²/day to 90 μg/m²/day. The maximum duration of exposure has been 196 days at a dose of 5/15 μg/m2/day. Treatment-emergent adverse events (TEAEs) were reported for similar percentages of subjects across the R/R ALL, MRD-positive ALL, and NHL studies. The most frequently reported (those reported in ≥ 20% of subjects overall) TEAEs were pyrexia, headache, nausea, anemia, hypokalemia, and diarrhea. Across studies, 55.8% of subjects had AEs of grade ≥ 3 that were considered by the investigator to be related to treatment. The most frequently reported treatment-related events of grade ≥ 3 were lymphopenia (8.2%), neutropenia (13.7%), febrile neutropenia (16.5%), and leukopenia (7.7%). Across studies, 37.1% of subjects had serious adverse events

that were considered by the investigator to be related to treatment. The most common treatment-related serious adverse events overall were lymphopenia (5.3%), pyrexia (8.2%), encephalopathy (3.0%), and tremor (2.5%). The incidence of many TEAEs was 2-fold or more higher in the NHL population than in the other indications, which was likely attributable to the increased dose of blinatumomab used in the NHL studies. Most permanent treatment discontinuations were due to nervous system events. Approximately 16.4% of subjects across all studies had TEAEs leading to study discontinuation that were considered by the investigator to be related to treatment. The most frequently reported treatment-related events leading to study discontinuation were encephalopathy (2%), aphasia (1.1%), and tremor (1.1%). Similarly, the most common cause of dose interruptions due adverse events were those ascribed to nervous system events. Of note, in the adult R/R ALL studies, any grade ≥ 3 CNS event required treatment interruption. Approximately 29% of subjects had TEAEs leading to treatment interruption across all studies. The most frequently reported treatment-related events leading to treatment interruption were pyrexia (4.0%), cytokine release syndrome (1.8%), encephalopathy (1.7%), and, and tremor (1.6%).[24]

2.2.1.2.4 Pharmacodynamics/Biomarkers[24]

Pharmacodynamic (PD) measures included lymphocyte subsets and cytokines. The response to blinatumomab was characterized by peripheral B-cell depletion, T-cell activation and redistribution as well as transient cytokine elevation. Consistent PD profiles were observed across clinical trials following the cIV regimen.

T-cell dynamics: Following blinatumomab cIV infusion, peripheral T-cell counts initially declined within 1 to 2 days to very low levels, a phenomenon described as redistribution from periphery to tissues. After the initial decline, T-cells started to increase and reached baseline levels in about 14 days. Increase of T-cell counts above baseline was found in some subjects. There was no difference between the dynamics of $CD4^+$ and $CD8^+$ T cells. A high inter-individual variability was observed in T-cell baseline levels. The time to return to baseline was variable across subjects (10 to 30 days).

B-cell Dynamics: B-cell counts in peripheral blood decreased rapidly and became undetectable during treatment at doses ≥ 5 $\mu g/m^2$/day (or 9μg/day) in most subjects. No recovery of B-cell counts was observed during the drug-free period between treatment cycles. Incomplete depletion of B cells was observed at doses of 0.5 and 1.5 $\mu g/m^2$/day. High inter-individual variability was found in baseline B-cell counts. Associated with the B-cell depletion, decreases in immunoglobulin (Ig) levels have been observed. Long-term Ig levels were measured in 6 MRD-positive ALL subjects treated with blinatumomab. Partial to complete recovery of IgM and IgG levels and incomplete recovery of IgA were observed during the long-term safety follow-up period.[34]

Cytokine Dynamics: The measured cytokines were TNF-α, IL-2, IL-6, IL-8, IL-10, IL-12, IL-4, and IFN-γ. Transient elevation of cytokines was observed in some subjects in the first 2 days following the blinatumomab infusion. The elevated cytokine levels returned to baseline within 24 to 48 hours during the infusion period. In subsequent treatment cycles, cytokine elevation was only observed in few subjects with much less intensity. The magnitude of cytokine elevation

trended higher at higher doses. The inter-subject variability in cytokine elevation was large.

See Section 8.1.1 for drug information.

2.2.2 **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.[35] 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.[36]

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, cellular 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, 2014). 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.[37] 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,[38] renal,[39-41] esophageal,[42] gastric,[43] ovarian,[44] pancreatic,[45] lung,[46] and other cancers.[35]

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.

2.2.2.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated.[35] 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.

2.2.2.2 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.[35] 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.

2.2.2.2.1. 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.[35] 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.

2.2.2.2.2. 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.[47] 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.[48] In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year.[49]

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).[36] 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.[50] 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.

2.2.2.2.3. Toxicology

A MTD of nivolumab was not defined.[51] 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,[36] grade 3 or 4 treatment-related events were noted in 53% of patients. Skin rash

represents the majority of these events.

2.2.2.2.4. 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.[36] 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 PD-L1 expression and responses may not be present in patients treated with the combination. Tissue expression of PD-L2, 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.

See Section 8.1.2 for drug information.

2.2.3 **Ipilimumab**

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4, Yervoy™) is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells.[52] CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and inhibits its interaction with ligands on antigen-presenting cells (APCs). The proposed mechanism of action for ipilimumab's effects in subjects with melanoma is indirect, possibly through T-cell potentiation and mediation of antitumor immune responses.

Ipilimumab has been approved for the treatment of unresectable metastatic melanoma in over 40 countries including the United States (US, March 2011), the European Union (July 2011), and Australia (July 2011). An initial dose finding study of ipilimumab in metastatic melanoma (0.3 mg/kg, 3 mg/kg, and 10 mg/kg every three weeks X 4) demonstrated that responses and AEs were dose-dependent.[53] In a subsequent phase III study in metastatic melanoma, ipilimumab was given every 3 weeks at 3 mg/kg, and 10-15% of patients suffered grade 3 or 4 irAEs including 7 such events that resulted in death among 540 participants (1.3%) who received ipilimumab. The most common grade 3 and 4 irAEs were in part similar to those seen with nivolumab including diarrhea and endocrine abnormalities, and could generally be managed by withholding treatment and giving steroids.[54] When compared to nivolumab, side effects from ipilimumab tend to be more common and more serious but remain manageable.

BMS and Medarex (acquired by BMS in Sep-2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing >13,800 subjects in several cancer types in completed and ongoing studies, including a compassionate use program.[52] The focus of the

clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies.

CTEP's clinical development of ipilimumab focuses on cervical, gastrointestinal, ovarian, prostate cancer, chronic lymphocytic leukemia, head and neck squamous cell carcinoma, solid tumors, Hodgkin and non-Hodgkin lymphomas, melanoma, and myelodysplastic syndrome. While the toxicity and clinical responses overlap, mechanisms of immune activation and range of responses appear to be different for each of the single agents. In a phase I study of patients with myelodysplastic syndrome (MDS) refractory to hypomethylating agents led by JHU, 27.3% of the patients maintained stable disease for >6 months, and three of eleven patients were able to undergo allogeneic HSCT without additional toxicities.[55] The lack of increased transplant-related toxicities following ipilimumab administration is particularly relevant in ALL, as allogeneic HSCT after a CR remains the preferred treatment paradigm in relapsed/refractory patients.

Preclinical data support the combinations of nivolumab and ipilimumab.[56]

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.[36]

For RCC results have been reported.[57]

The success of PD-1 and CTLA-4 targeted agents as monotherapy has stimulated intense interest in combination therapy including recent studies demonstrating the feasibility and efficacy of dual nivolumab and ipilimumab treatment in advanced melanoma. A double-blind phase III trial randomized patients with advanced melanoma to combination therapy with 1 mg/kg of nivolumab and 3 mg/kg of ipilimumab every 3 weeks or standard monotherapy with either nivolumab or ipilimumab. This study demonstrated a significant increase in objective responses (57.6% for the combination vs. 19.0% for ipilimumab vs. 43.7% for nivolumab) and median progression-free survival (11.5 months for the combination vs. 2.9 months for ipilimumab vs. 6.9 months for nivolumab) for patients treated with the combination of ipilimumab and nivolumab. The incidence of grade 3 and 4 AEs was significantly higher in the combination arm (55.0%) than in the nivolumab (16.3%) or ipilimumab (27.3%) monotherapy arms.[58] In an attempt to mitigate these toxicities, the combination is now primarily being evaluated in other disease settings with an increased dose of nivolumab (3 mg/kg) and a reduced dose of ipilimumab (1 mg/kg) q 3 weeks x 4 induction doses. Clearly, the side effects from combined immune checkpoint blockade are not trivial; however, in a review of 288 patients treated with hyper-CVAD, a standard adult ALL induction regimen, 54% of patients required hospitalization for treatment-related side effects.[59] This suggests that the treatment-related side effects of combined immune checkpoint blockade are acceptable when compared to traditional chemotherapy regimens for hematologic malignancies. Furthermore, the majority of irAEs are managed with steroids. Importantly, steroids are also indicated for the management of cytokine release syndrome (CRS) during blinatumomab administration, and it has been shown that patients are able to achieve remissions with blinatumomab monotherapy in spite of concurrent steroid

administration.[60] Thus if a patient receiving the combination of blinatumomab and immune checkpoint inhibitor(s) develops an irAE requiring steroids, then the initiation of steroids would not be expected to compromise the efficacy of continued treatment with blinatumomab.

See Section 8.1.3 for drug information.

2.3 Rationale

2.3.1 Immune Checkpoint Blockade and Leukemia

Immune inhibitory pathways, known as immune checkpoints, are upregulated in cancer cells and the tumor microenvironment leading to immune tolerance.[61] Major advances in immune checkpoint blockade over the last decade have resulted in impressive responses in solid tumors, but these novel agents have yet to gain traction in the treatment of leukemia. In a phase I trial, checkpoint blockade with an anti-programmed death ligand 1 (PD-L1) antibody, demonstrated objective responses in melanoma, non-small cell lung cancer (NSCLC), ovarian cancer, and renal cell carcinoma (RCC) with nearly 35% of patients demonstrating stable or improved disease after 24 weeks.[62] Based on this and subsequent studies, checkpoint inhibitors targeting the immune checkpoints PD-1 and CTLA-4 are approved for use in melanoma, RCC, and NSCLC; while recent studies have demonstrated promising results in Hodgkin's and Non-Hodgkin's lymphomas.[63,64]

Studies investigating the role of immune checkpoint pathways in leukemia are limited.[65] Clearly, the immune system plays a critical role in controlling leukemia as evidenced by the potent activity of a graft-versus-leukemia (GVL) effect following allogeneic HSCT in curing ALL and AML.[66,67] This suggests that leukemia cells are responsive to T cells and that anti-leukemia immunity may be susceptible to augmentation by checkpoint co-signaling molecule modulation. Preclinical studies using two different murine models of AML have shown that signaling through the PD-1/PD-L1 axis impairs anti-leukemic immunity and the *in viv*o blockade of this pathway improved outcomes.[68-70] Across different human studies, the frequency of PD-L1-expressing AMLs varied from 18% to more than 50%, increased after *ex vivo* stimulation by IFN-γ, after chemotherapy or at relapse.[71-73] We recently studied T-cell dynamics and inhibitory receptor (iR) expression in peripheral blood (PB) and bone marrow (BM) of AML patients. In our studies, we have identified several dominant expression patterns of iRs suggesting that iR signatures are consistent with immune recognition of AML and their role in sculpturing the effector T-cell responses against AML cell populations.[74] Furthermore, the analysis of $CD8^+$ T-cell gene expression profiles using microarrays revealed signatures of "T-cell exhaustion" at the time of diagnosis. Analysis of differentially-expressed genes and corresponding fold-changes through the use of Ingenuity pathway analysis identified downregulation of several co-stimulatory signaling pathways in $CD8^+$ T cells retrieved from patients with leukemia in comparison to healthy controls, underscoring the relevance of immune inhibitory pathways in controlling T-cell-mediated anti-leukemia responses. After chemotherapy, the gene expression pattern in $CD8^+$ T cells differed substantially in patients that achieved a response in comparison to those that did not enter remission and became similar to healthy controls.

Recent reports suggest that immune checkpoint pathways are also operational in ALL as evidenced by the expression of multiple co-inhibitory molecules on leukemia blasts and T cells from ALL

patients, including PD-L1 and PD-1, respectively.[75,76] The CD80/CD86 – CD28/CTLA-4 pathway provides key second signals that can regulate the activation or the inhibition of T cell responses. Both CD86 expression on ALL blasts and a soluble form of CTLA-4 (sCTLA-4) are found at a significantly higher frequency in ALL patients than healthy controls, and higher levels of each correlate with relapse risk and probability of survival following treatment.[77,78] Experimental evidence suggests that current anti-CTLA-4 antibodies bind both sCTLA-4 and membrane-bound CTLA-4 and as such that their administration may improve the immune recognition of ALL cells.[79] Furthermore, immune inhibitory pathways appear to be susceptible to upregulation within pro-inflammatory milieu such as the one created by blinatumomab, thereby promoting resistance to blinatomumab therapy and providing a unique opportunity for therapeutic intervention.

2.3.2 **Immune Checkpoint Inhibition Combined with BiTE Antibodies in Leukemia**

Recent laboratory findings suggest a role for combining BiTE antibody constructs with immune checkpoint blockade to enhance BiTE antibody efficacy in both AML and ALL. In regard to AML, the CD33/CD3 BiTE antibody construct AMG330 efficiently eliminated 19 of 38 primary AML samples in an *ex vivo* cytotoxicity assay.[80] The kinetics of AMG330-mediated lysis of primary AML cells was dependent on the initial E:T ratio, presence of central and effector memory T cells in the culture, and the intensity of CD33 expression on leukemia cells. Interestingly, the addition of AMG330 to primary AML cultures and the resulting pro-inflammatory environment led to the significant upregulation of PD-L1 on leukemia cells and PD-1 on the proliferating $CD45RA^{-}/CCR7^{+/-}$ memory T cell subset. The importance of this finding was further illustrated by the subsequent mechanistic studies which revealed that blockade of the PD-1/PD-L1 axis can significantly augment AMG330-induced leukemia cell lysis. This effect was particularly pronounced at lower E:T ratios and accompanied by a significant increase in T-cell proliferation and interferon-γ secretion.[80] These findings provide important pre-clinical rationale for combining BiTE antibodies and PD-L1/PD-1 pathway inhibitors in AML.

Several lines of evidence suggest that PD-1/PD-L1 axis is operational in ALL and may represent a potential mechanism of blinatumomab resistance. First, serial BM samples from a patient who failed to respond to blinatumomab demonstrated significant upregulation of PD-L1 on tumor cells after blinatumomab administration (2% at baseline vs. 40% after blinatumomab) without evidence of loss of CD19 expression on the tumor cells, which is an alternative mechanism of blinatumomab resistance. Second, an increase in PD-1 expression on BM lymphocytes was noted at the time of therapeutic failure. The primary patient's $CD3^{+}$T cells were unable to lyse patient's leukemia cells *ex vivo* in the presence of blinatumomab while the cells were effectively killed by healthy lymphocytes.[75] Finally, Feuchtinger et al. analyzed 20 primary ALL $CD10^{+}/CD19^{+}$ BM cells, and found that PD-L1, but also other co-inhibitory molecule such as HVEM, and co-stimulatory molecule such as CD86, were upregulated compared to healthy $CD10^{+}CD19^{+}$cells. PD-L1 upregulation on ALL blasts was noted upon exposure to Th1 cytokines such as interferon-γ or TNF-α. More importantly, PD-L1 was consistently more highly expressed on $CD19^{+}CD10^{+}$ BM cells in blinatumomab non-responders compared to responders and in relapsed ALL compared to initial diagnosis. Similarly, PD-1 but also other co-inhibitory molecule such as Tim-3 were more highly expressed (and co-expressed) on T cells from ALL patients when compared to healthy controls. In functional studies, the blinatumomab effect on T cells (proliferation and interferon-y production) *in vitro* appeared to be target cell-dependent and correlated with the target cell-

expression of co-signaling molecules. Combined inhibition of the PD-1 and CTLA-4 pathways with blinatumomab enhanced effector T cell function of healthy donors and patient's T cell*s* against ALL target cells more than PD-1 inhibition alone while inhibition of co-stimulatory CD28-CD80/86 interactions downregulated T cell effector function and proliferation. These findings led to the treatment of a single 12 year-old refractory ALL patient with the combination of pembrolizumab and blinatumomab, which resulted in strong T cell expansion and a significant decrease in ALL blasts.[76] Overall, the implication of these studies is that blinatumomab combined with co-inhibitory molecule blockade (PD-1/PD-L1, CTLA-4, or both) could be a rational strategy to overcome blinatumomab resistance in $CD19^+$ ALL.

2.3.3 **Improving Outcomes in ALL after Blinatumomab Treatment**

Though the initial study of blinatumomab in relapsed/refractory Pre-B ALL demonstrated a CR rate of 69%, a subsequent larger study in patients at high risk for poor outcomes led to a more modest response rate of 43%.[31,32] This response rate was comparable to that seen with cytotoxic chemotherapy in prior studies in relapsed Pre-B ALL.[4] Furthermore, this study also showed the limited durability of responses to blinatumomab with a median RFS of just 6.9 months among those who achieved MRD negative status following treatment. Thus while blinatumomab has shown significant activity with an acceptable safety profile in patients with relapsed or refractory Pre-B ALL, many patients still fail to achieve a response or relapse shortly after achieving a response, including MRD responders. Given the short duration of remission and high risk of relapse following blinatumomab, consolidation with an allogeneic HSCT has been shown to be a feasible strategy; however, only a subset of patients who achieve remission with blinatumomab will be candidates for allogeneic HSCT and post-transplant relapse remains a problem.[32,81] Thus, there is a need to develop additional strategies to increase and prolong responses after blinatumomab treatment. Monitoring of T-cell kinetics in relapsed and refractory patients treated on the initial phase II study identified that the expansion of $CD3^+$ T cells during the first 2 cycles correlates with the response, with long-term survivors having a higher degree of T-cell and T_{EM}-cell expansion. Interestingly, T-cell expansion was limited in MRD responders with shorter survival and was not seen in MRD non-responders. B cell kinetics revealed their rapid and persistent clearance in MRD responders while detectable return of peripheral B cells was noted before cycle 2 in non-responders.[81] Given the correlation between T-cell expansion, MRD clearance and subsequent long-term survival after blinatumomab treatment, augmenting the initial or early anti-leukemia T-cell response following blinatumomab is a rational strategy to improve response rate and extend its durability.

2.3.4 **Dose Selection**

The initial doses of blinatumomab, nivolumab, and ipilimumab to be used in this trial have been selected based on the results of prior trials demonstrating the safety of these drugs at the selected doses as well as the efficacy of blinatumomab at the selected dose in the treatment of ALL. All participants in this study will receive blinatumomab using the standard dosing regimen that has been FDA approved for the treatment of relapsed and/or refractory ALL based on the results of two Phase II trials.[31,32] As blinatumomab is the only agent in this trial that is known to have significant activity in ALL, it is critical that its dose remain unchanged. Nivolumab will initially be given in combination with blinatumomab at a flat dose of 240 mg every two weeks.

Population pharmacokinetics support the flat dose of 240 mg as being identical to 3 mg/kg for subjects weighing 80 kg, which was identified as a safe dose of nivolumab in the initial phase I trial.[35,51] Furthermore, data from the phase I trial of nivolumab suggest that its side effects are not dose-dependent, so lower doses may not mitigate toxicities even when combined with blinatumomab. However, if significant toxicities should arise at a flat dose of 240 mg every 2 weeks of nivolumab, then further patients will be treated at a de-escalated nivolumab dose of 80 mg every 2 weeks. In combination with nivolumab and blinatumomab, ipilimumab will first be given at a dose of 1 mg/kg every 6 weeks with escalation in subsequent patients to a dose of 3 mg/kg every 6 weeks, if the initial dose proves tolerable in combination with nivolumab and blinatumomab. This differs from a recent phase III study combining nivolumab and ipilimumab in metastatic melanoma that used an ipilimumab dose of 3 mg/kg every 3 weeks with 1 mg/kg every 3 weeks of nivolumab.[58] However, in the initial phase I study combining ipilimumab and nivolumab for the treatment of metastatic melanoma, nearly all treatment-related adverse events were reduced in patients who received 1 mg/kg of ipilimumab with 3 mg/kg of nivolumab every 3 weeks as compared to those who received 3 mg/kg of ipilimumab with 1 mg/kg of nivolumab every 3 weeks, although the objective response rate was comparable.[36] Furthermore, the reported half-life of ipilimumab is 15.4 days, and the combination with nivolumab does not seem to effect the clearance of ipilimumab.[52] Thus the decreased dose density of ipilimumab proposed in this study has strong biologic rationale and should help to mitigate the toxicities of ipilimumab.

2.4 Correlative Studies Background

The goal of the correlative studies in this trial will be to characterize the effect of treatment on T cells particularly on their re-distribution, in vivo expansion, activation, and effector differentiation in both the BM and PB compartments over time. This has recently been studied extensively by our group in AML, but there is currently a paucity of data in ALL. Thus the correlative studies are designed to gain insight about T-cell function in ALL patients prior to treatment, during blinatumomab monotherapy, following the addition of immune checkpoint inhibitors to blinatumomab, at the conclusion of treatment, and at the time of relapse. These studies will also characterize the effects of these treatments on co-signaling molecule expression and the expression of transcription factors in T-cell subpopulations. These investigations will not be limited to the immune inhibitory pathways for which there are currently commercially available inhibitors (i.e. PD-1/PD-L1 and CTLA-4/CD80/CD86), as inhibitors of many other pathways are currently in development, and we expect that interrogation of other pathways will identify potential future targets for immune checkpoint inhibition.

2.4.1 Dynamics of lymphocyte subpopulations in both peripheral blood and the bone marrow microenvironment pre- and post-blinatumomab and immune checkpoint inhibitor(s) treatment.

Current data from patients treated with blinatumomab suggests tri-phasic T-cell kinetics: T-cell counts decline within hours after the start of blinatumomab infusion, recover to baseline within a few days followed by T-cell expansion with, on average, more than doubling of T-cell counts at 2-3 weeks under continued infusion of blinatumomab, and lastly T-cell contraction in between cycles. Patients who achieved MRD clearance following blinatumomab and were long-term survivors had a higher degree of T-cell and T_{EM}-cell expansion after the first two treatment

cycles compared to patients having MRD response but short survival or non-responders.[34] This implies that T-cell expansion might be a key factor for improving response and survival of relapsed/refractory ALL patients after blinatumomab immunotherapy. In terms of T-cell subpopulations, T-cell expansion was associated with increases in $CD3^+$ T_{EM}, $CD8^+$ T_{EM}, $CD8^+_{TEMRA}$, $CD4^+$ T_{CM} cells.[82] There are limited data on the relevance of other T-cell subpopulations except a suggestion from a small study of ALL patients refractory to blinatumomab therapy that a high percentage of Tregs can predict a lack of response to therapy.[83] In terms of checkpoint inhibitors, the absolute lymphocyte count (ALC) has been found to be a specific pharmacodynamic biomarker of ipilimumab, and the degree of increase in the ALC has been associated with survival in patients with melanoma.[84-86] Ipilimumab administration is followed by enhanced T-cell proliferation and a rise in $CD8^+$ T_{CM} and $CD4^+$ T_{EM} cells.[87,88] Ipilimumab-induced changes in Tregs have been more controversial across different studies with both decreases and increases in circulating $FoxP3^+$Tregs observed in patients responding to treatment.[84,89] In contrast to ipilimumab, data on the predictive role of immune cell subsets and changes in ALC in cancer patients during PD-1 or PD-L1 blockade are lacking. In addition, no association between ALC and clinical response was observed when nivolumab and ipilimumab were administered in combination, suggesting that the combination of these two antibodies generated immune responses with unique features compared to their administration as monotherapy.[36]

We anticipate that the addition of nivolumab or nivolumab/ipilimumab to blinatumomab will be associated with increased T-cell effector differentiation and proliferation and improved post-treatment antitumor responses. Thus we propose monitoring of T-cell dynamics during treatment as an integrated biomarker to support the collection of mandatory PB and BM specimens.

The overarching goals of these studies are to: a) characterize the effect of blinatumomab in combination with checkpoint inhibition on T cells particularly on their re-distribution, *in vivo* expansion, activation, and effector differentiation in both PB and BM compartment over time and in relation to the response to therapy. We will also examine functional dynamics of Tregs and their fractions, B cells, and NK cells; b) identify potential predictive biomarker candidates (in combination with studies in Section 2.4.2) to guide improved selection of ALL patients who are more likely to benefit from addition of checkpoint inhibitors to blinatumomab therapy; c) characterize the mechanisms of relapse / refractoriness to blinatumomab combined with checkpoint inhibition such as T-cell dysfunction.

Our expectation is that the treatment strategy being investigated in this study will enable ALL patients who would not respond to a single-agent blinatumomab to respond to the combination of blinatumomab and immune checkpoint inhibition, and even enhance the depth and durability of response in those patients who may be responders to blinatumomab alone.

2.4.2 Changes in T-cell co-signaling receptors and canonic transcription factor expression in defined T cell subpopulations in both peripheral blood and bone marrow pre- and post-blinatumomab and immune checkpoint inhibitor(s) therapy.

Studies performed by us and others suggest the presence of multiple aberrations in T-cell co-

signaling receptors and canonic transcription factor expression in defined T-cell subpopulations in patients with AML but this has never been studied in depth in patients with ALL. Our group has shown that co-expression of a number of inhibitory receptors (iRs), such as CTLA-4, PD-1, TIM-3, 2B4 or BTLA, on T cells in patients with AML differs between PB and BM and changes through the course of the treatment.[74] Recent reports provided several lines of evidence that immune checkpoint pathways are also operational in ALL and may represent a potential mechanism of therapeutic resistance, including to blinatumomab. First, PD-1 but also other co-inhibitory molecule such as Tim-3 were more highly expressed (and co-expressed) on T cells from ALL patients compared to healthy controls.[76] Second, an increase in PD-1 expression on BM lymphocytes was noted at the time of therapeutic failure.[75] Finally, in functional studies, the blinatumomab effect on T cells (proliferation and interferon-y production) *in vitro* appeared to be target cell-dependent and correlated with the target cell-expression of co-signaling molecules. Combined inhibition of the PD-1 and CTLA-4 pathways with blinatumomab enhanced effector T-cell function of healthy donors and patient's T cells against ALL target cells more than PD-1 inhibition alone while inhibition of co-stimulatory CD28-CD80/86 interactions downregulated T-cell effector function and proliferation.[76]

Thus we anticipate that administration of blinatumomab in combination with checkpoint inhibitors should result in strong T-cell differentiation and expansion and potent anti-leukemia activity. Whether the expression of iRs differs in patients with ALL at presentation and relapse and what is the effect of therapy is unknown. As a part of this proposal we will conduct comprehensive analysis of signaling receptors and canonic transcription factor expression in defined T-cell subpopulations in both PB and BM before and post-blinatumomab and immune checkpoint inhibitor therapy. This represents a very relevant unmet need for future integration of checkpoint inhibitors or any other immunotherapy approaches seeking to overcome T-cell exhaustion, increase the response rates to therapies, and/or maintain remission in ALL. Thus, in addition to studies proposed in Section 2.4.1, we propose monitoring the dynamics of signaling receptors and canonic transcription factors expression in defined T-cell subpopulations during treatment as an integrated biomarker to support the collection of mandatory PB and BM specimens. As discussed in Section 2.4.1, the main goals of these studies are to characterize the effect of blinatumomab in combination with checkpoint inhibition on T-cell dynamics (re-distribution, in vivo expansion, activation, and effector differentiation) but also on expression of co-signaling molecules in both PB and BM compartment over time, to identify potential predictive biomarker candidates to guide better selection of ALL patients who are more likely to benefit from the addition of checkpoint inhibitors to blinatumomab therapy, and to characterize the mechanisms of relapse / refractoriness after blinatumomab combined with checkpoint inhibition such as T-cell dysfunction.

2.4.3 Expression of co-signaling molecules on leukemia blasts ($CD10^+/CD19^+/CD34^+$) before and after treatment with blinatumomab and checkpoint inhibitors.

Upregulation of programmed death-ligand 1 (PD-L1) on tumor cells in response to endogenous anti-tumor immunity inhibits adaptive immune responses by inducing T-cell dysfunction. Expression of PD-L1 on tumor cells has been associated with poor outcome in solid cancers as well as hematologic malignancies. Overall, cytokine-induced PD-L1 expression on tumors represents evasion of tumor immunity termed "adaptive resistance." Several lines of evidence

suggest that the PD-1/PD-L1 axis is operational in ALL and may represent a potential mechanism of blinatumomab resistance. Feuchtinger et al. analyzed 20 primary ALL CD10+/CD19+ BM cells, and found that PD-L1, but also other co-inhibitory molecule such as HVEM, and co-stimulatory molecule such as CD86, were upregulated compared to healthy CD10+CD19+ cells. PD-L1 upregulation on ALL blasts was noted upon exposure to Th1 cytokines such as interferon-γ or TNF-α. They observed that surface expression of the PD-L1 on the CD10+CD19+ cells was significantly higher in patients at the time of relapse than at the time of primary diagnosis and at both time points the expression was higher than in healthy controls. More importantly, PD-L1 was consistently more highly expressed on CD19+CD10+ BM cells in blinatumomab non-responders compared to responders.[76] Kohnke et al. analyzed serial BM samples from a patient who failed to respond to blinatumomab and demonstrated significant upregulation of PD-L1 on tumor cells after blinatumomab administration (2% at baseline vs. 40% after blinatumomab) without evidence of loss of CD19 expression, which is an alternative mechanism of blinatumomab resistance.[75]

We anticipate that administration of nivolumab or nivolumab/ipilimumab following blinatumomab will inhibit PD-L1/PD-1 and CTLA-4/CD80/86 axis, respectively, and result in increased responses. Thus we propose flow cytometric studies on leukemia blasts (CD19/CD10/CD34 plus other leukemia specific marker) to examine for the expression of co-signaling molecules (PD-L1, B7-H3, VISTA, Gal-9, CD86, PVR, PVR2, OX40L, ICOSL) at baseline, within ten days of blinatumomab treatment (before immune checkpoint inhibitor administration), in non-responders after cycle 1 and/or 2 and at the time of relapse. These serial studies are proposed to identify alternative resistance mechanisms that could be employed by ALL cells to evade blinatumomab/checkpoint inhibition and thus will provide critical information on the selection of best combinations in the future.

2.4.4 **Cytokine studies**

Blinatumomab treatment has been associated with transient release of cytokines such as IL-2, IL-6, IL-10, IFN-γ and TNF-α. However, there was not a clear pattern of cytokine release and response.[82] Administration of novel immunotherapeutics, including immune checkpoint inhibitors, alone or in combination with chemotherapy have resulted in systemic immune-related AEs.[90] Whether administration of nivolumab and nivolumab/ipilimumab following blinatumomab will increase cytokine expression and if increases in cytokines will be associated with toxicity is unknown. Thus, we will be collecting and storing serial plasma samples (at the time of PB collection) and if there is a strong rationale we will conduct measurement of cytokines to assess blinatumomab and checkpoint inhibitors-induced global T-cell activation. We will examine the levels of cytokines before and after treatment with blinatumomab and checkpoint inhibitors, including the levels of sCTLA-4. In particular, sCTLA-4 is found at a significantly higher frequency in ALL patients than healthy controls, and higher levels of sCTLA-4 correlate with relapse risk and the probability of survival following treatment.[77,78] Experimental evidence suggests that current anti-CTLA-4 antibodies bind both sCTLA-4 and membrane-bound CTLA-4 and as such that their administration may improve the immune recognition of ALL cells.[79] We anticipate that the levels of sCTLA-4 may decrease upon ipilimumab treatment.

2.4.5 Immune profiling of T cell repertoire and characterize T cell transcriptional signature before and after treatment

2.4.5.1 Immune Profiling of T-cell repertoire

High throughput deep sequencing of the TCRV-β CDR3 region is very valuable technology to better characterize the T-cell repertoire changes after checkpoint inhibitor administration. The best metric of TCR repertoire diversity are changes in clonality index and repertoire diversification. This strategy is being increasingly used to assess T-cell repertoire changes in both periphery and intra-tumor environment providing critical mechanistic clues. Previous studies have shown that ipilimumab leads to TCR diversification and the induction of new tumor-antigen specific T-cell responses as early as two weeks after first administration.[91-93] However, it remains unclear how simultaneous use of multiple immunotherapeutics will affect the TCR repertoire and if standardized metrics will correlate with toxicity or clinical response. We anticipate that treatment with blinatumomab will lead to diversification of the TCR repertoire that would be further modeled by checkpoint inhibitors. The studies proposed in this protocol will evaluate TCR diversity in T cells isolated from PB and BM from patients before and after treatment with blinatumomab and checkpoint inhibitors on the parent clinical trial. We will determine TCR diversity and clonal composition using a molecular and computational approach based on high-throughput DNA sequencing of rearranged TCRβ CDR3 regions from T-cell genomic DNA. The findings derived from these analyses are meant to generate hypotheses and will be expanded upon with additional studies.

2.4.5.2 Characterization of T-cell transcriptional signature before and after treatment

We have substantial experience with this approach from our ongoing analysis of T-cell dysfunction in patients with AML (data not shown). We now have evidence at the transcriptional level that CD8+ T cells from AML patients have significant up-regulation of genes involved in T-cell co-inhibition and transcription factors associated with T-cell exhaustion when compared to healthy controls. Serial gene expression analysis of CD8+ T cells (at diagnosis and after induction chemotherapy) using the Human Prime View Gene Expression Array (http://www.affymetrix.com) revealed that CD8+ T-cell gene signatures differ in patients who achieve complete response versus non responding (NR) patients at the time of response assessment. Most strikingly, the set of genes up-regulated in responding patients clustered together with healthy controls, whereas NR patients clustered separately.[74] Gene expression will be analyzed with focus on “pretreatment” and “response to treatment” analyses. We predict based on our previous work that several key co-stimulatory signaling pathways will be downregulated in CD8+ T cells retrieved from patients with ALL in comparison to healthy controls and that these pathways will differ after blinatumomab, nivolumab ± ipilimumab administration. We will also examine whether there is differential-expression of genes between CD8+ T cells in BM relative to those in circulation. These studies are likely to provide important insights into the T-cell transcriptome in patients with ALL in particular as it relates to the immunomodulatory strategies to modulate their function. The goal of this analysis is to provide the biological understanding necessary for future clinical studies of blinatumomab and checkpoint inhibitors in ALL including improved selection of patients who may benefit from this therapeutic strategy.

2.5 Summary

Outcomes in relapsed or refractory ALL as well as outcomes for older patients who receive traditional induction chemotherapy remain poor with a significant risk of relapse and death.[3-6,8,10] The novel BiTE antibody blinatumomab has recently demonstrated efficacy in both Ph-positive and Ph-negative Pre-B ALL that has relapsed or is refractory to treatment, but only half of the patients respond and the duration of remission from this treatment is typically short.[33,94] Pre-clinical studies suggest that inhibition of PD-1/PD-L1 immune regulatory axis could augment the blinatumomab-initiated T-cell response, thereby overcoming a potential mechanism of blinatumomab resistance.[75,76] There is also evidence that the CTLA-4/CD80/CD86 immune regulatory pathway is upregulated in ALL, suggesting that inhibition of this pathway in combination with blinatumomab could yield a more robust immune-mediated attack on $CD19^+$ ALL cells.[77,78] Combinations of the anti-PD1 antibody nivolumab and anti-CTLA4 antibody ipilimumab have demonstrated remarkable efficacy in melanoma with sustained responses and an acceptable side effect profile.[58] Thus there is strong rationale for combining blinatumomab with both single and dual immune checkpoint blockade in relapsed or refractory Pre-B ALL in the interest of increasing the frequency and duration of remissions by improving the T cell-mediated immune response to the underlying malignancy.

3. PATIENT SELECTION

3.1 Pre-Registration Eligibility Criteria

3.1.1 Patients must have suspected refractory or relapsed pre-B cell ALL or mixed phenotype acute leukemia (MPAL), or if newly diagnosed, the patient must be 60 years of age or older.

3.1.2 Bone marrow and/or peripheral blood specimens will be submitted for correlative studies as outlined in Section 9. Patients who have a dry tap will still be eligible.

3.2 Registration Eligibility Criteria

3.2.1 Patients must have histologically or cytologically confirmed by the local institution $CD19^+$ Precursor B-acute lymphoblastic leukemia (Pre-B cell ALL) OR $CD19^+$ mixed phenotype acute leukemia (MPAL): a) with relapse following or refractory to at least one prior line of therapy if older than 21 years; b) in second or higher relapse or refractory to at least two prior lines of therapy if 21 years old and younger (16-21); c) or they must have a new diagnosis of Pre-B cell ALL or $CD19^+$ MPAL but are ≥60 years old and are either not a candidate for or do not wish to receive traditional induction chemotherapy.

3.2.2 The evidence of $CD19^+$ expression on leukemia cells must be confirmed by pathology review of the bone marrow and/or peripheral blood specimens (flow cytometry and/or immunohistochemistry) collected at the time of current relapse and prior to the initiation of therapy.

3.2.3 Patients with Ph+ Pre-B cell ALL OR Ph+ MPAL will be eligible if they have been refractory to or intolerant of treatment with at least 1 second-generation or third-generation tyrosine kinase inhibitor (TKI).

3.2.4 Patients who were treated with blinatumomab in the past will be allowed on the study as long as they have persistent CD19 expression on leukemia cells and did not experience unacceptable toxicities with prior blinatumomab administration. Patients who were treated with chimeric antigen receptor (CAR)-modified T cells targeting CD19 in the past will be allowed on the study as long as they have persistent CD19 expression on leukemia cells.

3.2.5 Patients with a history of allogeneic HSCT will be eligible if they are more than 90 days removed from the date of stem cell infusion, have no evidence of acute graft-versus-host disease (GVHD) or active chronic (grade 2-4) GVHD, and are off of all transplant-related immunosuppression for at least 2 weeks.

3.2.6 Age ≥16 years. Because no dosing or adverse event data are currently available on the use of blinatumomab in combination with nivolumab ± ipilimumab in pediatric patients, children are excluded from this study, but will be eligible for future pediatric trials.

3.2.7 ECOG/Karnofsky performance status of 0-2 (Karnofsky ≥60%, see Appendix A).

3.2.8 Life expectancy of greater than 12 weeks.

3.2.9 Patients must have normal organ function as defined below:

- total bilirubin ≤2.0 mg/dL (except patients with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)
- AST(SGOT)/ALT(SGPT) ≤5× ULN
- Serum creatinine ≤1.5× ULN

OR

- creatinine clearance (CrCl) ≥50 mL/min (if using the Cockcroft-Gault formula below):

Female CrCl = (140 - age in years) x weight in kg x 0.85 72 x serum creatinine in mg/dL

Male CrCl = (140 - age in years) x weight in kg x 1.00 72 x serum creatinine in mg/dL

3.2.10 The effects of nivolumab, ipilimumab, and blinatumomab 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. 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 treatment on the study. 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 ± ipilimumab and blinatumomab 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.

Note: 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.

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.

3.2.11 Adequate pulmonary function as assessed by oxygen saturation ≥90% when ambulating and not requiring supplemental oxygen.

3.2.12 Patients with a known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS) will be eligible if:

1. They are generally healthy from an HIV perspective and on a stable antiretroviral regimen for >6 months.
2. They have had no AIDS-defining conditions in the past 12 months other than historically low CD4+ cell counts.
3. They have an undetectable viral load on standard assays.

3.2.13 Patients with a known history of hepatitis C (HCV) will be eligible if they have an undetectable viral load. If the patient received treatment for HCV, then that treatment must have been completed at least three weeks prior to enrollment.

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

3.3 Exclusion Criteria

3.3.1 Patients who have had chemotherapy or other systemic therapy or radiotherapy, or those who have not recovered from adverse events due to prior administered agents as follows: chemotherapy, radiotherapy or surgery ≤ 3 weeks prior to entering the study, targeted therapy (e.g., TKI) ≤ 1 week prior to entering the study; autologous HSCT ≤ 6 weeks prior to entering the study; investigational drug or immunotherapy (e.g. rituximab) ≤ 4 weeks prior to entering the study. Prophylactic intrathecal chemotherapy within one week of enrollment allowed. Patients will be allowed to receive cytoreduction with hydroxyurea, 6-mercaptopurine, corticosteroids (dexamethasone, prednisone or similar) or cyclophosphamide provided that it is discontinued at least 24 hours prior to the initiation of study treatment. Pre-phase treatment with dexamethasone 10 mg/m^2 (maximum total 24 mg per day) for up to 5 days is required for patients with bone marrow blasts more than 50%, peripheral blood blasts of 15,000/μL or higher, or elevated lactate dehydrogenase suggesting rapidly progressing disease as per investigator's assessment. Pre-phase treatment must be stopped at least 24 hours prior to the initiation of blinatumomab.

3.3.2 Patients who are receiving any other investigational agents.

3.3.3 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.

3.3.4 Patients with active central nervous system leukemia are excluded from this clinical trial because they may develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with a history of CNS leukemia but no active disease at the time of enrollment are eligible. The absence of CNS disease must be confirmed by flow cytometric and cytologic examination of the cerebrospinal fluid (CSF) within 7 days of study enrollment.

3.3.5 Active leukemia in the testes or isolated extramedullary relapse. Patients with a history of

treated leukemia in testes but no active disease at the time of enrollment are eligible.

3.3.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab, ipilimumab, or blinatumomab.

3.3.7 History of severe hypersensitivity reaction to any monoclonal antibody.

3.3.8 Uncontrolled intercurrent illness including, but not limited to, active, uncontrolled infection; symptomatic congestive heart failure; unstable angina pectoris; cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements. Patients with infection under treatment and controlled with antibiotics are eligible.

3.3.9 Pregnant women are excluded from this study because nivolumab and ipilimumab have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab and ipilimumab, breastfeeding should be discontinued if the mother is treated with nivolumab and ipilimumab. These potential risks may also apply to blinatumomab.

3.3.10 History of any chronic hepatitis including alcoholic, non-alcoholic steatohepatitis (NASH), drug related, autoimmune, chronic viral positive tests for hepatitis B virus surface antigen (HBsAg), hepatitis B core antibody (anti-HBc) in the absence of hepatitis B surface antibody (anti-HBs), or a positive hepatitis C (HCV) viral load. These patients are excluded due to the risk for autoimmune hepatitis with immune checkpoint inhibitors exacerbating their known liver disease as well as the unknown risk for hepatitis B and/or C reactivation with blinatumomab and immune checkpoint inhibitors.

3.3.11 Subjects with active autoimmune disease, a history of known or suspected autoimmune disease or a history of a syndrome requiring systemic corticosteroids (>10 mg daily of prednisone equivalent) except for the treatment of malignancy with the exception of:

a. Isolated vitiligo
b. Resolved childhood atopy
c. History of a positive ANA titer without associated symptoms or history of symptoms of an autoimmune disorder
d. Controlled thyroid disorders
e. Type I diabetes mellitus
f. Psoriasis, Sjögren's syndrome, and arthropathies not requiring systemic treatment

Autoimmune diseases: 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, autoimmune vasculitis, 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.

3.3.12 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), or as prephase treatment for cytoreduction as specified in 3.2.1 is permitted. Patients will receive steroids with blinatumomab to reduce cytokine release syndrome (CRS) as specified in the protocol.

3.3.13 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.

3.3.14 Patients who have a history of clinically relevant CNS pathology such as epilepsy, seizure, paresis, aphasia, stroke, severe brain injuries, dementia, Parkinson's disease, cerebellar disease, psychosis, or other significant CNS abnormalities. A history of treated CNS leukemia will be allowed if recent CNS studies confirm the absence of active CNS disease at the time of study entry (screening).

3.3.15 Patients with a known concurrent malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or carcinoma in situ of the cervix.

3.3.16 Subjects with interstitial lung disease that is symptomatic or may interfere with the detection or management of suspected drug-related pulmonary toxicity.

3.4 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.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

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. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	**IVR**	**NPIVR**	**AP**	**A**
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval.

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm

For questions, please contact the RCR ***Help Desk*** by email at <RCRHelpDesk@nih.gov.>

4.1.1 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 required to access all CTEP applications and, if applicable (*e.g.*, all Network trials), all Cancer Trials Support Unit (CTSU) applications and websites.

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

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

4.1.2 Investigator Brochure Access for CTEP IND agents

The current version of the Investigator Brochure (IB) will be accessible to site investigators and research staff 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 and a "current" password. Questions about IB access may be directed via email to IBcoordinator@mail.nih.gov or by phone (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET).

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 IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether

a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the #10030 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 *LAO-MD017*, and protocol #10030.
- 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 load to RSS as described above.)

4.2.2 Requirements For 10030 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- A Study Initiation Visit (SIV) is required for each participating site prior to activation. The local site PI must participate on the call as well as their Research

Nurse, Study Coordinator, and Pharmacist. To schedule a SIV, please email the LAO Protocol Contact and reference the protocol in the subject line of the email.

4.2.3 Submitting Regulatory Documents

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

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission

When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

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

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 Pre-Registration Procedures

In order to submit the baseline bone marrow aspirate and biopsy and peripheral blood samples, the patient must be pre-registered and assigned a subject ID. The following should be confirmed by the site prior to pre-registering the patient:

- **Informed consent:** the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Current human protection committee approval

of this protocol and a consent form is required prior to patient consent and registration.

- **Dose Level and Accrual:** prior to discussing protocol entry with prospective patients, site staff must contact the Johns Hopkins Regulatory contact to check the status of the dose level accrual.
- **Correlative Studies:** bone marrow and/or peripheral blood will be submitted for correlative studies for all patients pre-registered to this study. Note: If the procedure is a dry tap, the patient is still eligible. The peripheral blood must be submitted.

4.3.2 Registration Procedures through 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.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.3 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. Registrars must hold a minimum of an AP registration type.
- 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.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.4 General Guidelines

Following registration, patients should begin protocol treatment as rapidly as possible. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be initiated on an inpatient 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. The cycle length is 42 days for all dose levels.

Treatment will consist of the combination of blinatumomab and nivolumab, with or without ipilimumab per the dose escalation schedule below. A run-in of blinatumomab (10 days with planned dose escalation on day 8) prior to combination therapy will be incorporated at all dose levels to help determine the effect of blinatumomab on immune regulatory pathways in leukemia and as a means of minimizing the potential for overlapping toxicities, as many of blinatumomab's most significant side effects occur within the first few days of administration or after a dose increase. Nivolumab with or without ipilimumab will start on day 11 of cycle 1. When nivolumab and ipilumumab are scheduled on the same day, nivolumab should be given first followed by ipilimumab as described in Section 5.3.4

Pre-treatment phase (pre-phase): Pre-phase treatment with dexamethasone 10 mg/m^2 (maximum total 24 mg per day intravenously) for up to 5 days for patients with bone marrow blasts more than 50%, peripheral blood blasts of 15,000/μL or higher, or elevated lactate dehydrogenase suggesting rapidly progressing disease as per investigator's assessment is required unless other cytoreductive measures as outlined below have already been successfully implemented. Pre-phase treatment should be completed at least 24 hours before starting blinatumomab (study treatment). Other pre-phase treatment for cyto-reduction including cyclophosphamide, 6-MP, hydroxyurea, or corticosteroids (dexamethasone, prednisone or similar) (in patients not meeting the aforementioned criteria) may be used at the investigator's discretion but must be completed at least 24 hours prior to blinatumomab infusion.

5.2 Overall Study Design

This is a multi-site, open-label, phase 1 study of blinatumomab and nivolumab with or without ipilimumab in patients with CD19$^+$ Pre-B ALL or CD19$^+$ MPAL. The DLT evaluation window for all dose levels is the first cycle of treatment comprising 42 days from the initiation of blinatumomab. The considerations for opening a new dose level may only occur after the required number of subjects has passed the DLT observation period.

5.2.1 Dose Escalation

A standard 3+3 dose escalation design for blinatumomab and nivolumab will be used to ascertain the safety and tolerability of this combination. The first 3 patients will be enrolled at dose level DL A1 and monitored for the first cycle of treatment (42 days) for DLTs before enrolling additional patients. If ≤1 patient experience a DLT, an additional 3 patients will be enrolled at DL A1 to better define the safety and tolerability before proceeding to the combination of

blinatumomab with nivolumab and ipilimumab. If ≤1 of 6 patients experience a DLT, dose escalation will proceed to DL B1 with the next 3 patients. If ≥2 of 3 or ≥2 of 6 patients experience a DLT at DL A1, then dose de-escalation may be implemented BUT ONLY after thorough evaluation of toxicities, discussion with CTEP, investigators and CTEP agreement with the next three patients enrolled at DL A-1. If ≤1 of 3 patients experience a DLT, an additional 3 patients will be enrolled at DL A-1 to better define the safety and tolerability, before proceeding to the combination of blinatumomab with nivolumab and ipilimumab. If ≤1 of 6 patients experience a DLT, dose escalation will proceed to DL B-1 with the next 3 patients. If ≥2 of 3 or ≥2 of 6 patients experience a DLT at DL A-1, then dose escalation will be stopped and the combination of blinatumomab and nivolumab will not be further pursued in this patient population unless specific modifications to the treatment plan could be made to ameliorate toxicities. This would require review of all the toxicities, discussion with CTEP, investigators, and their agreement, and official amendment to the study.

When the safety of blinatumomab in combination with nivolumab has been established as above, the trial will proceed to either DL B1 or DL B-1, and a standard 3+3 dose escalation design for blinatumomab and nivolumab with ipilimumab will be used to ascertain the safety of this combination. Two dose levels (DL B-1, DL B1) may be used to evaluate this combination. Depending on the results from the combination of blinatumomab and nivolumab as outlined above, patients will first be enrolled at either DL B1 or DL B-1. If ≤1 of 3 patients experiences a DLT at DL B1, an additional 3 patients will be enrolled at DL B1. If ≤1 of 6 patients experience a DLT, then this will be defined as the MTD. If ≥2 or more of 3 or 6 patients experience a DLT at DL B1, then dose de-escalation may be implemented BUT ONLY after thorough evaluation of toxicities, discussion with CTEP, investigators and CTEP agreement and the next three patients may be enrolled at DL B-1. If the triplet combination starts at DL B-1 based on the results from the combination of blinatumomab and nivolumab alone, then three patients will be enrolled at DL B-1. If ≤1 of 3 patients experiences a DLT at DL B-1, an additional 3 patients will be enrolled at DL B-1. If no more than 1 of 6 patients experience a DLT at DL B-1, then this dose level will be defined as MTD. If 2 or more of 3 or 6 patients experience a DLT at DL B-1, then dose escalation will be halted and the combination of blinatumomab, nivolumab and ipilimumab will not be further pursued in this patient population unless additional data become available supporting different doses or schedules of nivolumab and ipilimumab administration. This will require review of all toxicity, discussion with CTEP and official study amendment. The MTD of blinatumomab in combination with nivolumab and ipilimumab is defined as the highest dose at which no more than 1 out of 6 patients experience a DLT. When the MTD of blinatumomab in combination with nivolumab and ipilimumab is determined, an additional 6 patients will be enrolled in an expansion cohort to further characterize safety, tolerability and any preliminary signs of biological or clinical activity. An expansion cohort of an additional 6 patients at the MTD of blinatumomab and nivolumab without ipilimumab will be also pursued to further characterize safety, tolerability and any preliminary signs of biological or clinical activity for this combination. The expansion cohort at the MTD of blinatumomab and nivolumab will be open for enrollment while dose-escalation continues for blinatumomab, nivolumab and ipilimumab. Enrollment priority: Patients will be enrolled in dose-escalation cohort for blinatumomab, nivolumab and ipilimumab first. However, if no slots are available, patients will be allowed to enroll in dose expansion cohort for blinatumomab and nivolumab. Once the MTD for blinatumomab, nivolumab and ipilimumab is defined, the enrollment in dose expansion cohorts

for blinatumomab/nivolumab and blinatumomab/nivolumab plus ipilimumab will be 1:1. This amendment was discussed with CTEP on 03/12/2019. Based on safety and efficacy data, the dose level A1 was defined as MTD for blinatumomab and nivolumab combination (1 of 6 patients with DLT; 5 of 6 patients with complete remission).

Dose Escalation Schedule[#]			
Dose Level	**Dose**		
	Blinatumomab[&]	**Nivolumab**	**Ipilimumab**
All Dose Levels	Nivolumab and ipilimumab (where indicated) will be initiated on Day 11 of Cycle 1		
Level A-1[*]	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	80 mg IV every 2 weeks	None
Level A1[*]	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	240 mg IV every 2 weeks	None
Level B-1	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	80 mg IV every 2 weeks	1 mg/kg IV every 6 weeks
Level B1	9 µg/day IV continuous infusion on Days 1-7 28 µg/day IV continuous infusion on Days 8-28	240 mg IV every 2 weeks	1 mg/kg IV every 6 weeks
Safety run-in of blinatumomab and nivolumab: Up to 6 subjects will be treated at dose level A1 and dose level A-1 (if necessary) prior to addition/dose escalation with ipilimumab. [#] Dose expansion of blinatumomab, nivolumab, and ipilimumab: An expansion cohort of 6 subjects will be treated at the MTD. # Dose expansion of blinatumomab and nivolumab: An expansion cohort of 6 subjects will be also treated at the MTD for blinatumomab and nivolumab only. [&] In Cycle 2 and subsequent cycles blinatumomab will be administered at 28 µg/day IV continuous infusion Days 1-28. Blinatumomab cycles are administered every 42 days.			

Regimen Description					
Agent	**Premedications ; Precautions**	**Dose****	**Route**	**Schedule****	**Cycle Length**
Blinatumomab	20 mg IV dexamethasone 1 hour prior to initiation in each cycle and at step dose escalation in cycle 1	9 µg/day on Days 1-7 28 µg/day on Days 8-28	Continuous IV infusion	Days 1-28 of each cycle up to 5 cycles	42 days (6 weeks)
Nivolumab	None	80 or 240 mg IV	IV, 30-minute infusion	Day 11, and then every 2 weeks for 1 year[&]	
Ipilimumab	None	1 mg/kg	IV, 90-minute infusion	Day 11, and then every 6 weeks for 1 year[&]	
** Doses and schedule as appropriate for assigned dose level. In Cycle 2 and subsequent cycles, blinatumomab will be administered at 28 µg/day IV continuous infusion on Days 1-28. [&] Patients will be eligible to continue receiving nivolumab and ipilimumab for up to 1 year					

5.2.2 **Dose Expansion**

An additional 6 patients will be treated at the MTD for both combinations: blinatumomab and nivolumab alone; and blinatumomab/nivolumab plus ipilimumab to gain additional safety information and to explore preliminary biological and clinical activity of these combination therapies. When the MTD of blinatumomab in combination with nivolumab and ipilimumab is determined (either DLB1: Nivolumab 240 mg plus Ipilimumab 1 mg/kg or DLB-1: Nivolumab 80 mg and Ipilimumab 1 mg/kg), an additional 6 patients will be enrolled in an expansion cohort to further characterize safety, tolerability and any preliminary signs of biological or clinical activity of nivolumab and ipilimumab in combination with blinatumomab. However, if the combination of blinatumomab, nivolumab and ipilimumab is found to be intolerable, then the expansion cohort of an additional 6 patients will be only pursued at the MTD of blinatumomab and nivolumab alone (either DLA1: Nivolumab 240 mg or DLA-1: Nivolumab 80 mg) to further characterize safety, tolerability and any preliminary signs of biological or clinical activity for blinatumomab and nivolumab combination.

Based on the review of safety and efficacy data, in discussion with CTEP on 03/13/2019, a separate expansion cohort of an additional 6 patients at the MTD of blinatumomab and nivolumab alone was added to the study in addition to the expansion cohort of nivolumab, ipilimumab and blinatumomab (triple combination) to further characterize safety, tolerability and

any preliminary signs of biological or clinical activity for this combination.

Continuous evaluation of adverse events will be performed throughout enrollment in the expansion cohort. If, at any time, the aggregate rate of treatment-related toxicities meeting DLT criteria exceeds 30% across all subjects treated in the expansion cohort, the safety data will be discussed and further enrollment may be suspended.

5.3 Study Drugs

5.3.1 **Blinatumomab**

Blinatumomab will be administered as a continuous IV infusion over four weeks followed by a two-week treatment-free interval. It is recommended that patients are hospitalized at least during the first 14 days of the first cycle and the first two days of the subsequent cycles. The hospitalization time depends on the investigator´s judgment, as well as safety and tolerability of blinatumomab.

Cycle 1: The planned initial dose of blinatumomab is 9 μg/day administered via continuous intravenous infusion on days 1-7 of the first 42-day cycle. If patients tolerate the initial infusion without significant adverse events, then the dose of blinatumomab will be escalated to 28 μg/day administered via continuous intravenous infusion on days 8-28 of cycle 1. There will be a minimum blinatumomab treatment-free interval of 14 days following the completion of blinatumomab infusion prior to the initiation of the next cycle. Patients will be eligible for up to five cycles of blinatumomab if they achieve complete remission (CR) or CR with incomplete hematologic recovery (CRh) following 1-2 cycles of treatment. Given that this is a group of patient with a very high risk ALL, patients will be eligible to proceed to allogenic HSCT at the treating physician's discretion at any time after a response is documented. For patients receiving **cycles 2-5**, the planned dose of blinatumomab is 28 μg/day to be administered via continuous infusion on days 1-28 of each 42-day cycle.

Blinatumomab should be infused at a constant flow rate using an infusion pump. The pump should be programmable, lockable, non-elastomeric, and have an alarm. Blinatumomab infusion bags should be infused over 24 or 48 hours according to the instructions on the pharmacy label on the bags (Section 8.1.1). The solution should be administered using IV tubing that contains a sterile, non-pyrogenic, low protein-binding, 0.2 micron in-line filter. The IV line should never be flushed, especially when changing bags or at the completion of infusion, due to the risk of excess dosage. Blinatumomab should be infused through a dedicated lumen. Nivolumab +/- ipilimumab will be infused through a separate line from blinatumomab to allow continuous blinatumomab infusion when nivolumab +/- ipilimumab are administered.

Blinatumomab is known to cause cytokine release syndrome (CRS). To reduce the incidence of CRS associated with blinatumomab administration, all patients will be pre-medicated with dexamethasone 20 mg to be given intravenously one hour prior to the initiation of blinatumomab on day 1 of each cycle, prior to a step dose escalation (such as cycle 1 day 8), or when restarting an infusion after an interruption of 4 hours or more.

5.3.2 **Nivolumab**

Nivolumab will be given every two weeks (±2 days) at a dose of 80 or 240 mg for up to 1 year. Nivolumab administration starts on day 11 of cycle 1; however, the first dose of nivolumab may be delayed up to 4 days (until day 15) in cycle 1 if necessary to allow resolution of any adverse events associated with blinatumomab administration. If the delay is more than 4 days then the nivolumab should be administered at the next scheduled time (day 25±2 days of cycle 1). Patients may be dosed no less than 12 days from the previous dose of drug. Patients may continue on nivolumab for up to one year from the start of treatment on the study if they achieve CR or CRh following 1-2 cycles of treatment.

Nivolumab is to be administered as IV infusion over approximately 30 minutes, using a volumetric pump with a 0.2/1.2-micron low protein binding (polyethersulfone membrane) in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline or D5W for delivery but the total drug concentration of the solution cannot be below 0.35 mg/mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline. Nivolumab will be administered through a separate line from blinatumomab to allow continuous blinatumomab infusion during nivolumab administration. A flat dose of 240 mg can be infused undiluted or diluted so as not to exceed a total infusion volume of 120 mL.

For nivolumab and ipilimumab combination studies see section 5.3.4.

5.3.3 **Ipilimumab**

Ipilimumab will be given at a dose of 1 mg/kg every 6 weeks. Patients may be dosed no less than 38 days from the previous dose of drug. Ipilimumab administration starts on day 11 of cycle 1; however, the first dose of ipilimumab may be delayed up to 4 days (until day 15) in cycle 1 if necessary to allow resolution of any adverse events associated with blinatumomab administration. If the delay is more than 4 days and as the goal is to administer ipilimumab and nivolumab on the same day, then the ipilimumab should be administered at the next scheduled time of nivolumab administration (day 25 ± 2 days). Patients may continue on ipilimumab for up to one year from the start of treatment on the study if they achieve CR or CRh following 1-2 cycles of treatment.

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

Study drug is to be administered as an IV infusion using a volumetric pump through an in-line, sterile, non-pyrogenic, low-protein-binding filter with a pore size of 0.2 micrometer to 1.2 micrometer.

The infusion should be administered over approximately 90 minutes, followed by a normal saline

(NS) flush with an adequate amount of NS to completely flush the residual fluid (dead space) in the administration set (approximately 30 - 50 mL). Ipilimumab will be administered through a separate line from blinatumomab to allow continuous blinatumomab infusion during ipilimumab administration.

5.3.4 **Nivolumab with Ipilimumab**

Nivolumab and ipilimumab should be given on the same day, whenever possible. If one medication needs to be held/delayed, the other medication should ideally be held as well to allow concurrent dosing.

When infusions of ipilimumab and nivolumab are given on the same day, the preferred treatment is to give nivolumab followed by ipilimumab.

Toxicity management for the combined agents follows the same template guidelines and algorithms that are provided in Section 6.3 and Appendix D for single agent nivolumab.

Follow the same infusion timing guidelines: nivolumab over 30 minutes and ipilimumab over 90 minutes. The length of each cycle and the number of cycles or doses should be explained clearly.

When both nivolumab and ipilimumab are to be administered on the same day, separate infusion bags and filters must be used for each infusion. Nivolumab is to be administered first. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion.

5.4 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) will be assessed for the first cycle of treatment (6 weeks/42 days) at all dose levels.

A DLT is considered a treatment-related toxicity. Subjects who are withdrawn from the study before the completion of the DLT evaluation period in any phase for a reason other than a DLT will be replaced (NOTE: This includes subjects who may be removed during the blinatumomab-only run-in phase, prior to addition of nivolumab/ipilimumab). A toxicity with a clear etiology NOT related to a study treatment agent may not be considered a DLT; the Protocol Chair should be consulted in all cases.

DLTs include any grade 3 or higher non-hematologic toxicities which requires a subject to permanently discontinue protocol treatment during the DLT period as defined in Section 6.2.2 and 6.3.4 with the following exceptions:

A) Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality that can be managed with electrolyte replacement, hormone replacement, insulin or that does not require treatment discontinuation
B) Any grade 3 or higher toxicities that are frequent in ALL population such as fever, infection, bleeding, fatigue, bone pain, disseminated intravascular coagulation, tumor

lysis syndrome. Grade 4 infections which are felt by the PI to be primarily related to nivolumab ± ipilimumab administration or may have been exacerbated by nivolumab ± ipilimumab administration will be considered DLT. The PI and study team will closely monitor the cumulative rate of infections through the trial and in the expansion cohort to see whether the rate is higher than expected with blinatumomab alone.

C) Any grade 3 or 4 event that occurs during the blinatumomab run-in period prior to the initiation of nivolumab or ipilimumab and requires permanent treatment discontinuation will not be considered a DLT. During the phase II trial based on which blinatumomab received FDA approval, 10% of patients permanently discontinued blinatumomab due to adverse events that were thought to be treatment-related and 18% of patients discontinued treatment due to adverse events.[32] Thus adverse events requiring treatment discontinuation due to blinatumomab monotherapy are common and expected, so they should not be defined as DLTs in a trial to assess combination therapy.

For hematologic toxicity, the only adverse event that will be considered dose limiting is:

Grade ≥4 neutropenia or thrombocytopenia with a hypocellular bone marrow and no evidence of residual leukemia lasting for >42 days. Given that severe anemia, neutropenia and thrombocytopenia are features of advanced ALL and commonly encountered in this patient population, they will not be used to define DLT except if associated with prolonged treatment-associated aplasia as described above.

Management and dose modifications associated with the above adverse events are outlined in Section 6. Dose delay would be permitted as indicated in Section 6.

Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting toxicity (DLT) is defined above.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level.**
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if 0 or 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level

	if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.
**For dose levels A1 and A-1 (if necessary), six total patients will be enrolled even if there are no toxicities among the first three patients	

5.5 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of blinatumomab, nivolumab, and ipilimumab with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix B (Patient Drug Information Handout and Wallet Card) should be provided to patients if available.

5.5.1 Supportive Care Guidelines

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed, provided their use is documented in the medical records and meets the dose modification guidelines outlined in Section 6. The administration of any other therapies intended to treat the primary condition including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed.

5.5.2 Permitted Concomitant Medications

• **Corticosteroids:** Inhaled or topical steroids and adrenal replacement doses ≤10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Subjects 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), for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by contact allergen), treatment of toxicities (e.g., cytokine release syndrome or neurologic toxicity due to blinatumomab or autoimmune toxicities caused by ipilimumab or nivolumab), and for treatment of infusion reactions and premedication (i.e. 20 mg of dexamethasone prior to blinatumomab initiation and step doses, and prior to re-initiating infusion if interrupted for ≥4 hours) is permitted. Additionally, prephase treatment with dexamethasone 10 mg/m^2 (maximum total 24 mg per day) for up to 5 days is required with bone marrow blasts more than 50%, peripheral blood blasts of 15,000/μL or higher, or elevated lactate dehydrogenase suggesting rapidly progressing disease as per investigator's assessment to reduce the tumor burden and mitigate the potential for cytokine release syndrome unless other acceptable cytoreductive measures, as written in

3.2.1 and 5.1 have previously been employed. There is a published case report suggesting that for cases of life-threatening cytokine release syndrome for which dexamethasone and supportive care measures are not adequate, consideration may be given to the administration of tocilizumab, the anti-IL-6 monoclonal antibody.[90]

5.5.3 **Antiemetics**

Antiemetics will be used according to standard practices. Any 5-hydroxytryptamine (5-HT3) receptor inhibitor (ex. ondansetron) combined with prochlorperazine or similar can be used during treatment, as needed.

5.5.4 **Antimicrobial Prophylaxis**

Patients should receive prophylaxis directed against gram-negative gastrointestinal infections (GI), Candidiasis/mold, Pneumocystis pneumonia (PCP), and/or herpes simplex virus (HSV), according to individual institutional practices.

5.5.5 **Intrathecal Prophylaxis**

Intrathecal prophylaxis must be used according to institutional standard practice for all patients with the choice of agent at the discretion of the investigator. Maximum doses of up to 15 mg of methotrexate, up to 100 mg of cytarabine, or 40 mg of cytarabine and 12 mg of methotrexate in triple therapy should be employed.[95-97] Hydrocortisone 50 mg intrathecally can optionally combined with methotrexate if desired to lessen the risk of arachnoiditis. To reduce the risk of CNS leukemia relapse, intrathecal prophylaxis should be administered during screening and on day 29-35 of at least the first two cycles at the conclusion of blinatumomab infusion as activity of blinatumomab has not been established in patients with CNS leukemia. This schedule of CNS prophylaxis administration has been adopted from prior blinatumomab trials to avoid confounding neurologic events related to intrathecal chemotherapy administration during blinatumomab administration.[31]

5.5.6 **Colony Stimulating Factors**

The routine use of colony stimulating factors is disallowed. The use of colony stimulating factors in the presence of severe neutropenic infection should be used in accordance with ASCO guidelines and discussed with the study chair before implementation.[98]

5.5.7 **Management of Tumor Lysis Syndrome**

Tumor lysis occurs as part of initial cytoreductive therapy. The most extreme form, TLS, is characterized by hyperuricemia, hyperphosphatemia, increased lactate dehydrogenase (LDH), coagulopathy, and a potential cytokine release syndrome. Since the occurrence of TLS is magnified in the presence of large tumor burdens, we will require prephase treatment as specified in 3.2.1 and 5.5.2 for any patient who has > 50% bone marrow blasts, ≥15,000/μL peripheral blood blasts, or an elevated LDH suggestive of rapidly progressive disease as per the investigator's assessment.

- To prevent hyperuricemia, all patients without known allergy will receive allopurinol 300-600 mg PO qday continuing through the period of maximal tumor lysis or other anti-uricemia regimen per institutional practice. Rasburicase may be used per institutional policy for hyperuricemia. Screening for G6PD deficiency should be obtained in susceptible populations before beginning Rasburicase.
- To decrease the risk of hyperphosphatemia, an oral phosphate binder per institutional practice (ex. sevelamer 400-800 mg) may be administered orally every 4-6 hours beginning on with prephase or protocol treatment and continued as tolerated until maximum tumor lysis has occurred.
- Cytokine release syndrome (CRS) can occur shortly after completion of blinatumomab infusion, accompany severe TLS, and present with fever, bronchospasm with dyspnea and/or respiratory distress, altered blood pressure, myalgias, arthralgias, tumor pain and/or urticarial rash. Any patient with cytokine release syndrome should receive 20 mg dexamethasone (or equivalent steroid) IV immediately. Tocilizumab may also be used at the discretion of the investigator for CRS as per section 5.5.2. and 5.5.8.1.

5.5.8 **Hypersensitivity/Infusion Reactions**

5.5.8.1 Blinatumomab

Reactions may manifest as fevers, chills, rigors, headache, rash, urticaria, angioedema, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

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

Remain at bedside and monitor subject until recovery from symptoms.

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

Continue therapy with supportive care, as per local institutional guidelines.

• **For Grade 2 symptoms**: Moderate reactions require therapy or infusion interruption but respond promptly to symptomatic treatment (e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids). The infusion of blinatumomab must be stopped immediately.

Supportive care, as per institutional guidelines.

If the interruption is four hours or longer, re-start of the infusion should be performed in the hospital, under the supervision of the investigator. The subject should be observed overnight for possible side effects after the re-start, either in the hospital or in the outpatient setting as applicable.

Within one hour prior to start of treatment, or re-start of treatment if interruption is four hours or longer, and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously.

• **For Grade 3 symptoms:** (Severe reactions)
The infusion of blinatumomab must be stopped immediately.

Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) up to three days. If symptoms persist, then tocilizumab may be used at the investigator's discretion as per 5.5.2.

The dexamethasone dose will then be reduced step-wise over up to four days.

Hold blinatumomab until toxicity resolves to grade ≤1, then resume drug at 9 µg/day for one week. If toxicity remains grade ≤1, increase dose to 28 µg/day to complete the 28-day cycle of therapy.

Within one hour prior to re-start of treatment and within one hour prior to any increases, patients must receive 20 mg of dexamethasone intravenously.

If grade 3 recurs after blinatumomab is resumed, stop drug permanently.

If the initial adverse event last for ≥ 2 weeks without improvement, then blinatumomab will be permanently discontinued.

• **For Grade 4 symptoms:** (Severe reactions)
The infusion of blinatumomab must be stopped immediately and permanently.

Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) up to three days. If symptoms persist, then tocilizumab may be used at the investigator's discretion as per 5.5.2.

The dexamethasone dose will then be reduced step-wise over up to four days.

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

5.5.8.2 Nivolumab and Ipilimumab

NOTE: The same infusion reaction guidelines apply to ipilimumab. Any reference to nivolumab below should be used also for considerations of ipilimumab-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.

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

Remain at bedside and monitor subject until recovery from symptoms.

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

Infusion rate may be slowed or interrupted and restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely.

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, and no further nivolumab will be administered at that visit.

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur, re-administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and (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 reactions)

    - 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): Nivolumab will be permanently discontinued.

  Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur.

  Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

  In the case of late-occurring hypersensitivity symptoms (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). Additional treatment prior to next dose as per guidelines above.

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

5.5.9 **Prohibited and Restricted Therapies**

Subjects in this study may use standard vaccines with certain exceptions. Where possible, routine vaccination for influenza and pneumoccal pneumonia should be given prior to the start of therapy but may be administered during treatment when clinically indicated. Vaccination should be given when there is enough separation to distinguish any vaccine reactions from drug toxicity. There is no experience using live attenuated vaccination during blinatumomab, nivolumab, and ipilimumab therapy in patients with ALL, so live vaccine should not be given during treatment.

Subjects may not use any of the following therapies during the study (unless utilized to treat a drug-related adverse event):

- Any non-study anti-cancer agent (investigational or non-investigational).
- Any other investigational agents
- Any other CTLA-4 inhibitors or agonists OR any other PD-1 inhibitors or agonists
- CD137 or other immunologic activation agonists
- Immunosuppressive agents
- Chronic systemic corticosteroids at supraphysiologic doses

5.6 Duration of Therapy

In general, treatment may continue for up to 5 cycles of blinatumomab and up to 1 year of nivolumab/ipilimumab or until one of the following criteria are met:

- Disease relapse, progression, or a lack of a complete remission by the end of the 2nd cycle of treatment
- Intercurrent illness that prevents further administration of treatment
- Adverse event(s) which require(s) permanently going off study treatment (see also Section 6 and specific algorithms in Appendix D):
  Any dosing interruption lasting >6 weeks for nivolumab or ipilimumab or >2 weeks for blinatumomab, with the following exceptions:
  - Dosing interruptions 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 greater than the specified time intervals, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Dosing with blinatumomab, nivolumab and/or ipilimumab may be individually held or discontinued with other study treatment(s) continuing if drug-related toxicity can be definitively attributed to an agent(s) and other individual agent re-treatment criteria are met as outlined in Sections 6.2.1, 6.3.1., and 5.9. Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Pregnancy
  - All WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.
  - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a subject participating in the study.
- Initiation of breast feeding by the patient
- Subject non-compliance
- Treatment required with other chemotherapeutic, investigational anti-neoplastic drugs, or allogeneic hematopoietic stem cell transplant. Patients should be off nivolumab therapy for at least 6 weeks prior to having allogeneic stem cell transplant.
- Delay of blinatumomab for more than 14 days within a cycle due to unresolved toxicities or delay of nivolumab or ipilimumab for more than six weeks due to unresolved toxicities.
- Death or lost for follow-up
- Completed treatment

Please see Section 6 for specific discontinuation criteria of blinatumomab, nivolumab, and ipilimumab individually due to toxicity.

5.7 Duration of Follow Up

Patients will be followed for overall survival and relapse every 3 months for 2 years after removal from study treatment or until death, whichever occurs first. Patients removed from study treatment for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Assessments for toxicity/adverse events are to continue for 100 days after the last dose of nivolumab/ipilimumab. Because many patients enrolled in this protocol will be candidates for a subsequent allo-HSCT, and there have been recent reports of increased toxicity among patients who undergo allo-HSCT following treatment with PD-1 inhibitors, all patients who go onto allo-HSCT following the completion of treatment on the study will undergo continuous monitoring. The clinical failures of acute grade 3-4 graft versus host disease and post-transplant non-relapse mortality will be monitored continuously for the first 100 post-transplant days. We will also collect data on development of additional cancers, subsequent therapy for their cancer, and survival. Medical records including laboratory, pathology, operative, and radiology reports will be obtained at the discretion of the Protocol Chair.

All reasons for discontinuation of therapy should be documented clearly in the medical record. If a subject discontinues or withdraws from the study, every attempt will be made to get a bone marrow biopsy and study bloods if the subject is able and willing to do so.

5.8 Criteria for Removal from Study

Patients will be removed from study treatment when any one of the applicable criteria listed in Section 5.6. is met. For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for 2 years from the end of study treatment for relapse and for survival (even if non-protocol therapy is initiated). Patient will be removed from study if any one of the following criteria is met: completion of follow up period, patient withdraws consent form study, death, or patients is lost for follow up. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.9 Criteria to Resume Treatment

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:

- 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 for nivolumab/ipilimumab or the fourteen day delay

period for blinatumomab, as per Section 5.6.

Dosing with blinatumomab, nivolumab and/or ipilimumab may be individually held or discontinued with other study treatment(s) continuing if drug-related toxicity can be definitively attributed to an agent(s) and other individual agent re-treatment criteria are met as outlined below. However, patients with renal, CNS, or pulmonary toxicity must be removed from study.

For patients treated with high dose steroids: Must resolve to baseline within 6 weeks of treatment. Subjects 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).

Must be off steroids for at least 2 weeks with no recurrence or new 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/ipilimumab.

Must have had no recurrence of symptoms or new symptoms during steroid taper.

5.10 Treatment Beyond Progression

Not applicable

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 General

Dosing with blinatumomab, nivolumab and/or ipilimumab may be individually held or discontinued with other study treatment(s) continuing if drug-related toxicity can be definitively attributed to an agent(s) and other individual agent re-treatment criteria are met as outlined below. All discontinuations, relationships to treatment, and treatment decisions must be clearly documented in the medical record. Any questions should be discussed with the Protocol Chair.

Although blinatumomab and immune checkpoint blockade have distinct toxicity profiles, they do share many adverse events. There is the possibility that one agent may potentiate the other and hence drug causality will not always be clear. In the event of uncertainty, dose reductions and/or delays will follow the most conservative approach.

Dose reductions due to toxicity are permanent. Dose escalation is not allowed.

NOTE: Tumor assessments should continue as per protocol even if dosing is delayed.

6.2 Blinatumomab

6.2.1 Re-Treatment Criteria

Prior to administration of first dose of blinatumomab in each cycle, subject's organ function and treatment-related toxicities must have recovered to the following values:

- total bilirubin ≤2.0 mg/dL (except patients with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)
- AST(SGOT)/ALT(SGPT) ≤5× ULN
- Serum creatinine ≤1.5 X ULN (Cr Cl of ≥50 mL/min)

6.2.2 Dose Modifications for Blinatumomab-Related Toxicity

Before any re-start, for an interruption of 4 hours or longer, pre-treatment with 20 mg of intravenous dexamethasone has to be administered. Re-start of the infusion should be performed under supervision of the investigator. The subject should be observed overnight for possible side effects after the re-start, either in the hospital or in the outpatient setting as applicable.

Please see Table 6.1 for determination of treatment cycle duration following treatment interruption for all adverse events and Table 6.2 for dose modifications for adverse events possibly, probably or definitely related to blinatumomab.

Table 6.1. Determination of Cycle Length Following Treatment Interruption for Adverse Events

Length of Dose Interruption Due to AE	Length of Treatment Prior to AE Onset	Investigator Action Once Blinatumomab is Restarted
≤ 7 days	--	Continue same cycle of blinatumomab for a total treatment duration of 28 days on blinatumomab.
8-14 days	≤ 14 days	Cycle of treatment will be re-started as if a new cycle and continue for an additional 28 days total.
8-14 days	> 14 days	This cycle of treatment will be terminated.
> 14 days	--	Permanent discontinuation of treatment.
NOTE: If the above adverse events resolve to a level allowing resumption of blinatumomab in less than 14 days, but logistical difficulties arise, restart of treatment can be postponed for up to five additional days without resulting in permanent treatment discontinuation		

If immune-related adverse events related to nivolumab or ipilimumab occur that require use of high dose steroids, the delay in blinatumomab cycle administration up to 6 weeks may be allowed to allow for tapering of steroids to prednisone 10 mg or less.

Table 6.2 Table of Dose Modifications for Adverse Events Possibly, Probably, or Definitely Related to Blinatumomab

Toxicity - Adverse Event CTCAE v4.0 terminology	AE Grade	Action
Events within the "Nervous system disorders" or "Psychiatric disorders" System Organ Class (SOC)	Grade 1	Continue at the same dose level
	Grade 2	Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) for up to three days. The dexamethasone dose will then be reduced step-wise over up to four days.
	Grade 3	Infusion of the blinatumomab must be stopped immediately. Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) for up to three days. The dexamethasone dose will then be reduced step-wise over up to four days. Hold blinatumomab until AE resolves to Grade ≤ 1 and for at least 3 days, then resume drug at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur and if it resolved within 7 days. If the toxicity takes more than 7 days to resolve, do not escalate to 28 mcg/day. If toxicity occurs at 9 mcg/day or it takes more than 14 days

Toxicity - Adverse Event CTCAE v4.0 terminology	AE Grade	Action
		to resolve, discontinue blinatumomab permanently. Infusion should be re-started in the hospital, under supervision of the investigator and the patient should remain hospitalized for at least two days. Within one hour prior to re-start of treatment and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously. In the first three days after re-start, vital sign measurements and writing tests (Appendix G) should be performed. If patient has already been dose reduced to 9 mcg/day dose for any reason and Grade 3 event occurs, blinatumomab should be discontinued permanently.
	Grade 4	Infusion of blinatumomab must be stopped immediately. Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) for up to three days. The dexamethasone dose will then be reduced step-wise over up to four days. Blinatumomab should be discontinued permanently.
Seizure	Grade 1-3	As per Grade 3 Nervous System/Psychiatric Disorders (above). Appropriate prophylactic / therapeutic doses of anticonvulsant treatment (e.g. phenytoin or levetiracetam) will be administered during subsequent infusions of blinatumomab. If seizure recurs discontinue blinatumomab permanently.
	Grade 4	Discontinue blinatumomab permanently
Cytokine release syndrome (CRS), Allergic reaction, Anaphylaxis, or Infusion related reaction	Grade 1	Continue therapy with supportive care, as per local institutional guidelines.
	Grade 2	The infusion of the blinatumomab must be stopped immediately. Supportive care, as per institutional guidelines. If the interruption is longer than four hours, re-start of the infusion should be performed in the hospital, under supervision of the investigator. Within one hour prior to re-start of treatment and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously.

Toxicity - Adverse Event CTCAE v4.0 terminology	AE Grade	Action
	Grade 3	The infusion of the blinatumomab must be stopped immediately. Dexamethasone should be administered at a dose of at least 24 mg per day (8 mg every 8 hours orally or IV) for up to three days. The dexamethasone dose will then be reduced step-wise over up to four days. Hold blinatumomab until the toxicity resolves to Grade ≤ 1, then resume drug at 9 mcg/day for one week. If toxicity remains Grade ≤ 1, increase dose to 28 mcg/day to complete 28-day cycle of therapy. Within one hour prior to the re-start of treatment and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously. If Grade 3 toxicity recurs after blinatumomab is resumed, stop drug permanently. If the initial adverse event lasts >14 days without improvement, then blinatumomab will be permanently discontinued.
	Grade 4	Blinatumomab should be discontinued permanently.
Liver functions Aspartate aminotransferase increased, Alanine aminotransferase increased (AST, ALT); bilirubin increased	Grade 1-2	Continue at same dose level.
	Grade 3-4	Hold blinatumomab until AE resolves to Grade ≤ 1, then resume drug at 9 mcg/day for one week. If toxicity remains Grade ≤ 1, increase dose to 28 mcg/day to complete the 28-day cycle of therapy. If Grade 3-4 toxicity recurs, stop the drug permanently. Within one hour prior to the restart of treatment and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously.
Disseminated intravascular coagulation	Grade 2	Continue at same dose level.
	Grade 3-4	Hold blinatumomab until AE resolves to Grade ≤ 2, then resume drug at 9 mcg/day for one week. If toxicity remains Grade ≤ 1, increase dose to 28 mcg/day to complete 28-day cycle of therapy. If Grade 3-4 toxicity recurs, stop drug permanently. If the initial adverse event lasts for > 14 days without improvement, then blinatumomab will be permanently discontinued. Within one hour prior to the restart of treatment and

Toxicity - Adverse Event CTCAE v4.0 terminology	AE Grade	Action
		within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously.
Thromboembolic event	Grade 1	Continue at the same dose level.
	Grade 2-4	Hold blinatumomab until clot and clinical situation stabilized, then resume drug at 9 mcg/day for one week. If no progression of thrombus, increase dose to 28 mcg/day to complete the 28-day cycle of therapy. If progression of existing thrombosis or new thrombosis or Grade 4 initially, stop drug permanently. Within one hour prior to the restart of treatment and within one hour prior to any dose increases, patients must receive 20 mg of dexamethasone intravenously.
Hematologic toxicities Lymphocyte count decreased	Grade 1-4	Continue at the same dose level.
Neutrophil count decreased	Grade 1-4	Continue at the same dose level.
Platelet count decreased	Grade 1-4	Continue at the same dose level.
All other AEs within the "Investigations" SOC and "Metabolism and nutrition disorders" SOC	Grade 1-4	Continue at the same dose level, provided AE is not medically consequential and has been readily corrected. If the abnormality is medically consequential, refer to guidelines for other non-hematologic AEs. Patients who develop Grade 3 or 4 electrolyte laboratory abnormalities may continue study treatment without interruption but should receive appropriate medical therapy.
Other non-hematologic AEs	Grade 1-2	Continue at same dose level.
	Grade 3-4	Hold blinatumomab until the AE resolves to Grade ≤ 1, then resume drug at 9 mcg/day for one week. If the toxicity remains Grade ≤ 1, increase the dose to 28 mcg/day to complete the 28-day cycle of therapy. If Grade 3 recurs, reduce the dose to 9 mcg/day and if after one week toxicity is Grade ≤ 2, continue at 9 mcg/day. If Grade 4 toxicity recurs, stop the drug permanently. If the toxicity takes more than 14 days to resolve, discontinue blinatumomab permanently. Within one hour prior to the restart of treatment and within one hour prior to any dose increases,

Toxicity - Adverse Event CTCAE v4.0 terminology	AE Grade	Action
		patients must receive 20 mg of dexamethasone intravenously.
Blinatumomab may be held or discontinued as needed for supportive care.		

6.3 Nivolumab and Ipilimumab

6.3.1 Re-Treatment Criteria

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:

- 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.

For patients receiving both nivolumab and ipilimumab who experience grade 2 or 3 adverse events requiring discontinuation of treatment with the combination, the treating investigator may consider continuing treatment with single agent nivolumab at the same dose when the event resolves to baseline. However, patients with renal, CNS, cardiac or pulmonary toxicity must be removed from study. For this protocol, if the treating investigator believes the subject is benefitting from treatment and feels it is in the best interest of the participant to continue on nivolumab single agent, this may be considered after discussion with the Protocol Chair.

For patients treated with high dose steroids:

- Adverse events must resolve to baseline within 6 weeks of treatment
- Must be off steroids for at least 2 weeks with no recurrence or new 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.
- Must have had no recurrence of symptoms or new symptoms during steroid taper

6.3.2 **Dose Delays**

If treatment is delayed >6 weeks for an adverse event, the patient must be permanently discontinued from study therapy.

Patients requiring high dose steroid treatment for autoimmune or inflammatory events should go off study treatment except for a short course of tapering steroids for infusion reaction, skin rash or endocrine events.

Patients with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Patients with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses of corticosteroids. Please note that 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.

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

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.

Please note that in some cases the treatment algorithms recommend steroids if symptoms do not resolve in 7 days. However, this recommendation is not meant to delay steroid treatment at any time it is clinically indicated.

Patients may be dose-delayed for evaluation and restarted depending on results. Any patient started on corticosteroids initially who is determined to not require steroid 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.

6.3.3 **Dose Modifications for Nivolumab-/Ipilimumab-Related Toxicity**

NOTE: No dose reductions apply to nivolumab or ipilimumab, only dose delays. The above dose delay criteria/time frames apply for nivolumab-/ipilimumab-related toxicity.

For information on specific toxicity and management plans for nivolumab or nivolumab/ipilimumab combination treatment, see below and reference management guidelines/algorithms in Appendix D, as noted.

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab and Nivo/Ipi Combination**
≤ Grade 1	No change in dose *
Grade 2	No change in dose *
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/pemphigoid. 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, ALT, Bilirubin	**Management/Next Dose for Nivolumab and Nivo/Ipi Combination**
≤ Grade 1	No change in dose.
Grade 2	Hold until UNL or baseline. Resume at same dose level.
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Continued treatment of active immune mediated hepatitis may exacerbate 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 Hepatic AE management algorithm	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab and Nivo/Ipi Combination**
≤ Grade 1	No change in dose
Grade 2	Hold until baseline. No change in dose
Grade 3	Off protocol therapy.
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 Amylase/Lipase	**Management/Next Dose for Nivolumab and Nivo/Ipi Combination**
Grade 2	Hold until baseline. Resume at same dose level if asymptomatic
Grade 3	Hold until baseline. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Grade 4	Hold until baseline. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or DM should be taken off treatment
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 and Nivo/Ipi Combination**
Grade 1	Consider holding dose pending evaluation and resolution to baseline including baseline pO2. Resume no change in dose after pulmonary and/or ID consultation excludes lymphocytic pneumonitis.
Grade 2	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation excludes nivolumab and associated lymphocytic pneumonitis as the cause of the pneumonitis. If nivolumab/ipilimumab related, hold until baseline. Patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation excludes nivoumab and associated lymphocytic pneumonitis as the cause of the pneumonitis. If nivolumab/ipilimumab related, 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.	

Cardiac Toxicities*	**Management/Next Dose for Nivolumab and Nivo/Ipi Combination**
≤ 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.	

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 and Nivo/Ipi Combination**
≤ Grade 1	No change in dose.
Grade 2	Hold dose pending evaluation and observation. # Hold until resolves to baseline. . 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 and Nivo/Ipi Combination**
≤ Grade 1	Asymptomatic TSH elevation* If asymptomatic continue therapy.
Grade 2	Hold until patients are on a stable replacement hormone regimen. If 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	

Renal	**Management/Next Dose for Nivolumab and Nivo/Ipi combination**
≤ Grade 1	Continue therapy; monitor creatinine weekly. If returns to baseline, resume routine monitoring per protocol. If worsens, treat as below.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level. If treated with steroids patients must be stable off steroids for two weeks.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level. If treated with steroids patients must be stable off steroids for two weeks.
Grade 4	Off treatment
Recommended management: See Renal Management Algorithm.	

Infusion reaction	**Management/Next Dose for Nivolumab and Nivo/Ipi combination**
≤ Grade 1	Evaluate patient and monitor per institution protocols. Continue at same dose level.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Off treatment.
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 5.5.8.2 infusion reactions	

Fever	**Management/Next Dose for Nivolumab and Nivo/Ipi combination**
≤ Grade 1	Evaluate and continue at same dose level
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 5.5.8.2. infusion reactions	

ALL OTHER EVENTS	**Management/Next Dose for Nivolumab and combination Nivolimab/Ipililmumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline(exceptions as noted below in Section 6.3.4)
Grade 3	Withold dose ≤ Grade 1 OR baseline. Off protocol therapy (exceptions as noted below in Section 6.3.4)
Grade 4	Off protocol therapy
Recommended management: As clinically indicated	

6.3.4 **Discontinuation of Nivolumab/Ipilimumab Treatment**

Nivolumab and ipilimumab treatment should be discontinued if any of the following criteria are met:

- Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
- Any 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. **NOTE:** Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality that can be managed with electrolyte replacement, hormone replacement, insulin or that does not require treatment **does not** require discontinuation.
- Any adverse event that requires discontinuation of treatment per Section 6.3.3.
- Any subjects who require additional immune suppressive treatment beyond steroids should go off study treatment.
- Subjects requiring >2 dose delays for the same type of event should go off protocol therapy.
- Any dosing interruption lasting >6 weeks, with the following exceptions:
    - Subjects 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 Protocol Chair. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Protocol Chair must be consulted.

6.4 Special Considerations

In the event that blinatumomab or nivolumab and/or ipilimumab are discontinued, the other study drugs may be continued per criteria above after discussion with the Protocol Chair.

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(s) (CAEPRs)

The Comprehensive Adverse Event 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 of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The 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.

> **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.

7.1.1 CAEPRs for CTEP IND Agents

7.1.1.1 CAEPR for Blinatumomab (AMG103, NSC 765986)

Frequency is provided based on 1276 patients. Below is the CAEPR for Blinatumomab (AMG 103).

Version 2.5, September 4, 2019[1]

Adverse Events with Possible Relationship to Blinatumomab (AMG 103) (CTCAE 5.0 Term) [n= 1276]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
	Blood and lymphatic system disorders - Other (coagulopathy)[2]		***Blood and lymphatic system disorders - Other (coagulopathy)[2] (Gr 2)***
		Blood and lymphatic system disorders - Other (hematophagic histiocytosis)	
		Blood and lymphatic system disorders - Other (lymphadenitis)	
		Blood and lymphatic system disorders - Other (lymphadenopathy)	

Adverse Events with Possible Relationship to Blinatumomab (AMG 103) (CTCAE 5.0 Term) [n= 1276]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Blood and lymphatic system disorders - Other (pancytopenia)	
	Disseminated intravascular coagulation[2,3]		***Disseminated intravascular coagulation[2,3] (Gr 2)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
CARDIAC DISORDERS			
	Sinus tachycardia		***Sinus tachycardia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
		Gastric hemorrhage	
		Gastrointestinal disorders - Other (pneumoperitoneum)	
	Mucositis oral		
Nausea			***Nausea (Gr 2)***
		Oral hemorrhage	
		Pancreatitis	
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[3]		***Chills[3] (Gr 2)***
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue[3]			***Fatigue[3] (Gr 2)***
Fever[3]			***Fever[3] (Gr 2)***
	Generalized edema		
	Non-cardiac chest pain		
	Pain		
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (hepatic function abnormal)[4]		***Hepatobiliary disorders - Other (hepatic function abnormal)[4] (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
	Cytokine release syndrome[3]		***Cytokine release syndrome[3] (Gr 3)***
	Immune system disorders - Other (immunodeficiency [immunoglobulin decreased])[5]		***Immune system disorders - Other (immunodeficiency [immunoglobulin decreased])[5] (Gr 2)***
INFECTIONS AND INFESTATIONS			
Infection[6]			***Infection[6] (Gr 4)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
		Injury, poisoning and procedural complications - Other (overdose)[7]	
INVESTIGATIONS			
		Activated partial thromboplastin time prolonged[2]	

Adverse Events with Possible Relationship to Blinatumomab (AMG 103) (CTCAE 5.0 Term) [n= 1276]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Alanine aminotransferase increased[4]		***Alanine aminotransferase increased[4] (Gr 3)***
	Alkaline phosphatase increased[4]		***Alkaline phosphatase increased[4] (Gr 2)***
	Aspartate aminotransferase increased[4]		***Aspartate aminotransferase increased[4] (Gr 4)***
	Blood bilirubin increased[4]		***Blood bilirubin increased[4] (Gr 2)***
	Blood lactate dehydrogenase increased		
		Creatinine increased[8]	
	GGT increased[4]		***GGT increased[4] (Gr 2)***
		Investigations - Other (blood fibrinogen increased)[2]	
	Investigations - Other (C-reactive protein increased)		***Investigations - Other (C-reactive protein increased) (Gr 2)***
	Investigations - Other (fibrin D dimer increased)[2]		
Lymphocyte count decreased			***Lymphocyte count decreased (Gr 4)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased[2]			***Platelet count decreased[2] (Gr 2)***
	Weight gain		***Weight gain (Gr 2)***
	Weight loss		
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyperglycemia		***Hyperglycemia (Gr 2)***
	Hyperuricemia		
	Hypoalbuminemia		
	Hypocalcemia		
Hypokalemia			***Hypokalemia (Gr 2)***
	Hypomagnesemia		
	Hypophosphatemia		
		Tumor lysis syndrome[9]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
	Bone pain		
	Generalized muscle weakness		
	Myalgia		
	Pain in extremity		***Pain in extremity (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Ataxia[10]		
	Cognitive disturbance[10]		
	Dizziness[10]		***Dizziness[10] (Gr 2)***
		Dysarthria[10]	
	Dysphasia[10]		
	Encephalopathy[10]		
		Facial nerve disorder[10]	

Adverse Events with Possible Relationship to Blinatumomab (AMG 103) (CTCAE 5.0 Term) [n= 1276]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
Headache[10]			***Headache[10] (Gr 2)***
		Intracranial hemorrhage	
		Leukoencephalopathy	
	Memory impairment[10]		
	Nervous system disorders - Other (apraxia)		
	Nervous system disorders - Other (cerebellar syndrome)[10]		
		Nervous system disorders - Other[10]	
	Paresthesia[10]		
		Reversible posterior leukoencephalopathy syndrome	
	Seizure[10]		
	Somnolence[10]		
		Transient ischemic attacks[10]	
	Tremor[10]		***Tremor[10] (Gr 2)***
PSYCHIATRIC DISORDERS			
		Agitation[10]	
	Anxiety[10]		
	Confusion[10]		
		Hallucinations[10]	
	Insomnia		***Insomnia (Gr 2)***
		Personality change[10]	
		Psychosis[10]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		
	Epistaxis		
		Hypoxia	
	Oropharyngeal pain		
		Pneumonitis	
	Voice alteration[10]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Hyperhidrosis		
	Pruritus		
	Skin and subcutaneous tissue disorders - Other (rash)[11]		***Skin and subcutaneous tissue disorders - Other (rash)[11] (Gr 2)***
VASCULAR DISORDERS			
		Capillary leak syndrome[3]	
	Flushing[3]		
	Hypertension[3]		***Hypertension[3] (Gr 2)***
	Hypotension[3]		***Hypotension[3] (Gr 2)***
	Thromboembolic event[2]		***Thromboembolic event[2] (Gr 2)***

[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]Blinatumomab (AMG 103) is known to cause a variety of adverse events associated with coagulopathy which may include: Activated partial thromboplastin time prolonged, Disseminated intravascular coagulation, Fibrinogen decreased, INR increased, Investigations - Other (blood fibrinogen increased), Investigations - Other (fibrin D dimer increased), Investigations - Other (activated partial thromboplastin time shortened), Investigations - Other (antithrombin III decreased), Investigations - Other (coagulation factor XII level decreased), Investigations - Other (coagulation factor XIII level increased), Investigations - Other (haptoglobin decreased), Investigations - Other (protein S decreased), Platelet count decreased, and Thromboembolic events.

[3]Symptoms of cytokine release syndrome (CRS) and/or allergic reaction may include chills, fever, fatigue, flushing, bronchospasm, and hypotension. In some cases, disseminated intravascular coagulation (DIC), capillary leak syndrome (CLS), and hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) have been reported in the setting of CRS.

[4]Symptoms of hepatic dysfunction may include Alanine aminotransferase increased, Alkaline phosphatase increased, Aspartate aminotransferase increased, Blood bilirubin increased, and GGT increased under the INVESTIGATIONS SOC.

[5]Immunodeficiency (immunoglobulin decreased) includes immunoglobulins decreased, blood immunoglobulin G decreased, blood immunoglobulin M decreased, and blood immunoglobulin A decreased.

[6]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[7]Overdoses have been observed. Overdoses resulted in adverse reactions, which were consistent with the reactions observed at the recommended therapeutic dose and included fever, tremors, and headache. In the event of overdose, interrupt the infusion, monitor the patient for signs of toxicity, and provide supportive care. Consider re-initiation of blinatumomab at the correct therapeutic dose when all toxicities have resolved and no earlier than 12 hours after interruption of the infusion.

[8]Acute kidney injury (acute renal failure) is associated with increased creatinine levels.

[9]Tumor lysis syndrome is defined as a massive overload of potassium, phosphate, uric acid, plus hypocalcemia, potentially causing lethal cardiac arrhythmias and/or renal failure.

[10]Blinatumomab (AMG103) is known to cause a variety of nervous system disorders which may include: Ataxia, Cognitive disturbance, Concentration impairment, Depressed level of consciousness, Dizziness, Dysphagia, Dysarthria, Dysesthesia, Dysphasia, Encephalopathy, Facial nerve disorder, Headache, Lethargy, Memory impairment, Paresthesia, Peripheral sensory neuropathy, Seizure, Somnolence, Syncope, Transient ischemic attacks, Tremor, Voice alteration, Nervous system disorders - Other (allodynia), Nervous Systems disorders - Other (cerebellar syndrome), Nervous system disorders - Other (dysgraphia), Nervous system disorders - Other (epilepsy), Nervous system disorders - Other (facial palsy), Nervous system disorders - Other (hemiparesis), Nervous system disorders - Other (hypertonia), Nervous system disorders - Other (hypotonia), Nervous system disorders - Other (pleocytosis), and Nervous system disorders - Other (polyneuropathy). Additionally, symptoms of some nervous system disorders are adverse events under the PSYCHIATRIC DISORDERS SOC and may include: Agitation, Anxiety, Confusion, Hallucinations, Personality change, and Psychosis.

[11]Rash includes rash, rash maculo-papular, erythema, local erythema, erythematous rash, generalized rash, exanthema, allergic dermatitis, and palmar-plantar erythrodysesthesia syndrome.

Adverse events reported on Blinatumomab (AMG 103) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Blinatumomab (AMG 103) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Pericardial effusion; Sinus bradycardia; Supraventricular tachycardia
CONGENITAL, FAMILIAL AND GENETIC DISORDERS - Congenital, familial and genetic disorders - Other (aplasia)
EAR AND LABYRINTH DISORDERS - Vertigo
EYE DISORDERS - Blurred vision; Optic nerve disorder; Papilledema; Periorbital edema; Photophobia
GASTROINTESTINAL DISORDERS - Abdominal distension; Anal mucositis; Dyspepsia; Dysphagia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Gait disturbance; General disorders and administration site conditions - Other (thrombosis in device); Hypothermia; Malaise; Multi-organ failure
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Vascular access complication
INVESTIGATIONS - Cardiac troponin I increased; Electrocardiogram QT corrected interval prolonged; INR increased; Investigations - Other (hypoproteinemia); Investigations - Other (lipase decreased); Lipase increased; Lymphocyte count increased
METABOLISM AND NUTRITION DISORDERS - Acidosis; Dehydration; Hypercalcemia; Hyperkalemia; Hyperphosphatemia; Hyponatremia; Metabolism and nutrition disorders - Other (fluid overload)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Muscle cramp; Neck pain
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Amnesia; Facial muscle weakness; Muscle weakness left-sided; Nervous system disorders - Other (difficulty following commands); Neuralgia
PSYCHIATRIC DISORDERS - Delirium; Depression; Psychiatric disorders - Other (altered mental status); Psychiatric disorders - Other (sleep disorder); Restlessness
RENAL AND URINARY DISORDERS - Acute kidney injury[7]; Hematuria; Proteinuria; Urinary incontinence
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Genital edema
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Bronchospasm[3]; Pleural effusion; Productive cough; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (chronic obstructive pulmonary disease)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Purpura; Skin and subcutaneous tissue disorders - Other (skin irritation)
VASCULAR DISORDERS - Hematoma

Note: Blinatumomab (AMG 103) 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.

7.1.1.2 CAEPR for Nivolumab (BMS-936558, MDX-1106)

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	
	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			

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%)**	
		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		
		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]	

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%)**	
		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)	
	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.

7.1.1.3 CAEPR for Ipilimumab

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 2678 patients. Below is the CAEPR for Ipilimumab (MDX-010).

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 2.10, March 29, 2019[1]

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
		Blood and lymphatic system disorders - Other (acquired hemophilia)	
CARDIAC DISORDERS			
	Atrial fibrillation		
		Myocarditis[2]	
		Pericardial effusion	
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
	Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
	Testosterone deficiency[2]		
EYE DISORDERS			
	Eye disorders - Other (episcleritis)[2]		
	Uveitis[2]		
GASTROINTESTINAL DISORDERS			

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Abdominal pain		
	Colitis[2]		***Colitis[2] (Gr 3)***
		Colonic perforation[3]	
	Constipation		
Diarrhea			***Diarrhea (Gr 3)***
	Enterocolitis		
	Esophagitis		
		Ileus	
Nausea			***Nausea (Gr 3)***
	Pancreatitis[2]		
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
		General disorders and administration site conditions - Other (Systemic inflammatory response syndrome [SIRS])	
		Multi-organ failure	
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (hepatitis)[2]		
IMMUNE SYSTEM DISORDERS			
	Autoimmune disorder[2]		
		Immune system disorders - Other (GVHD in the setting of allotransplant)[4]	
INFECTIONS AND INFESTATIONS			
		Infections and infestations - Other (aseptic meningitis)[2]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
		Lymphocyte count decreased	
	Neutrophil count decreased		
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		
	Hyperglycemia		
		Metabolism and nutrition disorders - Other (exacerbation of pre-existing diabetes mellitus)	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Arthralgia		
	Arthritis		
		Generalized muscle weakness	
	Musculoskeletal and connective tissue disorder - Other (polymyositis)[2]		
NERVOUS SYSTEM DISORDERS			
		Ataxia	
	Facial nerve disorder[2]		
	Guillain-Barre syndrome[2]		
	Headache		
	Myasthenia gravis[2]		
		Nervous system disorders - Other (immune-mediated encephalitis)[2]	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
	Trigeminal nerve disorder		
PSYCHIATRIC DISORDERS			
		Psychiatric disorders - Other (mental status changes)	
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
	Renal and urinary disorders - Other (granulomatous tubulointerstitial nephritis)		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis		
		Respiratory failure	
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)	
		Respiratory, thoracic and mediastinal disorders - Other (lung infiltration)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	
	Pruritus		***Pruritus (Gr 3)***
Rash maculo-papular			***Rash maculo-papular (Gr 3)***
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
	Urticaria		
VASCULAR DISORDERS			
	Hypotension		

[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]Ipilimumab can result in severe and fatal immune-mediated adverse events probably due to T-cell activation and proliferation. These can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune thyroiditis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, and adrenal insufficiency), ocular manifestations (e.g., uveitis, iritis, conjunctivitis, blepharitis, and episcleritis), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome. The majority of these reactions manifested early during treatment; however, a minority occurred weeks to months after discontinuation of ipilimumab especially with the initiation of additional treatments.

[3]Late bowel perforations have been noted in patients receiving MDX-010 (ipilimumab) in association with subsequent IL-2 therapy.

[4]Complications including hyperacute graft-versus-host disease (GVHD), may occur in patients receving allo stem cell transplant (SCT) after receiving Ipilimumab (MDX-010). These complications may occur despite intervening therapy between receiving Ipilimumab (MDX-010) and allo-SCT.

[5]In rare cases diplopia (double vision) has occurred as a result of muscle weakness (Myasthenia gravis).

[6]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[7]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on Ipilimumab (MDX-010) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Ipilimumab (MDX-010) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Blood and lymphatic system disorders - Other (pure red cell aplasia)[2]; Febrile neutropenia
CARDIAC DISORDERS - Conduction disorder; Restrictive cardiomyopathy
EYE DISORDERS - Extraocular muscle paresis[5]; Eye disorders - Other (retinal pigment changes)
GASTROINTESTINAL DISORDERS - Colonic ulcer; Dyspepsia; Dysphagia; Gastrointestinal disorders - Other (gastroenteritis); Gastrointestinal hemorrhage[6]; Proctitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure[2]
IMMUNE SYSTEM DISORDERS - Allergic reaction
INFECTIONS AND INFESTATIONS - Infection[7]
INVESTIGATIONS - Creatinine increased; Investigations - Other (rheumatoid factor); Lipase increased; Platelet count decreased; Serum amylase increased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Joint range of motion

decreased; Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Dizziness; Dysphasia; Ischemia cerebrovascular; Seizure
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia
RENAL AND URINARY DISORDERS - Proteinuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Cough; Dyspnea; Laryngospasm
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Skin hypopigmentation
VASCULAR DISORDERS - Flushing; Hypertension; Vascular disorders - Other (temporal arteritis)

Note: Ipilimumab (BMS-734016; MDX-010 Transfectoma-derived) 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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://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 Distribution of Adverse Event Reports

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 or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.
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. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." 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.

Pregnancy loss is defined in CTCAE as "Death in utero." Any pregnancy loss should be reported expeditiously, as **Grade 4 "Pregnancy loss"** under the Pregnancy, puerperium and perinatal conditions SOC. A pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.

A neonatal death should be reported expeditiously as Grade 4, "Death neonatal" under the General disorders and administration SOC.

Phase 1 and Early Phase 2 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.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

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 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[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

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting

mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution
General disorders and administration site conditions	Fatigue	3	+/-	Possibly/ Probably /Definite
Blood and Lymphatic System Disorders	Anemia Hypocellular Bone Marrow Febrile Neutropenia	3 and 4 3 and 4 3 and 4	+/-	Possibly/ Probably /Definite
Investigations	Lymphocyte count decreased Neutrophil count decreased Platelet count decreased White blood cell decreased	3 and 4 3 and 4 3 and 4 3 and 4	+/-	Possibly/ Probably /Definite
Infections and Infestations	All with the exception of unusual infections such as PML	3 and 4	+/-	Possibly/ Probably /Definite
Metabolism and Nutritional Disorders	Anorexia Hyperglycemia Hypokalemia Hypomagnesemia Tumor Lysis Syndrome	3 3 3 3 3 and 4	+/-	Possibly/ Probably/ Definite
Gastrointestinal Disorders	Oral Pain Mucositis	3 3	+/-	Possibly/ Probably/ Definite
Skin and Subcutaneous Tissue Disorders	Hyperhidrosis (sweating)	3	+/-	Possibly/ Probably/ Definite

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. For this trial the Adverse Event CRF is used for routine

AE reporting in 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 agents administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent(s)

8.1.1 Blinatumomab

Other Names: AMG103, MT103

Classification: Bispecific T cell engaging antibody

M.W.: ~ 55 kDa

Mode of Action: Through CD3 binding, blinatumomab recruits and engages T cells for redirected lysis of CD19-positive B cells, including those expressed with B-cell malignancies. T cells are bound by its anti-CD3 moiety, whereas B cells are bound by the anti-CD19 moiety. The subsequent serial lysis of multiple malignant cells by a single blinatumomab-activated T cell closely resembles a natural cytotoxic T cell reaction. Treatment with blinatumomab is associated with a rapid depletion of peripheral B cells, accompanied by T cell activation and a transient increase in cytokines.

Description*:* Blinatumomab is a fusion protein composed of two single-chain antibodies (scFv), murine anti-CD19 scFv and murine anti-CD3 scFv.

How Supplied: Amgen provides and NCI/DCTD distributes blinatumomab and IV solution stabilizer for blinatumomab.

1. **Blinatumomab** is available as a **38.5 mcg** preservative-free, white to off-white lyophilized powder for injection in 4 mL single-use vial. The agent is formulated with 3.68 mg citric acid monohydrate, 105 mg trehalose dihydrate, and 25.55 mg lysine hydrochloride, and 0.70 mg polysorbate 80, pH 7. The stopper of the vial is latex free.

2. **IV solution stabilizer for blinatumomab (NSC 773150)** is **not for reconstitution of blinatumomab.** The solution is available as a 10 mL single-use vial, preservative-free, clear, colorless-to-slightly yellow liquid solution. Each solution consists of 25 mM citric acid monohydrate, 1.25 M L-lysine hydrochloride, and 0.1% (w/v) polysorbate 80, pH 7. The stopper of the vial is latex free.

Preparation: NOTE: Only trained staff may prepare blinatumomab IV solution. Sites' standard procedure for compounding blinatumomab must be in compliance with the USP <797> guidelines (ISO Class 5 or better). Use aseptic technique and prepare blinatumomab IV solution under a qualified laminar flow hood.

a. You need an **empty IV bag** that is made of Polyolefin/polyethylene, ethylene vinyl acetate (EVA) or PVC non-DEHP. The **IV Infusion sets** must be a PVC Non-DEHP with 0.2 µm inline filter
b. Next, reconstitute blinatumomab lyophilized powder:
c. Add 3 mL of Sterile Water for Injection (SWI) to the vial to yield 3.08 mL of blinatumomab at **a final concentration of 12.5 mcg/mL.**
d. Rotate the vial to dissolve all powder. Do not shake.
e. The stability of the reconstituted vial is 4 hours at room temperature (22^0 C to 27^0 C) or 24 hours refrigerated at 2^0 to 8^0 C.
f. Then, further dilute blinatumomab prior to administration: Steps to prepare solution are in sequential order. The IV solution stabilizer for blinatumomab **must be added into the 0.9% NaCl bag before adding blinatumomab.**

Calculations:

24-hour IV bag (Inpatient)	Final Volume	Volume to be infused	Infusion rate
	270 mL	**240 mL**	**10 mL/hour**

Step 1. Add the calculated 0.9% NaCl volume into the approved empty IV bag

Calculated 0.9% NaCl (mL):
[Total volume to be prepared (270 mL)] - [Stabilizer solution volume (5.4 mL)] – [blinatumomab calculated dose volume (mL)]

Step 2. Add 5.4 mL IV solution stabilizer into the normal saline IV bag

IV Stabilizer solution (5.4 mL):
0.02 X total volume to be prepared (270 mL)

Step 3. Add the calculated blinatumomab dose volume into solution

Blinatumomab calculated dose volume (per 270 mL bag):
24-hour dose (mcg) ÷ **Volume to be infused (240 mL)** x total volume to be prepared (270 mL) ÷ 12.5 mcg/mL of blinatumomab

NOTE: the total volume to be made is 270 mL of which patient will receive a total volume of 240 mL over 24 hours at 10 mL/hour rate and 30 mL will remain in the IV line set. You may also prepare a 24-hour IV bag for outpatient use but the overfill volume for the IV line infusion may be different and must be changed accordingly in the calculation.

48-hour IV bag (outpatient)	Final Volume	Volume to be infused	Infusion rate
	250 mL	**240 mL**	**5 mL/hour**

Step 1. Add the calculated volume of 0.9% NaCl into approved empty IV bag:

[Total volume to be prepared (250 mL)] – [IV Stabilizer Solution volume (5 mL)] – [blinatumomab calculated dose volume (mL)]

Step 2: Add 5 mL IV stabilizer solution into the normal saline IV bag:

0.02 X total volume to be prepared (250 mL)

Step 3: Add the calculated dose volume of blinatumomab into solution:

48-hour dose (mcg) ÷ volume to be infused (240 mL) x total volume to be prepared (250 mL) ÷ 12.5 mcg/mL of blinatumomab.
NOTE: The total volume to be made is 250 mL of which patient will receive 240 mL over 48 hours at 5 mL/hour rate and 10 mL will remain in the IV line set.

Volume for the dead space of the IV line may be adjusted according to the size of the infusion IV line being used at each institution

a) Rotate IV bag to mix thoroughly the solution. Do not shake. Avoid foaming the IV bag.
b) Visually inspect for floating particles or discoloration of the IV solution. If that occurs, do not use the prepared solution.
c) **Prime the IV line with the prepared IV solution before administering it to patient.**

Infusion Pump:
- Use programmable pump that is approved by the appropriate regulatory authority for the country in which the subject is undergoing treatment.
- Pump alarm must be visual and auditory
- Pump must be lockable
- **Elastomeric pumps are NOT allowed**
- CADD Infusion pumps are allowed

IV bag label: Suggestion for the IV bag label
- Patient name and number
- Name of the drug
- Dose (mcg/day and volume/day)
- Infusion rate
- Expiration date and time

- CAUTION: NEW DRUG – Limited by United States law to investigational use.
- Bag number
- [Additional information may be provided on the label in accordance with state, local, and country pharmacy regulations.]

Storage: Store intact vials of blinatumomab and IV solution stabilizer of blinatumomab refrigerated at 2^0 – 8^0C (36^0- 46^0F), protect from light.

Stability: Shelf life stability studies of the intact vials of blinatumomab and stabilizer solution are on-going.
The stability of the prepared IV solution is 8 days when stored refrigerated at 2^0 to 8^0 C. The total storage and administration time must not exceed 8 days. Once at room temperature, discard the IV bag after 96 hours.

Route*(s)* of Administration: IV infusion

Method of Administration: Use a central line to administer the IV solution**. Do not flush the IV line** as it will create an IV bolus to be administered into the patient.

NOTE: Infusion interruption: Record all interruption. Technical or logistical interruption must be as minimal as possible and re-start the infusion as soon as possible. If an interruption is longer than four hours, the re-start of the infusion must take place in the hospital under supervision of the investigator. The subject should be observed overnight for possible side effects after the re-start, either in the hospital or in the outpatient setting as applicable. Monitor patients for potential adverse events as described in the protocol and the Investigator Brochure.

Patient Care Implications: The effect of blinatumomab on fertility has not been evaluated. Blinatumomab is not recommended in pregnant women and in women of childbearing potential not using contraception. It is not known whether blinatumomab or its metabolites are excreted in human milk. Women are not allowed to breastfeed while receiving blinatumomab. Monitor patients for cytokine release syndrome, tumor lysis syndrome, and infusion reaction. Refer to protocol for specific recommendation. Monitor patients for psychiatric events such as confusion, disorientation, and cognitive attention disturbances. Patients should not drive or operate dangerous machinery while receiving blinatumomab.

8.1.2 **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, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and 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. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 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 should 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 (up to 25°C, 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 over 30 minutes. 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.

Patient Care Implications: Women of childbearing potential (WOCBP) receiving nivolumab must continue contraception for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP must continue contraception for a period of 7 months after the last dose of nivolumab.

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).

8.1.3 **Ipilimumab** (NSC 732442)

Chemical Name or Amino Acid Sequence: 4 polypeptide chains, 2 identical heavy chains with 447 amino acids and 2 identical light chains consisting of 215 amino acids.

Other Names: Anti-CTLA-4 monoclonal antibody, MDX-010, Yervoy™

Classification: Human monoclonal antibody

M.W.: 147,991 Daltons

Mode of Action: Ipilimumab is specific for the CTLA4 antigen expressed on a subset of activated T-cells. CTLA4 interaction with the B7 molecule, one of its ligands expressed on professional antigen presenting cells, can down-regulate T-cell response. Ipilimumab is, thought to act by blocking the interaction of CTLA4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA4/B7 creates the interaction.

Description: Ipilimumab is a fully human immunoglobulin ($IgG_1\kappa$) with two manufacturing processes – ongoing trials have been using substances manufactured using Process B. New clinical trials will be using ipilimumab that is manufactured by Process C. The Process C has been developed using a higher producing sub-clone of the current Master Cell Bank, and modified cell culture and purification steps.

How Supplied: Bristol-Myers-Squibb (BMS) supplies Ipilimumab to the DCTD/NCI. Ipilimumab injection, 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, nonpyrogenic, single-use, isotonic aqueous solution that may contain particles.

Each vial is a Type I flint glass vial with gray butyl stoppers and sealed with aluminum seals.

	Process C
Component	**200 mg/ vial**[a]
Ipilimumab	213 mg
Sodium Chloride, USP	249 mg
TRIS-hydrochloride	134.3 mg
Diethylenetriamine pentacetic acid	1.67 mg
Mannitol, USP	426 mg
Polysorbate 80 (plant-derived)	4.69 mg
Sodium Hydroxide	QS to pH 7
Hydrochloric acid	QS to pH 7
Water for Injection	QS: 42.6 mL
Nitrogen[b]	Processing agent

[a]Includes 2.6 mL overfill.

[b]Nitrogen is used to transfer the bulk solution through the pre-filled and sterilizing filters into the aseptic area.

Preparation: Ipilimumab is given undiluted or further diluted in 0.9% NaCl Injection. Ipilimumab is stable in a polyvinyl chloride (PVC), non-PVC/non DEHP (di-(2-ethylhexyl) phthalate) IV bag or glass container up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

Storage: Store intact vials refrigerated at (2^0 to 8^0 C), protected from light. Do not freeze.

Stability: Shelf-life surveillance of the intact vials is ongoing. Solution as described above is stable up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

CAUTION: Ipilimumab does not contain antibacterial preservatives. Use prepared IV solution immediately. Discard partially used vials.

Route*(s)* of Administration: Intravenous infusion. Do not administer ipilimumab as an IV push or bolus injection.

Method of Administration: Can use a volumetric pump to infuse ipilimumab at the protocol-specific dose(s) and rate(s) via a PVC IV infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (0.2 micron to 1.2 micron).

Patient Care Implications: Monitor patients for immune-related adverse events, e.g., rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis and hypothyroidism. If you suspect toxicity, refer to the protocol guidelines for ruling out other causes.

Availability

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

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

8.1.4 Agent Ordering and Agent Accountability

8.1.4.1 NCI-supplied agents may be requested by the responsible 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.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Active CTEP-registered investigators and investigator-designated shipping designees

and ordering designees can 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 and a "current" password. 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.

8.1.4.2 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 PMB 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.

8.1.4.3 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: http://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)

8.2 Blinatumomab Administration-Inpatient and Outpatient Procedures

Inpatient

Blinatumomab will be administered as a continuous IV infusion over four weeks followed by a two-week treatment free interval. It is recommended that patients are hospitalized at least during the first 14 days of the first cycle and the first two days of each of the subsequent cycle. The hospitalization time depends on the investigator´s judgment, as well as the safety and tolerability of blinatumomab.

The infusion bags will be changed by site nursing personnel. Close monitoring during the first 48 - 72 hours of treatment in the first cycle will be indicated because of the potential AEs associated with T cell redistribution and potential cytokine release effects triggered by the administration of blinatumomab. Nurses/physicians trained in critical care medicine should be available for immediate intervention in case of complications. Afterwards the treatment may be continued on an outpatient basis, if it is judged to be safe and feasible by the investigator.

Outpatient

In the outpatient setting, several options are available for blinatumomab administration:

- The patient will return to the study site for all changes of infusion bags.
- If allowable by the treating physician's institution, infusion bag changes may be performed in the patient's home by an ambulatory/home care service provider. In this case, the ambulatory/home care service provider will be given the remaining bags for the week of treatment that have been prepared by the site investigational pharmacy in a validated shipper for transport. The validated shipper will maintain proper storage conditions during transport for the prepared infusion bags. The infusion bags are to kept/stored at the ambulatory/home care service provider in a controlled temperature monitored refrigerator used at the ambulatory/home care service provider. Patients will be visited by an ambulatory/home care service provider at specific intervals to change the infusion bag. The ambulatory/home care service will be trained and will receive written instruction for transport of drug from the site investigational pharmacy and from the ambulatory/home care service provider to the patient's home for infusion. If necessary shipment of the infusion bags from the site investigational pharmacy to the ambulatory/home care service provider for subsequent transport to the patient's home for infusion will be allowed via use of a validated shipper and a traceable method of shipment via an express courier.
- If all options above are not feasible, shipping the prepared infusion bag in a pre-qualified insulated shipper directly to patient's home via overnight courier delivery service for administration by home healthcare agency staff is acceptable. **Patients are not to open the box upon the receipt of the drug.**

  NOTE: The NCI Drug Accountability Record Form (DARF) must reflect all transaction for preparing and providing the infusion bags to an ambulatory/home care service provider. The ambulatory/home care service provider must also keep DARFs to log in/out date when receiving the infusion bags from the sites and when dispensing the infusion bags to patients as well as the times of all infusion bag changes.

NOTE: The pre-qualified shipper box will include a form of shipment of blinatumomab IV bag(s) to patient's home. That form will be completed by site/pharmacy prior to shipping the IV blinatumomab bags. The form is available as Appendix H. The home healthcare service will open the box at the time that the IV drug is to be administered to patient. The home healthcare service staff will complete the second portion of the form and any discrepancy must be reported immediately to the site pharmacy.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Overview

Mandatory research bone marrow aspirates (40 cc-4 green top tubes-sodium heparin) and research peripheral blood specimens (preferred 100 cc [minimum 60 cc]-10 (min 6) green top tubes-sodium heparin) will be obtained (preferentially on the same day): at baseline (1-7 days prior to the initiation of study treatment; up to 14 days prior to the initiation of study treatment allowed for patients requiring prephase treatment), prior to first dose of nivolumab / ipilimumab in cycle 1 (day 8-11 +/- 4 days), at the end of cycles 1 and 2 (day 29-35), and every 3-4 months up to 1 yr, or at the time of relapse or off study.

Additional research peripheral blood specimens (preferred 40 cc [minimum 30 cc]– 3 or 4 green top tubes) will be collected in cycle 1 (days 1, 15, and 22; all ±3 days), and prior to cycle 2 (day 40-42 of cycle 1).

9.1.1 Collection of specimens

All operating procedures for specimen collection, handling and storage are standardized. Common standardized operating procedures (SOPs) are described in Appendix E. The plan is to collect research PB (60-100 cc or 30-40 cc) and BM specimens (40 cc) from the patient at the time points indicated in Section 9.1 above and on the Study Calendar. Re-adjustment of the direction of bone marrow aspirate needle should take a place after each 5 cc is collected to prevent hemodilution. At the time of sample collection, tubes must be thoroughly mixed to prevent clotting. In the event that the baseline BM aspirate and biopsy was not successful or inevaluable, and subject cannot undergo a second BM aspirate and biopsy for any reason, including refusal, the subject will be allowed to be treated on study protocol as long as diagnosis can be confirmed by other means, including analysis of PB.

9.1.2 Handling and Shipping of Specimens

A unique subject identifier will be assigned to each subject by the coordinating center and will be used to label the samples. The protocol scientific investigator(s) analyzing the samples will be blinded as to the direct subject identifiers.

All specimens should be labeled with the patient's identification number (given at the time of registration), and study number. Sample collection date and time, and sample source (PB or BM) will be recorded on correlative studies collection worksheet (Appendix F) and copy included in delivery. All data will be kept in laboratory log.

Research blood and marrow aspirates will be sent to Johns Hopkins to the laboratory of Dr. Leo Luznik. Dr. Luznik laboratory: 1650 Orleans Street, CRB1RM 2M88, Baltimore MD 21287. Tel: (410) 955-8567. Dr. Luznik's e-mail: luznile@jhmi.edu

At each sampling interval, BM and PB mononuclear cells (PBMC) will be processed via Ficoll density gradient centrifugation. The washed cells will be counted and viably cryopreserved using

a controlled-rate freezer with transfer to the vapor phase of liquid nitrogen for long-term storage. Please refer to the JHU Protocol for PBMC/Plasma (Appendix E).

9.1.2.1 Specimen collection/processing (JHU): The specimens collected at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins should be delivered to the Luznik laboratory immediately after collection.

9.1.2.2 Specimen collection / processing (Participating Sites). Other participating sites should collect PB and BM specimens as outlined in the protocol above. Participating sites will have two options:

a) To process specimens following the SOP in Appendix E. Samples will be stored at each study site and will be batch shipped on dry ice based on arrangement with the participating site PI and Dr. Luznik and upon request of the Study Chair they will be batch shipped to SKCCC. Samples will be shipped or delivered to Dr. Luznik laboratory: 1650 Orleans Street, CRB1RM 2M88, Baltimore MD 21287. Tel: (410) 955-8567.

b) If a participating site does not have the capability of processing the specimens as detailed in Appendix E, then specimens can be shipped overnight to Dr. Luznik's laboratory using cold shipping packages (boxes) provided by Fedex. Please notify the PI-Dr. Gojo, coordinating center research nurse and Dr. Luznik's laboratory of specimen shipment.

9.2 Methods

The overarching goals of exploratory correlative studies are to: a) characterize the effect of blinatumomab in combination with checkpoint inhibition on T cells particularly on their re-distribution, in vivo expansion, activation, and effector differentiation in both PB and BM compartment over time and in relation to the response to therapy; b) identify potential predictive biomarker candidates to guide improved selection of ALL patients who are more likely to benefit from addition of checkpoint inhibitors to blinatumomab therapy in the future; c) characterize the mechanisms of relapse / refractoriness to blinatumomab combined with checkpoint inhibition such as T-cell dysfunction or to identify alternative resistance mechanisms that could be employed by ALL cells to evade blinatumomab/checkpoint inhibition. These studies should provide critical information on the selection of best immune-therapeutic combinations for futures studies in ALL. The proposed exploratory correlative studies are as follows:

9.2.1 Flow cytometry studies

9.2.1.1 **Exploratory/Ancillary Correlative Studies #1: T-cell subsets distribution ($CD4^+$, $CD8^+$, T_{regs}, T_{effs}), their differentiation status and expression of co-signaling molecules and canonic transcription factors, NK cells, and B cells before and post-blinatumomab, and immune checkpoint inhibitor(s) therapy in both peripheral blood and the bone marrow microenvironment (Serial PB and BM samples collected at all time points as indicated in Section 9.1).** We will perform multi-parameter flow cytometry on PB and BM specimens obtained at designated

time points indicated in the Section 9.1 and on the Study Calendar to examine the effect of blinatumomab and checkpoint inhibition on T, NK and B cell dynamics in two different compartments in ALL patients. The analysis will be performed using three pre-determined panels of mAbs including but not limited to those specific for CD3, CD4, CD8, PD-1, CD45RA, CCR7, CD25, CD27, CD28, CD57, CD69, Ki-67, T-bet, CD95, CD127, FoxP3, HLA-DR, CTLA-4, TNFRII, TIM-3, LAG-3, CD160, 2B4, BTLA, KLRG-1, Eomes, Blimp-1, CD16, and CD56. This multi-parametric flow cytometry approach allows examination of different $CD4^+$ and $CD8^+$ T-cell subpopulations (expressing CD45RA, CCR7, CD27, CD28 and CD57), their expression of co-signaling molecules, proliferation (% Ki-67 positive cells) and activation status (CD69, HLA-DR and TNFRII) as well as phenotypic separation of human $CD4^+FoxP3^+$ T cells into three distinct subpopulations. Natural killer cells (NK), NK-T cells will be enumerated using CD16 and CD56 (NK/NK-T cells). Additionally kinetics and durability of B cell depletion (CD19) will be assessed. Dr. Luznik (co-investigator) and his lab have extensive experience in characterizing different T cell subpopulations in leukemia patients at diagnosis, during and after chemotherapy, after allogeneic HSCT, and in follow up as published.[74] Our studies in AML clearly show differences in T cell distribution, activation status, and expression of different co-signaling molecules between the PB and BM and depending the disease status and response to therapy.[74]

NOTE: See BIOASSAY TEMPLATE IN Appendix C.

9.2.1.2 **Exploratory/ Ancillary Correlative Laboratory Studies #2: Characterize the expression of co-signaling molecules on BM or PB ALL blasts prior to, during and after blinatumomab and checkpoint inhibition (Pre-treatment, week 2 Cycle 1 if feasible, at response assessment following Cycle 1 and/or 2, BM)**. Flow cytometric studies will be performed on leukemia blasts (CD19/CD10/CD34 plus other leukemia specific marker) to examine for expression of co-stimulatory and co-inhibitory molecules such as PD-L1, B7-H3, VISTA, Gal-9, CD86, PVR, PVR2, OX40L, ICOSL at baseline, within 10 days of blinatumomab treatment (prior to first administration of checkpoint inhibitors), in non-responders following combination treatment after cycle 1 and/or 2, and at the time of relapse. Collected specimens will also serve to examine for other mechanisms of relapse or resistance such as CD19 loss.

9.2.2 Cytokine studies

9.2.2.1 **Exploratory/ Ancillary Correlative Laboratory Studies #3: Cytokine measurement**. As a part of PB and BM collection and mononuclear cell isolation we will collect plasma that will be used for the measurement of defined cytokines (IL-2, IL-6, IL-10, IFN-γ, TNF-α) to assess blinatumomab and checkpoint inhibitors-induced global T-cell activation. We will examine the levels of cytokines before and after treatment with blinatumomab and checkpoint inhibitors, including the levels of sCTLA-4 using a standard ELISA tests (Bio-Plex Pro Human Cytokine 8-Plex Immunoassay, Bio-Rad Laboratories, Inc; Human sCD152/CTLA-4 Platinum ELISA.

eBioscience).

9.2.3 Molecular studies

9.2.3.1 **Exploratory/ Ancillary Correlative Laboratory Studies #4: Immune Profiling of T cell Repertoire (pre-treatment, response assessment after Cycle 1 or 2 and at relapse, PB and BM).** The studies proposed will evaluate the TCR diversity in T cells isolated from PB and BM from patients before and after treatment with blinatumomab and checkpoint inhibitors. We will determine TCR diversity and clonal composition using a molecular and computational approach based on high-throughput DNA sequencing of rearranged TCRβ CDR3 regions from T cell genomic DNA from Adaptive Biotechnologies. This approach allows direct measurement of the TCRβ CDR3 region sequence diversity in any arbitrarily complex population of T cells, and also permits quantitative description of the clonal composition of the population. The Luznik laboratory in collaboration with the E. Warren laboratory from Fred Hutchinson Cancer Research Center (FHCRC) have performed extensive monitoring and tracking of the TCR repertoires in patients undergoing alloHSCT.[99] We are utilizing an established multiplex PCR strategy to amplify the CDR3 region of the TCR, spanning the variable region formed by the junction of the V, D and J segments and their associated non-template insertions followed by Illumina-based sequencing methodology, a well-characterized methodology developed by Adaptive Technology (http://www.adaptivebiotech.com/technology/). Sequencing is followed by comprehensive bioinformatics analyses focused on determining the diversity of the T cell and B cell repertoires as well as the entropy and clonality of each repertoire consistent with previous studies. Through these ongoing studies we have developed substantial experience not only in using and analyzing DNA retrieved from unsorted PBMCs but also from sorted T cells subpopulations (naïve vs memory vs regulatory), paired PB and BM samples and as well from DNA retrieved from FFPE archived tissues.

9.2.3.2 **Exploratory/ Ancillary Correlative Laboratory Studies #5: Characterization of T cell transcriptional signature before and after treatment (pre-treatment, response assessment after Cycle 1 or 2 and at relapse, PB and BM).** For the analysis of T-cell gene expression profiles we will use the Human Prime View Gene Expression Array (http://www.affymetrix.com). Differentially-expressed genes and corresponding fold-changes will be analyzed using standard statistics and through the use of Ingenuity pathway analysis as described previously. We predict based on our previous work that several key co-stimulatory signaling pathways will be downregulated in CD8+ T cells retrieved from patients with leukemia in comparison to healthy controls and that these pathways will differ after blinatumomab, nivolumab and ipilimumab administration. We will also examine whether there is differential-expression of genes between CD8+ T cells in BM relative to those in circulation. These studies are likely to provide important insights into the T cell transcriptome in patients with ALL in particular as it relate to the immunomodulatory strategies to modulate their function. While analysis will focus on CD8+ T cells, as part of T cell isolation we are routinely sorting CD8+, CD4+, B cells, NK cells and tumor cells;

these other populations will be stored for future analysis. Given that recent studies suggest that mutant neo-epitopes provide potent targets of checkpoint inhibitors, stored tumor specimens may be used in the future for neo-antigen discovery studies.

The workflow of specimens for TCR sequencing (Adaptive Biotechnologies) and gene expression analysis have been optimized in Luznik Laboratory. Briefly, CD8+ T cells from serial samples (at diagnosis and at the response assessment) will be FACS-purified from PB and or BM and DNA/RNA will be isolated and used for TCR deep sequencing and gene expression studies, respectively. To determine TCR diversity of T cells we are utilizing an established multiplex PCR strategy followed by Illumina-based sequencing methodology, a well-characterized methodology developed by Adaptive Technology (http://www.adaptivebiotech.com/technology). For gene expression studies, cDNA will be synthesized and amplified from total RNA will be hybridized to the Human Prime View Gene Expression Array (http://www.affymetrix.com) at JHU Deep Sequencing and Microarray Core.

10. STUDY CALENDAR

Notations/reminders for all calendars:

- Baselines assessments are to be conducted within 7 days prior to start of protocol therapy unless otherwise noted. If the patient will receive pre-phase treatment, then a BM aspirate and biopsy within 14 days of starting treatment on this study is allowed.
- On study physical exams to include medical history since last assessment, as appropriate.
- Pregnancy test (serum or urine) is required for women of childbearing potential ≤3 days prior to study enrollment and must be negative to initiate treatment.
- Correlative blood and bone marrow sample participation is mandatory.
- Additional tests may be performed at the discretion of the treating investigator or site as per routine practice, or as otherwise clinically indicated.
- The schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.) with the guidance of the Protocol Chair/designee, as appropriate, and will not be reportable as a deviation unless the endpoints of the study are affected.

Table 10.1	**Screening**	**Cycle 1**			**Cycles 2-5**			**Beyond Cycle 5**		
Treatment Cycle:	Main Study Screening	Week1	Weeks 2-4	Weeks 5-6	Week 1	Weeks 2-4	Weeks 5-6	Every 2 weeks for up to 1 year	Every 3 months for 2 years after completing study treatment	Off Study
Blinatumomab (D 1-28 every 42 days)		X	X		X (D1-D28)	X (D1-D28 only)				
Nivolumab (every 2 weeks starting on D11 of Cycle 1)[1]			X	X		X	X	X		
Ipilimumab (every 6 weeks starting on D11 of Cycle 1)[1]			X			X		X (Every 6 weeks)		
Informed Consent	X									
Inclusion/Exclusion Criteria	X									
Demographics and Medical History	X									
Concurrent Meds	X	X----------X								
History/Physical Exam[2]	X	X	X	X	X	X	X	X	X	X
Vital Signs including oxygen saturation[2]	X	X	X	X	X	X	X	X	X	
Height/Weight[2]	X	X			X			X (every 6 weeks)		
Performance Status[2]	X	X			X			X (every 6 weeks)		
CBC with	X	X	X	X	X	X		X	X	X

Differential[3]										
PT, aPTT, fibrinogen, D-dimer[4]	X	X	X		X					
Serum chemistry[5]	X	X	X	X	X	X	X	X	X	X
Amylase, lipase [6]	X		X	X		X	X	X		
TSH (with reflective Free T3 and FreeT4)[6]	X		X	X		X	X	X		
Hepatitis Serologies[7]	X									
Quantitative Immunoglobulin[8]	X				X[8] (cy 2 only)		X[8] (end of cy 5)	X[8] (every 3-6 months	X[8] (every 3-6 months)	
Urinalysis	X									
Serum or urine HCG for WOCBP only[9]	X				X			X (every 6 weeks)		
Writing Test[10]	X	X	X		X	X[10]				
EKG[11] and ECHOcardiogram	X									
BM Aspirate and Biopsy[12]	X		X (within 3 days prior to 1st nivolumab)	X			X[12] (end Cy 2; every 3-4 mos up to 1 year)	X[12] (every 3-4 months up to 1 year)		X[12] (off treatment or relapse)
MRD Monitoring [12]	X		X	X			X[12]	X[12]		X
LP and CSF analysis with Intrathecal Chemotherapy[13]	X			X			X[13] (cy 2 only required)	As per investigator's discretion	As per investigator's discretion	

Correlative Studies from BM (Section 9.1 and Appendix E for instructions)[14]	X		X (within 3 days prior to nivo)	X			X[14]	X[14]		X[14]
Correlative Studies from PB (Section 9.1 and Appendix E for instructions)[15]	X	X	X	X	X	X	X	X (every 3-4 months up to 1 year)		X[15] (off treatment or relapse)
Symptom/Toxicity Assessment[16]	X	X----------See specific requirements for monitoring after last dose of nivolumab and/or ipilimumab, and after allo-HSCT[16]----------X[16]								
Follow-up/Survival[17]									X[17]	X[17]

[1] Ipilimumab will be administered every 6 weeks and only to subjects treated on DL B1 (B-1) and B-2. Both Ipilimumab and Nivolumab can be given ± 2 days from the scheduled time and delayed in Cycle 1 up to day 25 as defined in Section 5.3.2 and 5.3.3.

[2] Interim history/physical exam and vital signs (heart rate, blood pressure, temperature, respiratory rate and 02 saturation) will be performed prior to each cycle, weekly during cycle 1 and 2, and every 2 weeks in cycle ≥3. During cycle 1 patient are expected to be hospitalized for 14 days and in subsequent cycles for the first 2 days and will have daily history/physical exam/vital signs (including S02) as per standard of care (SOC). Vital signs (including S02) will be monitored prior to start of each nivolumab and ipilimumab infusion and at the end of infusion or more frequently as indicated. Height will be recorded only prior to start of treatment on the study. Weight and performance status will be measured/assessed prior to each cycle (every 6 weeks).

[3] CBC and differential will be performed on day 1 of each cycle, daily for the first 4 days of cycle 1, weekly during cycle 1 and 2, and every 2 weeks thereafter for the duration of therapy. CBC and diff should be performed within three days of nivolumab and ipilimumab administration. The frequency of CBC/diff measurements may be higher and adjusted for individual patient's need as per the SOC requirements for monitoring and treatment of patients with ALL.

[4] PT/PTT, fibrinogen and d-dimer will be measured prior to each cycle of blinatumomab. In cycle 1, due to higher risk for DIC because of active disease, PT/PTT, fibrinogen and d-dimer should be checked at least twice a week in the first two weeks. The frequency of measurement may be higher and adjusted for individual patient's need as per the SOC requirements for monitoring and treatment of patients with ALL.

[5] Serum chemistry: albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, uric acid. Serum chemistry will be performed on day 1 of each cycle, daily for the first 4 days of cycle 1, weekly during cycle 1 and 2, and every 2 weeks thereafter for the duration of therapy. Serum chemistry should be performed within three days of nivolumab and ipilimumab administration. The frequency of serum chemistry measurements may be higher and adjusted for individual patient's need as per the SOC requirements for monitoring and treatment of patients with ALL.

[6] Amylase, lipase, and TSH should be checked within three days prior to each nivolumab administration. If TSH is abnormal, Free T3 (triiodothyronine) and T4 (thyroxine) should be assessed. Screening time point must be done within 28 days of registration.

[7] Hepatitis serologies will be performed as baseline and include: Hepatitis B surface antigen (HBV sAg), Hepatitis B core antibody (anti-HBc), and Hepatitis surface antibody (HBsAb). Hepatitis C antibody (HCV Ab) and/or Hepatitis C RNA (HCV RNA). Screening time point must be done within 28 days of registration.

[8] Quantitative immunoglobulin should be measured at baseline, at the end of cycle 1, at the end of the last blinatumomab cycle and every 3-6 months thereafter until off study. Screening time point must be done within 28 days of registration.

[9] For WOCBP (women of childbearing potential) only: A serum or urine pregnancy testing is required prior to study enrollment and prior to each cycle.

[10] Writing test (Appendix G) should be performed at baseline, prior to each cycle, and then weekly during blinatumomab administration (week1-4) in cycle 1 and 2 and every 2 weeks during blinatumomab administration (week 1-4) in cycle ≥3 of blinatumomab.

[11] All patients should have baseline ECG. Baseline ECHOcardiogram is required for patients with a history of CHF, or at risk because of underlying cardiovascular disease (e.g history of angina, heart attack, or prior cardiovascular interventions or findings), history of cardiac arrhythmias, or exposure to cardiotoxic drugs (e.g anthracyclines, cyclophosphamide, or others) as clinically indicated. Any patient with heart arrhythmia or ischemic changes on baseline ECG should have baseline ECHOcardiogram. In addition, any patient who develop symptoms or signs of CHF, MI, cardiomyopathy, or myositis in the course of the study, should have repeat EKG, ECHOcardiogram, as well as cardiac evaluation including lab tests, CPK, troponin, and cardiology consultations as clinically indicated.

[12] Bone marrow aspirate and biopsy to assess disease status and response to therapy will be performed at baseline (after pre-registration and prior to registration), at the end of cycle 1 and 2 (day 29-day 35), and every 3-4 months thereafter up to 1 yr, any time relapse is suspected or off treatment. Additional bone marrow aspirates for research purposes only will be performed in cycle 1 within 3 days of the first nivolumab/ipilimumab administration (between days 8-11 of cycle 1 but can be delayed if nivolumab and/or ipilimumab administration is delayed in Cycle 1 as per Section 5.3.2 and 5.3.3) prior to nivolumab administration. Each time bone marrow assessment is performed to assess disease status, the flow cytometry studies for MRD assessment will be performed.

[13] All patients should have baseline CSF assessment prior to enrollment on the study. Administration of intrathecal chemotherapy is required when LP is performed with a choice of agent (ara-C or MTX or both) and dose as per institutional standard of practice. Subsequent IT chemotherapy administration for CNS prophylaxis should be performed at the end of blinatumomab administration in cycles 1 and 2 (day 29-35) and the determination of need for subsequent CSF prophylaxis will be per investigator's discretion.

[14] Each time bone marrow is performed for disease status assessment additional 40 cc of the bone marrow should be collected for research studies. Additional bone marrow aspirate for research purpose only will be performed in cycle 1 (between days 8-11) prior to nivolumab administration.

[15] Peripheral blood 100 cc (min 60 cc) for research studies will be collected at baseline (after pre-registration and prior to registration), prior to first dose of nivolumab / ipilimumab in cycle 1 (-3 days), at the end of cycle 1 and 2 (day 29-35), and every 3-4 months up to 1 yr, or at the time of relapse or off treatment. Additional research peripheral blood 40 cc (min 30 cc) will be collected in cycle 1 (day 1, 15, 22; all ±3 days), on day 1 (-3 days) of cycle 2.

[16] Assessments for toxicity/adverse events are to continue for 100 days after the last dose of nivolumab or ipilumimab, whichever was administered last. These may occur by phone for subjects no longer being seen at the institution; however, an in-person clinic visit may be needed to confirm event resolution and/or to effectively evaluate possible immune-related events. In addition, all patients who go onto allo-HSCT following the completion of treatment on the study will undergo continuous monitoring for acute grade 3-4 graft versus host disease and post-transplant non-relapse mortality for the first 100 post-transplant days. Subjects must be told of follow-up requirements at study entry.

[17] Subjects will be followed about every 3 months for disease status and survival. Information about subsequent treatment received and response will be collected.

11. MEASUREMENT OF EFFECT

Although the clinical benefit of nivolumab and ipilimumab has not yet been established in ALL, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for leukemia response and symptom relief in addition to safety and tolerability. Patients will be assessed by standard criteria. For the purposes of this study, patients will be re-evaluated with a bone marrow biopsy at the conclusion of the first and second cycle of treatment, at the end of the last blinatumomab containing cycle, and every 3-4 months thereafter, and off study. A bone marrow biopsy should also be obtained at anytime there is clinical suspicion for relapse based on changes in symptoms, exam, or laboratory parameters.

11.1 Antitumor Effect – Hematologic Tumors

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with blinatumomab.

Evaluable for objective response. Patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

11.1.2 Disease Parameters

Complete Remission (CR)

1. No circulating blasts or extramedullary disease
    - No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement
2. Trilineage hematopoiesis (TLH) and <5% blasts
3. Absolute neutrophil count (ANC) >1,000/μL
4. Platelets >100,000/μL

CR with incomplete blood count recovery (CRi)

- Meets all criteria for CR except platelets and/or ANC

Refractory Disease

- Failure to achieve CR after 1-2 cycles of treatment

Progressive Disease (PD)

- Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease

Relapsed Disease

- Reappearance of blasts in the blood or bone marrow (>5%) or in any extramedullary site after a CR

Minimal Residual Disease (MRD)

- MRD in ALL refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow.

11.1.3 Methods for Evaluation of MRD

The high relapse rate seen in adult ALL indicates the lack of eradication of MRD despite morphologic remission. Several techniques, including monitoring of fusion gene transcripts; clonal immunoglobulin heavy chain gene rearrangement; or multicolor flow cytometry have shown reliability in estimating the risk of relapse in ALL patients.[100]

Given the equivalence of PCR techniques and flow cytometry in monitoring MRD in ALL patients,[101] a flow cytometric approach will be used for MRD assessment in this study. This approach has been adopted for all patients with ALL in our institution and for patients with NHL involving the bone marrow. Furthermore, the flow cytometry laboratory at Johns Hopkins is a reference laboratory for MRD monitoring in ALL for all COG studies. Those patients with unique gene fusions detectable by PCR will also be monitored for MRD by specific PCR tests following the completion of blinatumomab treatment, and then per the institutional standard-of-care. Collected samples may also be used for the assessment of MRD at a molecular level by high-throughput sequencing of IGH as previously described,[102] or by PCR for patients with a known fusion gene transcript.

11.1.4 Response Criteria

Responses will be assessed at the conclusion of cycles 1 and 2 and in follow-up given the response definitions provided above.

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time criteria are met for CR or CRi (whichever is first recorded) until the first date that relapsed disease is objectively documented.

11.1.6 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12. STUDY OVERSIGHT AND 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.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly, or more frequently, conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and adverse events and unanticipated problems.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via 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 (check at < https://ctepcore.nci.nih.gov/iam >) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA, or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

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/clinicalTechnologies/?National-Cancer-Institute-NCI-11. 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 CTMSSupport@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, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). 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

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 Overall

This is a multi-site, open-label, Phase 1 study of evaluating safety, tolerability, and preliminary antitumor activity of blinatumomab and nivolumab with or without ipilimumab in patients with CD19+ Pre-B ALL and CD19+ MPAL. The study consists of two parts: dose escalation for blinatumomab and nivolumab with and without ipilimumab and dose expansion at MTD. The dose expansion cohort will only be initiated once the MTD for the combination therapy has been established. In each part of the study, a ten-day run-in of blinatumomab prior to combination therapy will be incorporated to help determine the effect of blinatumomab on immune-related parameters of each patient's leukemia.

13.2 Analysis Population

- Safety Analysis Set: All subjects who receive at least one dose of study drug.
- Dose-Limiting Toxicity Evaluation Set: A subject must meet one of the following 3 criteria to be evaluable for dose-escalation decisions: receive all of the scheduled doses of combination therapy and has completed 6 weeks of treatment without a DLT; experience a DLT in the first 6 weeks of combination therapy; or withdrawn from the study prior to completing 6 weeks of combination treatment due to a DLT.
- Efficacy Evaluable Analysis Set: All subjects who receive at least one dose of study drug, have an adequate baseline disease assessment and at least one post-baseline response assessment.

13.3 Study Design/Endpoints

13.3.1 Primary Objectives

13.3.1.1 Adverse events and toxicities will be tabulated and reported by type and grade for all dose levels of all treatment combinations and the proportions reported with exact 95% binomial confidence intervals.

13.3.1.2 The maximum tolerated dose (MTD) of the combination of blinatumomab plus nivolumab, and blinatumomab plus both nivolumab and ipilimumab will be determined in subjects with poor-risk, relapsed or refractory $CD19^+$ Pre-B cell ALL or $CD19^+$ mixed phenotype acute leukemia (MPAL).

13.3.2 Secondary Objectives

13.3.2.1 The frequency distribution of responses, including CR, CRi or progressive disease (PD) by treatment arm and dose level for all patients on study will be determined and reported with exact 95% confidence intervals.

13.3.2.2 For patients who have a clinical response, the frequency of MRD+ vs. MRD- responses will be reported by treatment arm and dose level for all patients on study.

13.3.2.3 For patients who have a clinical response, duration of response (DOR) will be analyzed as a time-to-event outcome with the duration being measured as the time from measured response to progressive disease, death, or study end.

13.3.2.4 Overall survival will be measured from the first day of treatment on the study until death or last known follow up.

13.3.3 **Exploratory Objectives**

13.3.3.1 The absolute lymphocyte count and the distribution of T cell subsets ($CD4^+$, $CD8^+$, T_{regs}, T_{effs}) and their differentiation status, NK cells, and B cells before and post-blinatumomab, and immune checkpoint inhibitor(s) therapy in both peripheral blood and the bone marrow microenvironment will be determined.

13.3.3.2 Changes in T cell co-signaling receptors expression in defined T cell subpopulations and their canonic transcription factor expression in both peripheral blood and bone marrow before and post-blinatumomab, and immune checkpoint inhibitor(s) therapy will be recorded.

13.3.3.3 Changes in expression of co-signaling molecules on leukemia blasts ($CD10^+/CD19^+/CD34^+$) before and after treatment with blinatumomab and checkpoint inhibitors will be examined.

13.3.3.4 Serum levels of cytokines before and after treatment with blinatumomab and checkpoint inhibitors, including the levels of sCTLA-4, will be determined.

13.3.3.5 Immune profiling of T cell repertoire and characterization of T cell transcriptional signature before and after treatment will be determined.

13.4 Study Design

This study proposal will be a phase 1 trial of blinatumomab plus immune checkpoint inhibition in patients with relapsed/refractory ALL or those with a new diagnosis of ALL who cannot receive standard induction. The first part of the study will be a dose escalation following a 3+3 design for the combination of blinatumomab plus nivolumab. The study will start with the recommended dose of blinatumomab in relapsed/refractory ALL. Nivolumab will be started at the recommended doses for monotherapy for metastatic melanoma. This design is being used as an added safety measure and a more controlled start of the study to minimize the risk of unexpected toxicities or other issues that may occur in the combination treatment of blinatumomab with either ipilimumab or nivolumab.

The first three patients will be enrolled at dose level DL A1 and monitored for the first cycle of treatment (42 days) for DLTs before enrolling additional patients. If less than 2 patients

experience a DLT, an additional 3 patients will be enrolled at that dose level. If 0 or 1 out of 6 patients have a DLT, that dose level will be deemed safe. If 2 or 3 of the first 3 patients experience a DLT, that study arm may de-escalate to dose level DL A-1 (only after CTEP approval) and another 3 patients will be enrolled. If less than 2 patients experience a DLT in the de-escalated dose, an additional 3 patients will be enrolled at that dose. If 0 or 1 out of 6 patients have a DLT, the de-escalated dose will be deemed safe. If 2 or 3 patients experience a DLT, that study arm will be stopped for review.

When the safety of blinatumomab in combination with nivolumab is confirmed, the study will expand to treat patients with this combination plus ipilimumab. A standard 3+3 design will be used to assess the safety and tolerability of the combination. Two dose levels (DL B1, DL B-1,) are available. If DL A1 was deemed safe, then three patients will be enrolled at DL B1. If ≤1 of 3 patients experience a DLT during the first cycle of treatment (42 days), at DL B1, an additional 3 patients will be enrolled at DL B1. If no more than 1 of 6 patients experience a DLT, then it will be defined as the MTD. If 2 or more of 3 or 6 patients experience a DLT at DL B1, then dose de-escalation may be implemented BUT ONLY after CTEP approval and the next three patients may be enrolled at DL B-1. If ≤1 of 3 patients experience a DLT during the first cycle of treatment (42 days), an additional three patients will be enrolled at DL B-1. If 2 or more of 3 or 6 patients experience a DLT at DL B-1, then dose escalation will be halted and the combination of blinatumomab, nivolumab and ipilimumab will not be further pursued in this patient population unless additional data become available supporting different doses or schedules of nivolumab and ipilimumab administration. This will require review of all toxicity, discussion with CTEP and official study amendment.

When the MTD of blinatumomab in combination with ipilimumab and nivolumab is determined, an additional 6 patients will be enrolled in an expansion cohort to further characterize safety, tolerability and any preliminary signs of biological or clinical activity of the triplet blinatumomab/nivolumab plus ipilimumab. An expansion cohort of 6 subjects will be also pursued at MTD for doublet of blinatumomab and nivolumab only to further characterize safety, tolerability and any preliminary signs of biological or clinical activity in this cohort.

13.4.1 Dose Expansion

Continuous monitoring of safety will be completed during the enrollment and treatment period of each expansion cohort. If it becomes evident that the proportion of patients experiencing a treatment-related toxicity meeting DLT criteria convincingly exceeds 30%, the study will be halted for a safety consultation. The toxicity stopping rule will hold enrollment if the posterior probability of failure being larger than 0.30 is 70% or higher. The prior for this monitoring rule is beta (1,2). This means that our prior guess at the proportion of failures is 33%, and there is 90% probability that this proportion is between 2.5% and 77.6%. The monitoring rule and operating characteristics are given below.

- Stop if 2 out of 2 patients experience a DLT: Pr(Risk>0.3 | Data) = 0.916
- Stop if 2 out of 3 patients experience a DLT: Pr(Risk>0.3 | Data) = 0.837
- Stop if 2 out of 4 patients experience a DLT: Pr(Risk>0.3 | Data) = 0.744
- Stop if 3 out of 5 patients experience a DLT: Pr(Risk>0.3 | Data) = 0.874
- Stop if 3 out of 6 patients experience a DLT: Pr(Risk>0.3 | Data) = 0.806

Operating Characteristics of stopping rule for toxicity, based on 5000 simulations:

True Toxicity Rate	Probability of declaring treatment too toxic	Average sample size
20%	17%	3.9
25%	26.8%	3.8
30%	34.6%	3.7
35%	43.1%	3.6
40%	52.7%	3.5
45%	61%	3.4
50%	68.8%	3.2

13.4.2 Early Stopping for Safety in the Post-Transplant Setting

Because many patients enrolled in this protocol will be candidates for a subsequent allo-HSCT, and there have been recent reports of increased toxicity among patients who undergo allo-HSCT following treatment with PD-1 inhibitors, all patients who go onto allo-HSCT following the completion of the clinical protocol will undergo continuous monitoring. The clinical failures of acute grade 3-4 graft versus host disease and post-transplant non-relapse mortality will be monitored continuously for the first 100 post-transplant days. If it becomes evident that the proportion of acute grade 3-4 graft versus host disease is greater than 25% or the proportion of non-relapse mortality is greater than 20% then the study would be held for a safety consultation.

Acute Grade 3-4 GVHD

The acute GVHD grade 3-4 stopping rule will hold enrollment if the posterior probability of failure being larger than 0.25 is 70% or higher. The prior for this monitoring rule is beta(1,3). This means that our prior guess at the proportion of failures is 25%, and there is 90% probability that this proportion is between 1.7% and 63.2%. The following table shows the resulting rules.

Safety review triggered if number of patients with grade 3-4 GVHD is:	2	3	4	5	6	7	8	9	10	11
Number of patients is between:	2-4	5-8	9-11	12-15	16-18	19-22	23-26	27-30	31-33	34-36

For example, the rule will call for a safety review if 3 out of the first 5 patients experience acute grade 3-4 GVHD. The table below shows the percent of times the stopping rule will halt the study for a safety review and the average sample size under different hypothetical risks of grade 3-4 acute GVHD.

Operating Characteristics of stopping rule for GVHD, based on 5000 simulations:

True acute or chronic GVHD grade 3-4 rate	Probability of stopping for excess GVHD grade 3-4	Average Sample Size
10%	8.0%	31.7
15%	20.7%	28.4
20%	37.1%	24.6
25%	58.5%	19.4
30%	77.3%	14.9
35%	89.2%	11.3
40%	95.9%	8.4

Non-Relapse Mortality

The non-relapse mortality safety stopping rule will hold enrollment if the posterior probability of failure being larger than 0.20 is 70% or higher. The prior for this monitoring rule is beta(1,4). This means that our prior guess at the proportion of failures is 20%, and there is 90% probability that this proportion is between 0.60% and 60.2%. The following table shows the resulting rules.

Safety review triggered if number of patients with non-relapse mortality is:	2	3	4	5	6	7	8	9
Number of patients is between:	2-5	6-9	10-14	15-18	19-23	24-27	28-32	34-36

For example, the rule will call for a safety review if 4 out of the first 10 patients die without relapse. The table below shows the percent of times the stopping rule will halt the study for a safety review and the average sample size under different hypothetical risks of non-relapse mortality.

Operating Characteristics of stopping rule for non-relapse mortality, based on 5000 simulations:

True Non-Relapse Mortality Rate	Probability of stopping for too much non-relapse mortality	Average Sample Size
10%	13.0%	30.5
15%	31.0%	26.3
20%	53.7%	21.0
25%	74.3%	16.1
30%	89.6%	11.6
35%	96.2%	8.9

13.4.3 Statistical Analysis Plan

The primary objective of this study is to evaluate the safety and tolerability of blinatumomab given in combination with nivolumab, or in combination with both nivolumab and ipilimumab in subjects with poor-risk, relapsed or refractory $CD19^{+}$ Pre-B cell ALL or CD19+ biphenotypic

leukemia. All adverse events and toxicities will be tabulated and reported by type and grade for all dose levels of all treatment combinations and the proportions reported with exact 95% binomial confidence intervals. Any patient who receives at least one dose of any treatment will be included in toxicity reporting, regardless of eligibility or study drop out.

13.5 Sample Size/Accrual Rate

In the dose escalation phase of this study, the sample size is determined by the number of DLTs observed. The minimum sample size for this first part of the study will be 6 patients at DL1 and DL-1 (if necessary), and a maximum sample size of 12 patients. During the dose escalation phase of the combination arm, the minimum number of patients treated will be 3 and maximum will be 6 at each dose level. If the combination is deemed safe and the expansion cohort is enrolled, the sample size will be either 9 or 12 at that dose level. The maximum total sample for the entire study will be 36 patients.

The anticipated complete response (CR) rate in this patient population is approximately 40%. With a minimum sample size of 12 subjects in the combination arm, the probabilities of observing at least 5 responses out of 12 patients (41.7% CR rate) would be 0.42, 0.56, and 0.70 if the true CR rate is 35%, 40%, and 45%, respectively. If the true CR rate is less than 15%, the chance of observing 5 responses out of 12 would be 0.024 or less. This sample size also allows for estimation of the CR rate with precision (half the width of the 95% exact binomial confidence interval) being no wider than 25.5%.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	1	0	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1	2	0	0	**3**
White	7	13	3	3	**26**
More Than One Race	0	0	0	0	**0**
Total	**9**	**15**	**3**	**3**	**30**

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13.6 Stratification Factors

Not Applicable

13.7 Analysis of Secondary Endpoints

The secondary objectives of this study include describing preliminary antileukemia activity of blinatumomab and nivolumab, and blinatumomab plus both nivolumab and ipilimumab, including the effects on MRD, and assessing preliminary antileukemia activity in an expansion cohort of patients with poor-risk, relapsed or refractory $CD19^+$ Pre-B cell ALL. The frequency distribution of responses, including CR, CRi or progressive disease (PD) will be reported by treatment arm and dose level for all patients on study and reported with exact 95% confidence intervals. For patients who have a clinical response, duration of response (DOR) will be analyzed as a time-to-event outcome using the Kaplan Meier method with the duration being measured as the time from measured response to progressive disease, death, or study end. A sensitivity analysis may be performed to evaluate DOR while excluding patients who go on to have an allo-HSCT. Overall survival will be measured using the Kaplan Meier method as the time from the first day of treatment on the study until death or last known follow up.

13.8 Analysis of Exploratory Endpoints

13.8.1 Characterizing T cell populations

The analyses of pre- and post-treatment PB and BM specimens for immune parameters will be descriptive and graphical in nature. Data will be summarized for each patient group separately (blinatumomab plus nivolumab; blinatumomab plus nivolumab and ipilimumab). Flow values at each time point will be summarized using geometric means and standard deviation. Differences in baseline values between patient subgroups will be explored using linear regression models. We are specifically interested in determining what pretreatment signature is associated with outcome, such as having a complete response or becoming refractory. To this end, we will use logistic regression models with ROC analysis to describe which pretreatment parameters are potential predictors of outcomes following treatment. Values will be checked for skewness and log-transformed as appropriate. The change in flow values at each time point after blinatumomab and blinatumomab plus checkpoint inhibitor(s) relative to the baseline value will be modeled using mixed-effects, linear regression models that include fixed effects for time point and a random effect for the patient to account for within-patient correlation of measurements. Post-treatment changes in T cell populations will be correlated with clinical outcomes such as CR vs. No CR or MRD vs. No MRD using interaction tests. T cell subsets in the PB and BM defined by multicolor flow cytometry will be summarized before and after treatment with blinatumomab alone or plus checkpoint inhibitors by treatment group using summary statistics. Differences in T cells before and after treatment will be explored using non-parametric Wilcoxon signed rank tests or paired t tests as appropriate. The expression of co-inhibitory/-stimulatory molecules will also be examined in the same way.

13.8.2 Characterization of T cell transcriptional signature before and after treatment.

Expression levels will be summarized at pre-treatment and post-treatment using standard descriptive statistics. Differentially-expressed genes and corresponding fold-changes will be analyzed using standard statistics and through the use of Ingenuity pathway analysis as described previously. We will identify differentially-expressed genes by patient subgroups such as CR v. NR and MRD v. No MRD using interaction tests.

13.8.3 Leukemia Blasts

The analyses for changes in expression of co-signaling molecules on leukemia blasts ($CD10^+/CD19^+/CD34^+$) before and after treatment with blinatumomab and checkpoint inhibitors will be summarized using descriptive statistics and either Wilcoxon rank sum tests or paired t tests, as appropriate for a single time point, or using the mixed effects linear regression models for simultaneously modelling data from multiple time points as previously described for analyzing the T cell population data. We will explore the changes in blasts in terms of expression of co-signaling molecules over time and summarize any correlation in the pattern of blast changes over time using the regression models and interaction tests described above.

13.8.4 Cytokine Profiling

Changes in cytokine levels for all patients and within patient subgroups will be summarized using descriptive statistics and either Wilcoxon rank sum tests or paired t tests, as appropriate for a single time point, or using the mixed effects linear regression models previously described for simultaneously modelling data from multiple time points for the T cell population data.

13.8.5 Immune Profiling

Immune profiling of T cell repertoire and characterize T cell transcriptional signature before and after treatment will be analyzed using the approach outlined in a prior study.[99] Specifically, two distinct measures, clonality and Gini coefficient, will assess the degree of clonal skewing in the *TRB* repertoires. Clonality is a metric derived from the Shannon entropy of the frequency distribution of *TRB* sequences normalized by the $\log_2$(number of unique *TRB* sequences); it captures the clonal composition of a T cell population and has an adjustment for sequencing depth, thus making it less sensitive to this critical variable.[103] The Gini coefficient is a graphically defined metric of repertoire diversity in the sequence frequency distribution. These measures will be used to define clonality, which will be correlated with patient outcomes, such as CR v No CR and MRD v No MRD.

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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: PATIENT DRUG INFORMATION HANDOUT AND WALLET CARD

Information for Patients, Their Caregivers, and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient _____ is enrolled on a clinical trial using the experimental study drugs, blinatumomab and nivolumab with or without ipilimumab. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

These are the things that you as a healthcare provider need to know:

Blinatumomab, nivolumab, and ipilimumab are not known or expected to interact with certain enzymes in the body, the heart's electrical activity, or with other medications; however, there is the possibility of interactions.

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.

Blinatumomab, nivolumab, and ipilimumab may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. It is helpful to bring your medication bottles or an updated medication list with you.

Many health care providers can write prescriptions. You must tell all of your health care providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

These are the things that you and they need to know:

Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers, as needed, to review any medicines and herbal supplements that you are taking or may start during the study.

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine. Your study doctor's name is _____ and he or she can be contacted at _____.

STUDY DRUG INFORMATION WALLET CARD

You are enrolled on a clinical trial using the experimental study drugs blinatumomab and nivolumab with or without ipilimumab. This clinical trial is sponsored by the NCI. Blinatumomab, nivolumab, and ipilimumab may interact with other drugs. Because of this, it is very important to:

- Tell your doctors if you stop taking any medicines or if you start taking any new medicines.
- Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

Blinatumomab, nivolumab, and ipilimumab must be used very carefully with other medicines.

- Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that you are taking or may start during the study.
- Before prescribing new medicines, your regular health care providers should go to a frequently-updated medical reference for a list of drugs to avoid, or contact your study doctor.
- Your study doctor's name is _____

  and can be contacted at _____.

APPENDIX C: BIOASSAY TEMPLATES

Exploratory/Ancillary Biomarkers for the Assessment of T-cell Subset Distribution, Differentiation Status, and the Expression of Co-signaling molecules and Canonic Transcription Factors in both the peripheral blood and bone marrow microenvironment

1) **Name of Marker:** Surface expression of cluster of differentiation (CD) markers and intracellular transcription factors in peripheral blood and bone marrow T cells

2) **Role of the Biomarker Assay in the Trial: Exploratory Assay.**

- **Assay purpose:**

a) To determine phenotypic correlates of effective response to blinatumomab and checkpoint inhibitors (nivolumab with or without ipilimumab)
b) To assess phenotypic predictors of responsiveness to blinatumomab and checkpoint inhibitors (nivolumab with or without ipilimumab)
c) To determine which T cell populations are the targets of checkpoint inhibitors in combination with blinatumomab

- **Assay type:** Flow cytometry.

3) **Laboratory conducting study for the trial:** Dr. Leo Luznik's research laboratory.

4) **Assay:** Standardized tubes and procedures will be used for specimen collection, processing, cryopreservation and storage (Appendix E). Our workflow has been specifically designed to maximize information yield from smaller samples and is based on well developed and implemented standard operating procedures (SOPs). In our analysis we will utilize standardized pre-determined multi parametric flow-cytometry mAb panels that interrogate dynamic changes across comprehensive immune cell populations. In the development and use of the integrated biomarker assay in the context of clinical trial we have followed guidelines from the Biomarkers Taskforce of the NCI Investigational Drug Steering Committee (*Dancey JE et al. Clin Cancer Res 2010;16:1745-55*). We will also follow all recommendations and protocols for standardizing immunologic readouts such as published by SITC/FDA/NCI Workshop on Immunotherapy Biomarkers (*Butterfield LH et al. Clin Cancer Res 2011;17(10):3064-76*). All multicolor flow-cytometry panels will follow standard principles of panel design and follow recommendation published by the "Human Immune Phenotyping Consortium" on the standardization of flow cytometry immunophenotyping in clinical studies (*Maecker HT et al. Nat Rev Immunol 2012, 12:191–200*).

- ***Analyte(s) and sources of assay components:*** The directly conjugated monoclonal antibodies outlined in the Table 1 below. All antibodies will be obtained from commercial vendors.

Table. 1

Antigen	Fluorochrome	Clone	Antibody type	Cat No.	Company
CD3	AF700	HIT3a	Mouse IgG2a, κ	300324	BioLegend
CD3	APC	OKT3	Mouse IgG2a, κ	17-0037-42	eBioscience
CD3	evolve 605	OKT3	Mouse IgG2a, κ	83-0037-42	eBioscience
CD4	PerCP Cy5.5	OKT4	Mouse IgG2b, κ	45-0048-42	eBioscience
CD4	BV605	OKT4	Mouse IgG2b, κ	317438	Biolegend
CD8	PerCP-Cy5.5	RPA-T8	Mouse IgG_1, κ	45-0088-42	eBioscience
CD8	pacorange	3B5	Mouse IgG2a	MHCD0830	Thermo Fisher (Invitrogen)
CD45RA	APC-Cy7	HI100	Mouse IgG2b, κ	304128	BioLegend
CCR7	BV650	G043H7	Mouse IgG2a, κ	353234	BioLegend
CD25	PE-Cy7	BC96	Mouse IgG_1, κ	302612	BioLegend
CD27	BV605	O323	Mouse IgG1, κ	302830	BioLegend
CD27	AF700	M-T271	Mouse IgG1, κ	560611	BD
CD28	PE-Cy7	CD28.2	Mouse IgG1, κ	25-0289-42	eBioscience
CD127	e-fluor 450	eBioRDR5	Mouse IgG_1, κ	48-1278-42	eBioscience
BTLA (CD272)	AF647	MIH26	Mouse IgG2a, κ	344510	Biolegend
BTLA (CD272)	PE	MIH26	Mouse IgG2a, κ	344506	Biolegend
CD160	AF488	BY55	Mouse IgM	53-1609-42	eBioscience
CD244 (2B4)	FITC	C1.7	Mouse IgG_1, κ	329506	Biolegend
CD57	pacblue	HCD57	Mouse IgM, κ	322316	BioLegend
KLRG1	APC	REA261	Mouse IgG_1, κ	130-103-639	Miltenyi
CTLA-4 (CD152)	AF647	BNI3	Mouse IgG2a, κ	555855	BD
PD1 (CD279)	PE	EH12.1	Mouse IgG_1, κ	560795	BD
TIM3 (CD366)	PE	344823	Rat IgG2A	FAB2365P	R&D Systems
PDL1	Alexa Fluor 700	MIH1	Mouse IgG_1, κ	565188	BD
CD56	PECF594	B159	Mouse IgG_1, κ	562289	eBioscience
Ki-67	PerCP Cy5.5	B56	Mouse IgG_1, κ	561284	BD
CD69	PE	FN50	Mouse IgG1, κ	12-0699-42	eBioscience
T-bet	PE-CF594	04-46	Mouse IgG_1, κ	562467	BD
FoxP3	V450	259D/C7	Mouse IgG_1, κ	560459	BD
HLA-DR	FITC	G46-6	Mouse IgG2a, κ	555811	BD
TNFR2 (CD120b)	PE	3G7A02	Rat IgG2a, κ	358404	Biolegend
LAG-3 (CD223)	PE-Cy7	3DS223H	Mouse IgG1, κ	25-2239-42	eBioscience
Eomes	PE	WD1928	Mouse IgG1, κ	12-4877-42	eBioscience
Blimp-1	PE-CF594	6D3	Rat IgG2a, κ	565274	BD
CD16	PerCP Cy5.5	3G8	Mouse IgG_1, κ	302028	Biolegend
CD95	Pacific Blue (V450)	DX2	Mouse IgG_1, κ	305619	Biolegend
LIVE/Dead fixable Aqua Dead stain kit	405 nm			L34957	Life Technologies

- ***Technical platform:*** The Cancer Center flow cytometry facility is equipped with two Becton Dickinson (BD) FACSAria II cell sorters, a BD LSR Fortessa with capability of simultaneous analysis of up to 18 colors, and a BD LSR II with capability of simultaneous analysis of up to 15 colors are available for use in this project.

- ***Specimens:*** Peripheral blood samples will be obtained via phlebotomy while bone marrow will be collected through standard access to iliac crest. Peripheral blood should be drawn in 4-10 10 ml green-top vacutainer tube (sodium heparin) as per schedule defined in the protocol Section 9.1 and the Study Calendar (Table 10.1). Aspirated bone marrow cells will be collected in a 4-10 ml green-top vacutainer tube (sodium heparin). All collected specimens will be labeled with the patient's study number, sample collection date and time, and sample source (PB or BM). Specimen collection will be recorder on Correlative Studies Collection Worksheet (Appendix F). All data should be kept in laboratory log.

- ***Anticipated methods for specimen processing:*** At each sampling interval, BM and PB mononuclear cells (PBMC) will be processed by Ficoll density gradient centrifugation. Please refer to Appendix E: Protocol for Plasma/PBMC isolation (Ficoll Hypaque). Each sample is aliquoted in multiple vials of 5-20 x 10^6 cells/vial for PBMC and 25-75 x 10^6 for BM samples. This strategy for sample processing enables purification of PBMCs and the corresponding mononuclear cell fraction from BM, and of plasma, for separate experimental uses. Freezing enables sample collection over time, which allows analysis of multiple samples simultaneously in a single experiment (batching) for increased reproducibility and comparability of results. The cell viability upon freeze and subsequent thaw is >85% for the majority of the samples (lower viability can be seen in samples from patients with uncontrolled leukemia). The function and numbers of the lymphocyte populations of interest have been found to be mostly unaffected by freezing and thawing in recent study (small changes in the numbers of CD8+ T cells, naïve T cells and central memory T cells have been described, but are generally assessed as being of minor importance) (*Riedhammer et al. Methods in Molecular Biology 2015: 1304: 53–61*). Thawed cells will be washed in phosphate-buffered saline (PBS) before staining with appropriate concentrations of fluorochrome-conjugated monoclonal antibodies. We are also routinely allowing a short rest period before staining and closely follow the literature on optimal use of the markers on cryoprocessed specimens (*Ogunjimi B et al. J Immunol Methods 2013;392:63-67; Lemieux J et al. J Immunol Methods 2016;434:73-82*). Staining for extracellular antigens is performed directly after wash, while fixation and permeabilization is performed before staining for intracellular antigens, such as transcription factors and cytokines. For intracellular staining we are using commercially available reagents and protocols (BD and eBioscience)

- ***Data acquisition:*** Consistency of data acquisition will be ascertained by calibrating the flow cytometer with calibrating beads daily. For compensation of flow cytometry data we will prepare single stain controls with the appropriate antibodies (mainly using PBMCs, but commercially available compensation beads will be utilized for weakly expressed antigens), and perform compensation of fluorescent spillover with the compensation applications included in the flow cytometry analysis softwares DIVA (Becton Dickinson) and FlowJo (TreeStar). At least 10,000 cell events will be collected including appropriate isotype controls for cell surface markers and intracellular proteins. Viability dyes will be used to facilitate the gating of "live" cell populations.

- ***Data analysis:*** We will utilize standardized analysis and gating strategies for multi parametric flow-cytometry mAb panels (*Herzenberg LA et al. Nat Immunol 2006;7(7):681-5; Lugli E et al. Cytometry A 2010;77(7):705-713*). All samples will be analyzed by setting appropriate side and forward scatter gates to exclude cell clumps and debris, and to include the population of interest: lymphocytes. A "live" gate in the fluorescence channel containing the viability stain will be used to exclude dead cells. Fluorescence minus one (FMO, samples that include all antibodies but the one of interest) and isotype controls will be used to distinguish positive and negative populations for studied antigens. The results will be reported as percent of gated cells positive for each antibody. A BD FACSAria or BD LSR II flow cytometer with DIVA software (BD) will be used for data acquisition. The quantitative, continuously distributed data generated by this procedure will be analyzed using DIVA and/or FlowJo. Additional analysis with softwares for complex statistical and visual multivariable analysis, (available as freeware or at https://www.cytobank.org/platform.html) such as SPICE, bh-SNE and CITRUS will be performed to the best advantage. These novel and powerful analysis tools are especially suited for presentation of high-dimensional data sets, identifying rare biological subsets, and gating single-cell events across different samples (*Saeys Y et al. Nat Rev Immunol 2016; 16:449-62*).

5. Analytical performance of the assay and the metrics to determine the level of the change following the treatment: These studies exploring T cell populations have been extensively studied in Luznik's laboratory. All samples are coded and assays are performed in blinded fashion. Assays are batched and contain both positive and negative biological controls. Luznik laboratory routinely include PBMCs and BM mononuclear cells from healthy individuals as controls for all the flow-cytometry runs. The results from controls are stored and averaged to generate a range for normal individuals. In our analysis we are achieving a high reproducibility in measuring the effects of the treatment under investigation in regard to T cell subset(s) numbers and/or other measurable markers (co-signaling molecules, transcription factors, cytokines etc.) and the correlation between

repeated measurements from the same patients. Based on our performance analysis the correlation between repeatedly measured subsets is very high (>90%) with the lower bounds of the 95% confidence limits higher than 80% for majority of the markers measured, implying the high reproducibility of our assay. We have also developed criteria for measuring the magnitude of markers changes that take into the account differences between the baseline and post-treatment values (unpublished data). Specifically**,** for each marker, we modeled the log-scale percentage of cells expressing specific markers as a function of time and clinical effect. A linear mixed effects regression model with a random intercept for each patient was then used to estimate the differences between time points for each marker while accounting for the patient clustering. Standard linear regression models were used to estimate differences in log-scale percentages between patients at pre-treatment and healthy controls. For each marker and each model, we extracted the test statistic associated with the difference between time points (or groups) and plotted them using standard visualization approaches such as plots and heatmaps.

6. Clinical utility of the integrated assay: Flow values at each time point will be summarized using geometric means and standard deviation. Differences in baseline values between patient subgroups will be explored using linear regression models. We are specifically interested in determining what pretreatment signature is associated with outcome, such as having a complete response or becoming refractory. To this end, we will use logistic regression models with ROC analysis to describe which pretreatment parameters are potential predictors of outcomes following treatment. Values will be checked for skewness and log-transformed as appropriate. The change in flow values at each time point after blinatumomab and blinatumomab plus checkpoint inhibitor(s) relative to the baseline value will be modeled using mixed-effects, linear regression models that include fixed effects for time point and a random effect for the patient to account for within-patient correlation of measurements. Post-treatment changes in T cell populations will be correlated with clinical outcomes such as CR vs. No CR or MRD vs. No MRD using interaction tests. T cell subsets in the PB and BM defined by multicolor flow cytometry will be summarized before and after treatment with blinatumomab alone or plus checkpoint inhibitors by treatment group using summary statistics. Differences in T cells before and after treatment will be explored using non-parametric Wilcoxon signed rank tests or paired t tests as appropriate. The expression of co-inhibitory/-stimulatory molecules will also be examined in the same way.

References:

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3) Riedhammer C, Halbritter D, Weissert R. Peripheral Blood Mononuclear Cells: Isolation, Freezing, Thawing, and Culture. Methods in Molecular Biology 2015: 1304: 53–61.
4) Ogunjimi B, Hens N, Malfait R, Van Tendeloo V, Smitset E. Creating a robust framework for the analysis of cryopreserved samples in quantitative immunological experiments. J Immunol Methods 2013;392:63-67.
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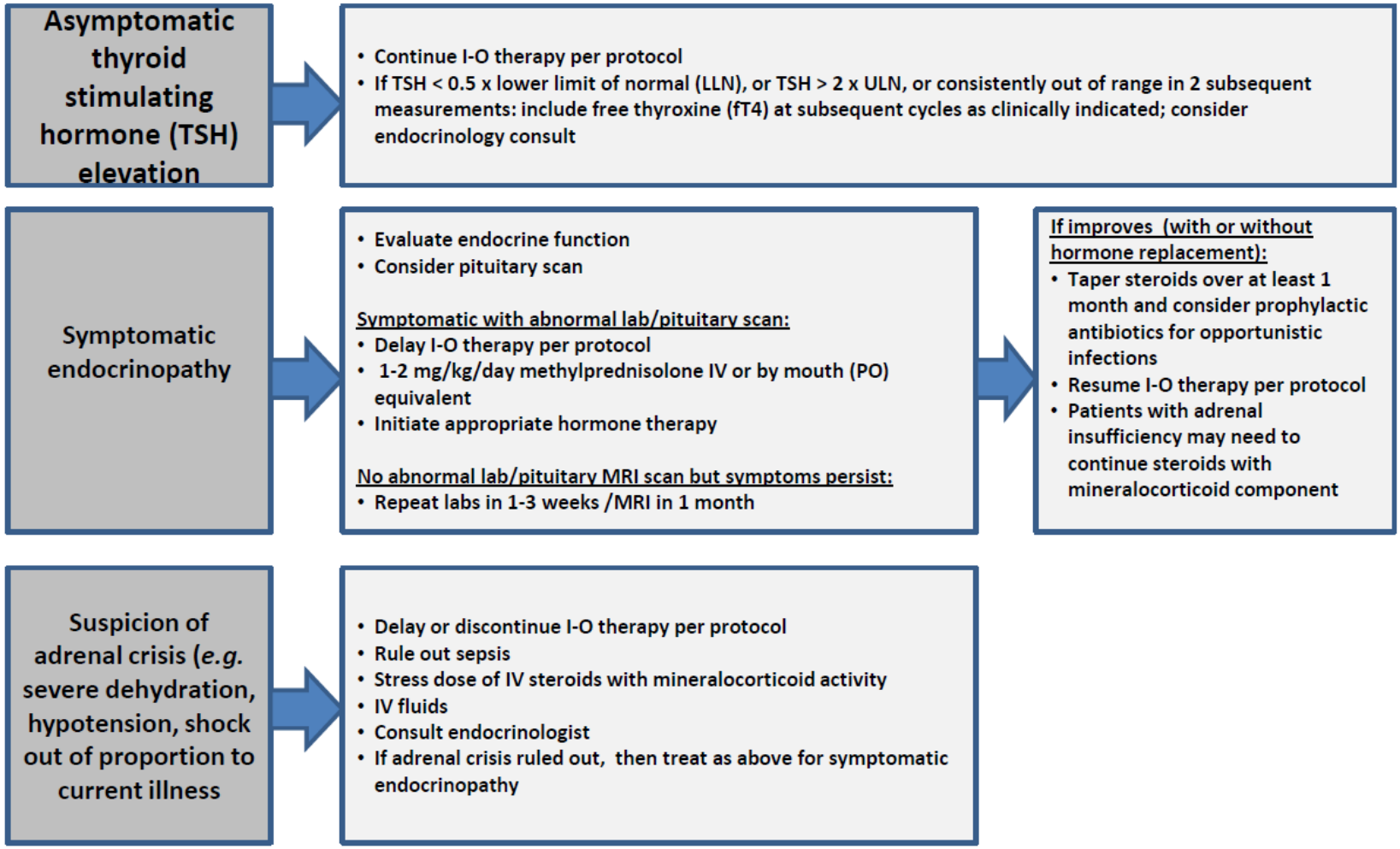
6) Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parkset DR. Interpreting flow cytometry data: a guide for the perplexed. Nat Immunol 2006;7(7):681-5.
7) Lugli E, Roederer M, Cossarizza A. Data analysis in flow cytometry: the future just started. Cytometry A 2010;77(7):705-713.
8) Saeys Y, Gassen SV, Lambrecht BN. Computational flow cytometry: helping to make sense of high-dimensional immunology data. Nat Rev Immunol 2016; 16:449-62.
9) Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol 2012, 12:191–200.

APPENDIX D: MANAGEMENT ALGORITHMS FOR NIVOLUMAB-/IPILIMUMAB-RELATED ADVERSE EVENTS: ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

Endocrinopathy Management Algorithm

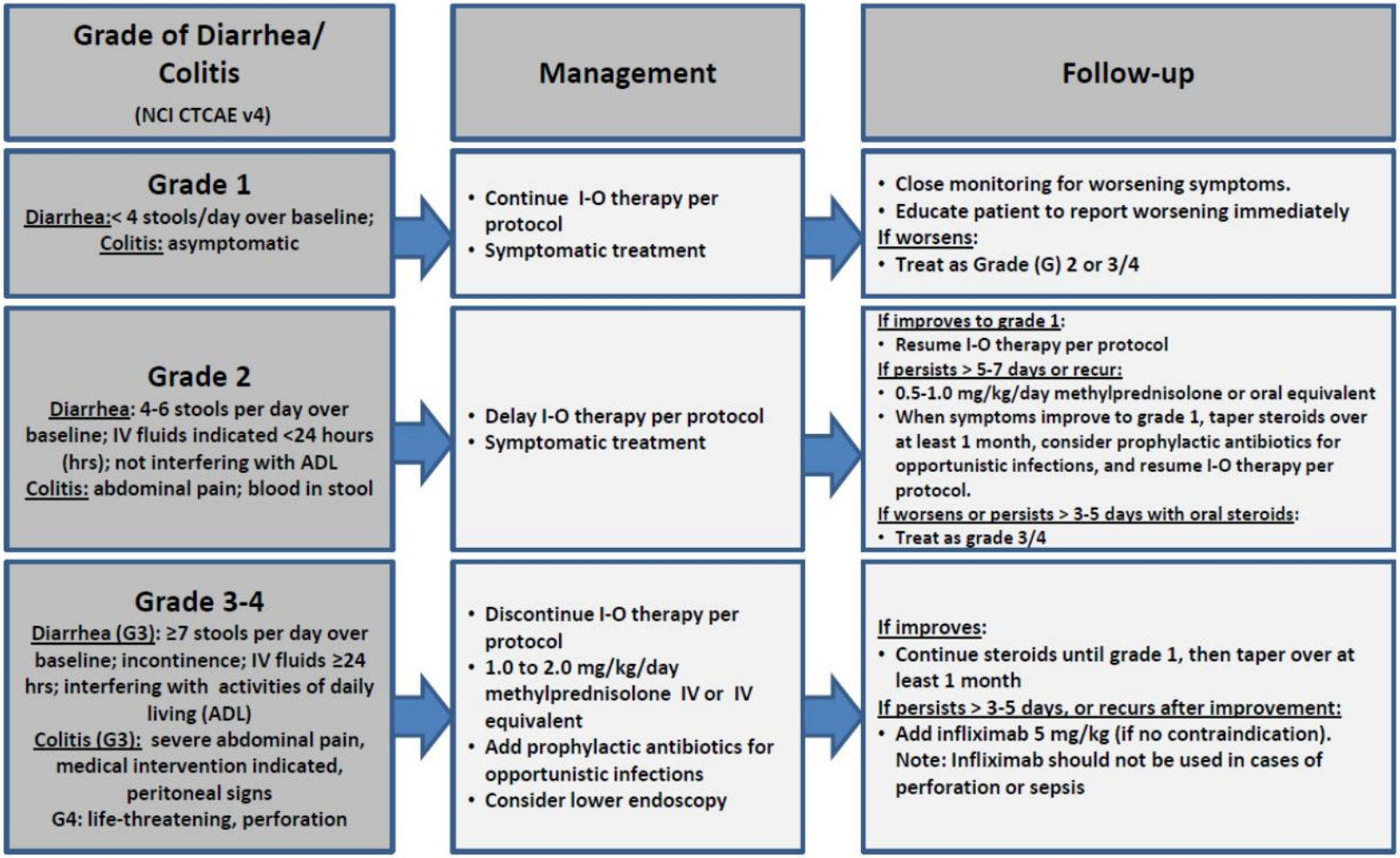
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy.
Consider visual field testing, endocrinology consultation, and imaging.



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.



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.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

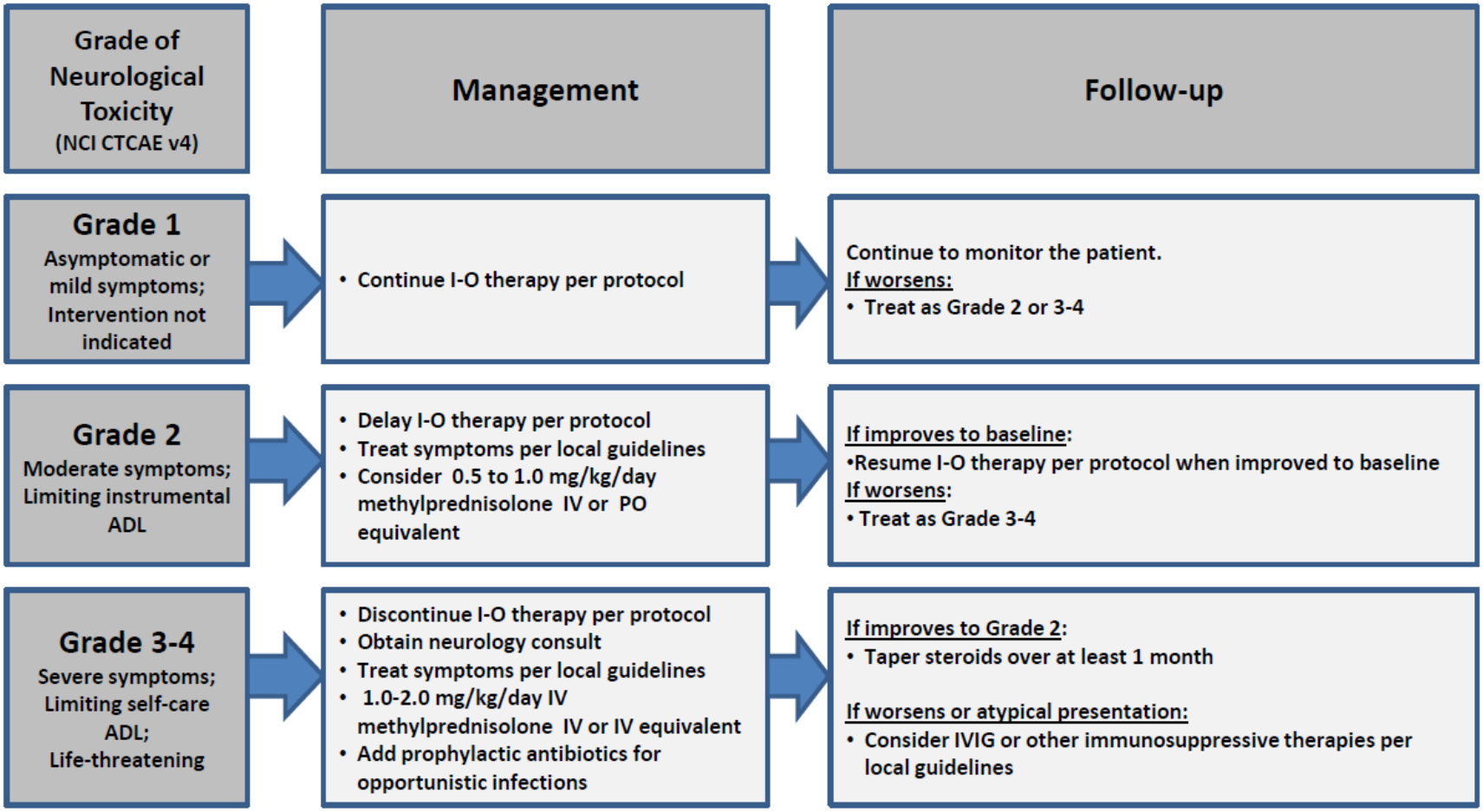
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

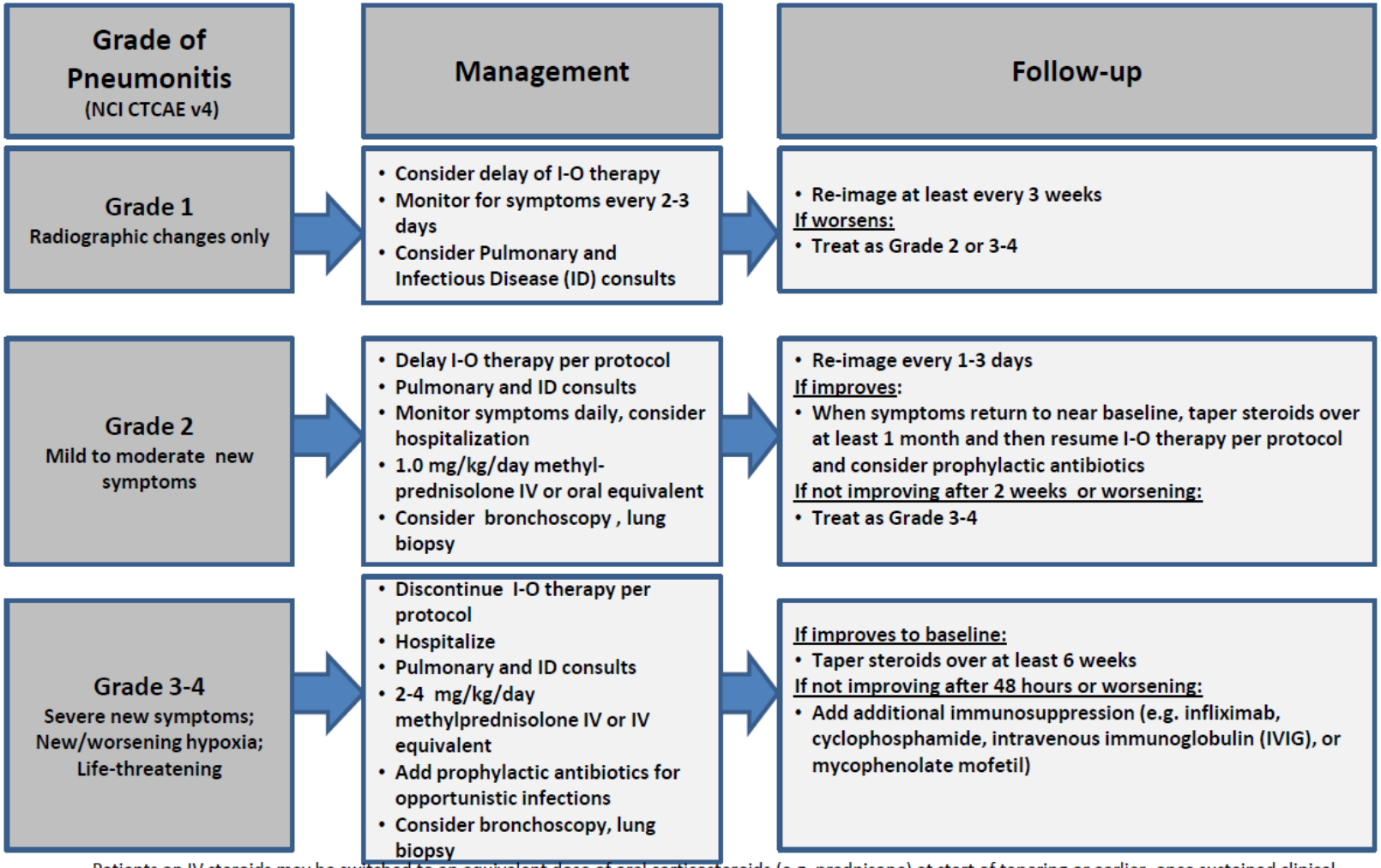
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

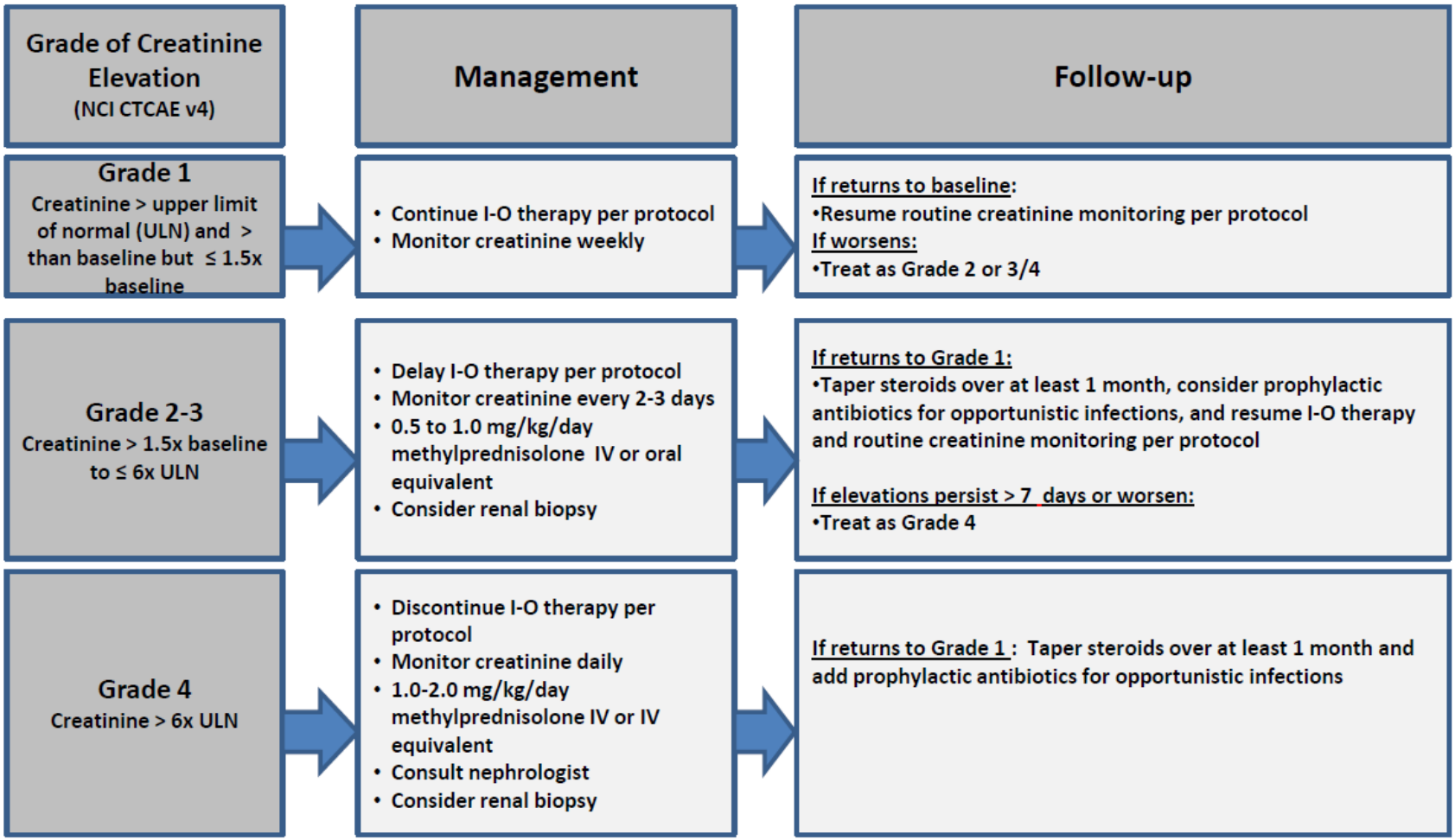
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

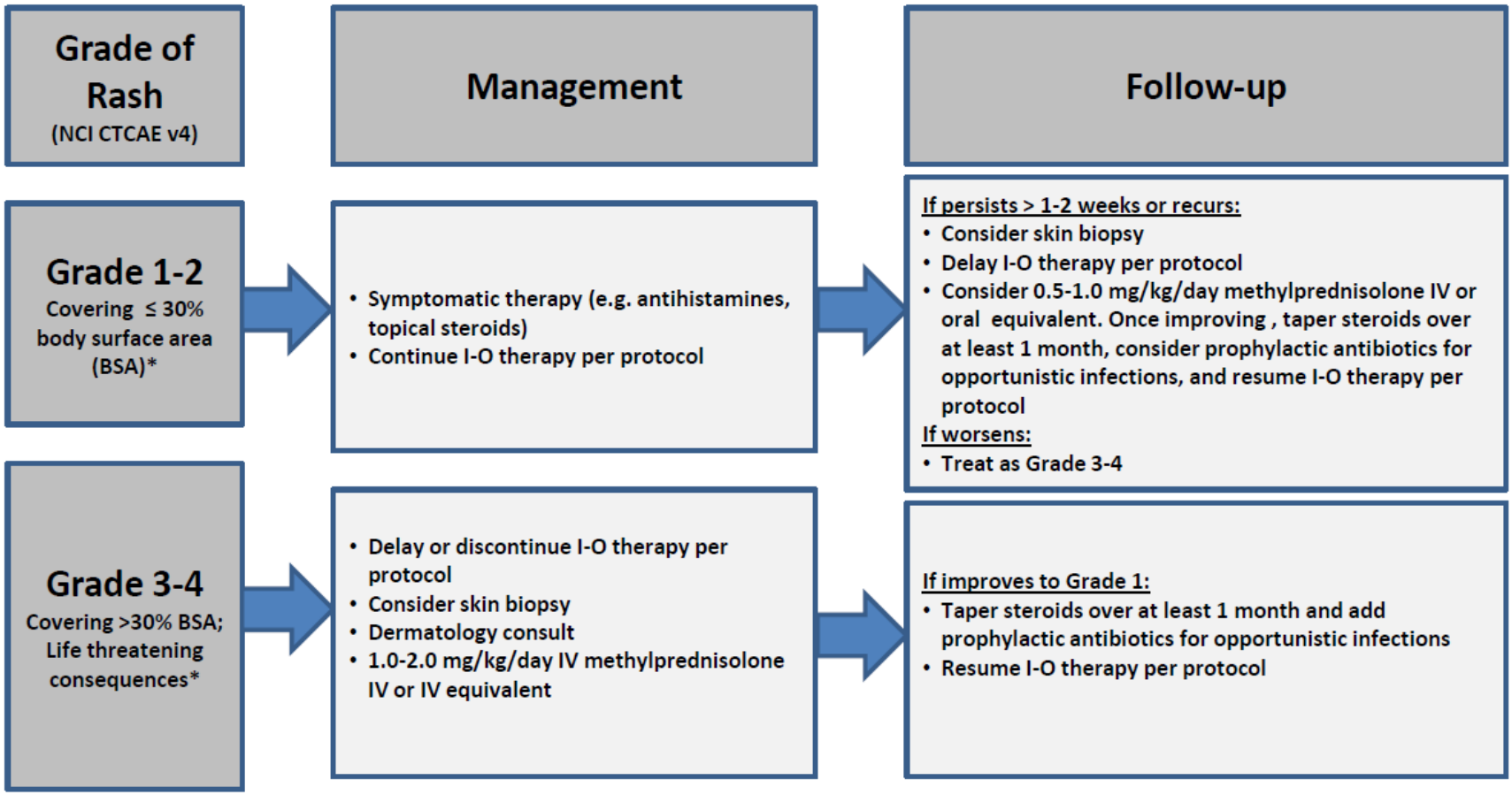
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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 E: OPERATING PROCEDURES FOR SPECIMEN COLLECTION

Peripheral blood and bone marrow specimen collection will be performed as delineated in the study calendar and in Section 9.1.

Bone marrow: 4 x 10 mL will be collected in green top (sodium heparin) tubes (~40 mL). Re-adjustment of the direction of bone marrow aspirate needle should take a place after each 10 cc is collected to prevent hemodilution. At the time of collection, green top tubes must be thoroughly mixed to prevent clotting.

Peripheral blood: 10 x 10 mL green top (sodium heparin) tubes of blood (~100mLs, minimum 60mLs) or 4 x 10 mL green top tubes (~40 mL, minimum 30 mL) will be collected at specific time points as indicated in Section 9.1 and the Study Calendar.

Specimens should be labeled with the patient's study number (given at the time of registration), study number, sample collection date and time, and sample source (PB or BM). Sample collection date and time, and sample source (PB or BM) will be recorded on the correlative collection worksheet and copy included with delivery. All data should be kept in the laboratory log. At each sampling time, BM and PB mononuclear cells (PBMC) will be processed via Ficoll density gradient centrifugation. The washed cells will be counted, and viably cryopreserved using a controlled-rate freezer with transfer to the vapor phase of liquid nitrogen for long-term storage.

The Sidney Kimmel Comprehensive Cancer Center (SKCCC) specimens should be delivered to the Luznik laboratory immediately after collection.

Specimen collection / processing (Participating Sites).
Participating sites should collect PB and BM specimens as outlined in the study calendar and Section 9.1. Participating sites have two options:

1) Process specimens following the SOP described below. Samples will be stored at each study site. Any samples collected under the clinical protocol will be batch shipped on dry ice based on discussions with the participating site PI, Study Chair and Dr. Luznik.

2) If the participating site does not have capability of processing the specimens as described below, then specimens can be shipped overnight to Dr. Luznik's laboratory using cold shipping packages (boxes) provided by Fedex. Please notify PI (Study Chair), study coordinator and Dr. Luznik's laboratory of specimen shipment.

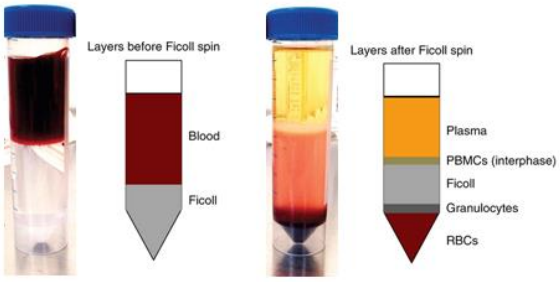
Shipping of Specimen(s)
The samples will be processed, stored and analyzed in Dr. Luznik's laboratory at the SKCCC at Johns Hopkins University. Upon request of the Study Chair they will be batch shipped to SKCCC. Samples will be shipped or delivered to Dr. Luznik laboratory: 1650 Orleans Street, CRB1 Rm 216, Baltimore MD 21287. Tel: (410) 955-8567

Freezing Protocol (SOP)

1. Prepare 50mL falcon tubes with Ficoll density grade media (Histopaque)
    - Ratio Ficoll : blood should be 1:1
    - Do not exceed 20mL Ficoll to 20mL blood (i.e. total volume of 40mL)

2. Slowly layer blood on top of Ficoll
    - Use whole blood or marrow aspirate, do not dilute with PBS or anything else
    - Be careful not to mix the two layers

3. Centrifuge: spin at the following settings:
    - 1240rpm (=364G)
    - 20 Celsius
    - Break off
    - 30min



4. Prepare 6 small Eppendorf tubes
    - Take off 6 x 500ul of plasma (top layer; blood only; not for BM aspirate) and put it into Eppendorf tubes (this is for cytokine studies); label tubes with the study #, patient ID #, and timepoint.
    - Be careful not to get into any layer but the plasma layer
    - Long term storage in -80 Celsius freezer

5. Soak up PBMC layer with transferring pipette and put it into a new 50mL Falcon tube
    - Fill up with PBS up to 45mL, vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

6. First wash
    - Dump supernatant
    - In case of visible RBC contamination add 1Ml of lysing buffer to the cell pellet, vortex, and leave for 1 minute. Then add 20mL of PBS
    - If there is no RBC contamination only add 20mL of PBS and vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

7. Second wash
    - Dump supernatant
    - just add 20mL of PBS and vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4 Celsius, break high, for 5 minutes

8. Count cells
    - Dump supernatant
    - add 1mL of PBS and vortex thoroughly
    - take 10ul out and mix with 90ul of trypan blue
    - count in hematocytometer

9. Prepare freezing tubes and media
    - Put cells in the meantime on ice
    - Freezing media: FBS containing 10% DMSO
    - Prepare 4 - 6 x 1.8mL vials labeled with study #, timepoint, cell count, date, type of specimen,

Study # _____
Pt ID# _____
Timepoint: _____
Cell count: _____
Date(time):

10. Final spin
    - Add 20ml of PBS to the cells, vortex
    - Centrifuge: Spin at 1240 rpm (=364G), 4°C, break high, for 5 minutes

11. Freeze cells - General rules
    - PBMCs: 1.5mL freezing media per 10 -20 million cells (=1 vial)
    - BMMCs: 1.5mL freezing media per 30 million cells (=1 vial)

12. Freezing
    - Put vials into Mr. Frosty® (container with Ethanol, that gradually cools down samples) and put into -80 Celsius fridge for about 24 hours
    - After 24 hrs (up to 48hrs) transfer vials into liquid nitrogen tanks (to minimize temperature fluctuations make sure you transport them there on dry ice)

Considerations regarding freezing and shipping

- Samples should optimally be processed the same day
- If there is a sample coming in late, keep it in the fridge (in 4◦C) overnight and process them early the next morning.
- Samples processed should be stored in -80 Celsius for 24-48 hours and get transferred into liquid nitrogen thereafter
- Samples should be shipped (Fedex) in batches on dry ice to:

    Luznik Lab
    Hopkins CRB1, Room 216
    1650 Orleans Street
    Baltimore, MD 21287

- If there are no liquid nitrogen tanks available, samples can be kept in -80 Celsius for up to 2 weeks, but no longer. Shipments to Hopkins should then be done more frequently.

Important considerations:
1. All work should be done using standard BSL2 procedures (blood and body fluid precautions).
2. Maintain a clean workspace and use a containment laminar airflow hood when possible.
3. Minimize the chance of contamination.
4. Work quickly but methodically.
5. Keep tubes closed as much as possible and work quickly.
6. Change gloves frequently and maintain situational awareness.

APPENDIX F: CORRELATIVE STUDIES COLLECTION WORKSHEET

Protocol Sample Time	Sample Due Date	Actual Sample Date	Peripheral Blood	Draw initials PB	Bone Marrow	Draw Initials (BM)	Comments	Shipped/Delivered
Baseline (within 7-14d of Day 1)			60-100 cc		40 cc			
C1 D1 (-3 d)			30-40 cc					
C1 D8 (+ 3 d)			60-100 cc		40 cc			
C1 D15 (±3 d)			30-40 cc					
C1 D22 (±3 d)			30-40 cc					
C1 D 29-35 (±3d)			60-100 cc		40 cc			
C2 D1 (-3 d)			30-40 cc					
C2 D 29-35 (±3d)			60-100 cc		40 cc			
3 months later			60-100 cc		40 cc			
6 months later			60-100 cc		40 cc			
9 months later or at 1 yr			60-100 cc		40 cc			
Off study treatment			60-100 cc		40 cc			
Relapse			60-100 cc		40 cc			

APPENDIX G: WRITING TEST

Subject ID _____
Instructions for the Subject: **Enter the date and time of day. Please write the simple phrase as indicated below. Rewrite the initial phrase at subsequent days.**

Study Day	Date	Time	***Sweet as apple pie***	Study Staff-Initial & Date

APPENDIX H: SHIPMENT OF BLINATUMOMAB IV BAG FROM SITE/PHARMACY TO PATIENT'S HOME

To be completed by Site/Pharmacy:

From: (Investigator Name, Address)	**To Patient:** (Patient Initials, Study ID No) _____ ; _____	**Protocol No.:**
Site Pharmacy contact	— — —	

Prepare shipment of IV bag at 2°C to 8°C in validated/pre-qualified insulated shipper as per manufacturer instructions (see shipping container instructions). Please take care to use the applicable instructions for summer or winter package preparation, respectively.

IV Bag number	**Date of packaging** [DD/MMM/YYYY]	**Time of packaging** [hh:mm]	**packed by** (initials)

Please tick the boxes and fill in the information below when preparing the IV bag shipment!

- □ **Validated/pre-qualified shipping container duration of time 2°C to 8°C temperature is maintained: _____ hours**
- □ **Cooling elements for provided box used according to manufacturer's instruction**

Confirmed by:_____ _____
(print name, signature) (date)

To be completed by Ambulant/Home Care Service Provider:

Shipment box unopened and content intact? **YES** □

NO □

IF NO, please comment _____

Date and time shipment box opened: _____ _____
(date) (time)

Confirmed by: _____ _____
(print name, signature) Amb. Care Service (date)

NOTE: **If content is not intact, please do not use IV bag and inform site pharmacy immediately! If time box opened minus time of packaging exceeds the time duration the shipping container maintains 2°C to 8°C, please do not use IV bag and inform site pharmacy immediately!**