Masonic Cancer Center
University of Minnesota

Nivolumab, Oral Cyclophosphamide, and N-803 for Relapsed/Refractory Acute Myeloid Leukemia (AML) and Higher-Risk Myelodysplastic Syndrome (MDS)

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Revision History

Revision #	Version Date	Detail of Changes	Consent change? (Y/N)
	10/03/2017	Original version for CPRC and BMS (earlier version sent)	n/a
	11/01/2017	In response to CPRC stips: Expand nivolumab background information in Section 2.3 and Section 2.4 (new) Add wording to Section 6.2 regarding use of hydroxurea and immunosuppression during the study treatment. Other edits: Cover page - update Co-I's Section 4.2.1 – clarify negative pregnancy test with 7 days of study administration Section 13 and Section 14.2 – provide more detail pertaining to study endpoints Section 14.3 – expand definition of evaluable Other minor edits and updates	n/a
	02/23/2018	Original version to the IRB and FDA: To align versions approved by CPRC and the older version approved by BMS (no changes from BMS affecting the 11/01/2017 version approved by the CPRC) Update in protocol template language: • Update Section 5 Patient Registration/Study Enrollment to current template language • Section 12.3 – delete wording about submitting copy of IRB continuing review to CPRC • Replace CTCAE v 4.03 with v 5.0 • Replace study coordinator with newer designation of Primary Clinical Research Coordinator (PCRC) • Add co-investigators Other minor edits and clarifications throughout.	n/a
	03/20/2018	In response to FDA's potential hold and non-hold comments: • Synopsis, schema, Section 3 and Section 6.3 – clarify and make consistent that an increase in absolute blast count is ≥ 25% in peripheral blood or bone marrow from baseline is criteria for stopping treatment due to disease progression • Synopsis, Section 4.1.2 and Appendix I - Add risk score of >4.5 to the definition of higher risk MDS, separate relapsed AML and MDS eligibility requirements • Synopsis, Section 4.1.4 and Appendix I – add bilirubin ≤ 3 x ULN to eligibility and require both AST and ALT results (previously or) • Section 3, Section 11.2 and Section 14.4 – add selected Grade 3 nonhematologic events that result in the permanent discontinuation of nivolumab (diarrhea, colitis, pneumonitis, transaminitis, myocarditis, adrenal insufficiency) • Section 6.1.1 – clarify nivolumab will be administered in the outpatient setting, add monitoring language. • Add IND number to the title page	yes
1	05/17/2018	• Per BMS for patient safety - Nivolumab may induce graft versus host disease in patients who have had a prior allo-HSCT. To reduce this risk, patients who have had a prior alloHSCT will receive a lower dose of nivolumab (1 mg/kg instead of 3 mg/kg). • Increase research sample blood volume as an insufficient amount was originally written into the protocol • Minor edits to match current protocol template	Yes

2	09/13/2018	• Updated Principal Investigator/IND Sponsor to Fiona He, MD	Yes

Revision #	Version Date	Detail of Changes	Consent change? (Y/N)
		• Section 4.1.2 & Eligibility checklist: Updated eligibility criteria (disease status) • Minor clarification of research blood draw procedure • Section 14.4 updated sample size • Protocol synopsis: Updated for clarification including monthly peripheral blood blast count disease evaluation • Section 1.3 correlative objectives added neo-antigen identification on blasts • Section 8.1 bone marrow biopsy evaluations, changed screening bone marrow biopsy to within 21 days of study registration, and that bone marrow biopsies may be omitted if not medically safe to perform	
3	03/26/2019	• Removed bone marrow biopsy response assessment from final treatment visit • Cyclophosphamide will now be provided by BMS • Updated study committee to remove Drs Cooley & Ustun	Yes
4	12/04/2019	• Updated study committee to add Najla El Jurdi and Joseph Maakaron • Schema, Synopsis, Section 1, , Section 3, Section 6.1, Section 7.3, Section 10.3,Section 14.3, Appendix V, Appendix VI: Added SC N-803 once every 3 weeks in treatment schema, changed Nivolumab dosing frequency to once every 3 weeks • Schema, Section 3, Section 5.2, Section 6, Section 8.1, Section 8.2, Section 14.1: Changed total number of cycles from 4 to 5 (each cycle defined as 3 weeks instead of 4 weeks) • Synopsis, Section 1.1, Section 3, Section 6.4, Section 13, Section 14: Changed bone marrow evaluation schedule from D30 and D90 to D21 and D42 • Section 2.6, Section 2.8: Added background information for N-803 • Section 8.2: Added research blood draw at C1D8, C1D15, C2D1, C2D8, C2D15, C3D1, C4D1, C5D1, end of treatment visit • Synopsis, Section 4.1, Section 8.2: Added disease eligibility criteria of extramedullary leukemia that is biopsy-confirmed in absence of bone marrow involvement; added CT or PET-scan for disease restaging • Section 4.1: Added to inclusion criteria: Ability to be off prednisone and other immunosuppressive drugs (>10 mg daily prednisone equivalent) for at least 14 days prior to and while receiving N-803. Inhaled or topical steroids, and adrenal replacement steroid doses < 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease • Section 4.2: Added prior N-803 administration and cellular therapy product within 30 days as exclusion • Added Appendix V and Appendix VI • Section 6.1, Section 7, Section 11, and Section 14.4 reverted protocol back to CTCAE v 4.03 in order to match protocol build in Oncore • Section 1.1, Section 14.1: Changed timepoint for primary endpoint of ORR to day 42	Yes

5	06/08/2020	Per FDA Stipulations: • Synopsis, Schema, Section 3, Section 5.2, Section 6.1, Section 14.1: Clarified that original treatments as closed Arms 1 and 2, treatment with N803 will be called Arms 3 and 4 • Synopsis, Section 3; Clarified that Day 42 BMBX will be used to help determine additional cycles • Synopsis, Section 1.3: added correlative objective to assess Tcell infiltration through immunohistochemical testing in the bone marrow • Synopsis, Section 4.1: clarified eligibility for relapsed MDS • Synopsis, section 4.2: added exclusion for allo HSCT receiving calcineurin inhibitor within 4 weeks • Section 6.1: updated eligibility for additional cycles (moved information previously in section 6.1.5) • Section 6.1.5: updated dose administration guidelines for N803 • Section 6.2: Updated GVHD management guidelines	No
Revision #	**Version Date**	**Detail of Changes**	**Consent change? (Y/N)**
		• Section 6.3: updated definition of disease progression • Section 11.2, section 14.4: updated stopping rule events Per IRB Stipulation: • Section 3 – Added N803 as part of endpoint Minor edits and clarifications: • Section 7.1, section 8.1 – added "N-803" • Section 8.2: updated research tests performed on lab samples • Throughout protocol updated internal HTML and section links	
6	9/18/2020	Per FDA Stipulations: Synopsis, Section 1.1, Section 3.0, Section 5.2, Section 14.1 - Removed stage II from trial design Synopsis, Section 1.2, Section 14.1 - defined response rate in secondary objectives Synopsis, Section 3.0, Section 14.1- updated accrual goal Section 3.0, Section 11.2, Section 14.4- Updated stopping rules Section 6.1 – clarified eligibility to begin additional treatment courses Section 6.2 – Updated study management in the case of new onset GVHD Additional Edits: Section 7.1, Section 10.1, Section 10.3.6, Section 10.4 Updated the risk language for N-803 and Nivolumab, based information from other trials using study product and latest Investigator Brochures	Yes
7	04/28/2021	Synopsis, Schema, Section 3.0, Section 5.2, Section 14.3, Section 14.4, number of patients changed from 24 to 20 – Rationale: improve feasibility of recruitment while maintaining goals for statistical power Synopsis, section 1.3, Section 8.2, added and clarified correlative studies Rationale: add immune-related gene expression profiling of bone marrow to evaluate other pathways that may confer sensitivity or resistance to therapy Section 8.2 added bone marrow sample. Rationale: for RNA gene expression studies Section 13.0, Section 14.1 changed primary endpoint to dose determination, moved original primary endpoint to secondary. Rationale: prior amendment removed stage II from study design. Primary endpoint changed to reflect dose finding rather than efficacy.	Yes

7A	05/12/2021	Synopsis, Schema – corrected number of patients. Rationale – error correction (number of patients was amended in previous version)	No

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Table of Key Abbreviations

ALT	alanine aminotransferase (aka SGPT)
alloHSCT	allogeneic hematopoietic stem cell transplant
AML	acute myeloid leukemia
AST	aspartate aminotransferase (aka SGOT)
BM	bone marrow
CNS	central nervous system
CR	complete remission
CRi	complete remission with incomplete blood count recovery
CRp	complete remission with incomplete platelet count recovery
CTCAE v4.03	Common Terminology Criteria for Adverse Events version 4.03
CTX	cyclophosphamide
DC	dendritic cell
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
HCT	hematopoietic cell transplant
HI	hematologic improvement
IDO	indolamine 2,3 dioxygenase
IPSS	International Prognostic Scoring System
irAE	immune related adverse event
IV	intravenous
MDS	myelodysplastic syndrome
MDSC	myeloid derived suppressor cells
NK	natural killer
OR	objective response
ORR	overall response rate
OS	overall survival
PO	per os
PR	partial remission

PFS	progression-free survival
SOC	standard of care
WOCBP	women of childbearing potential

Protocol Synopsis

Nivolumab, Oral Cyclophosphamide and N-803 for Relapsed/Refractory Acute Myeloid Leukemia (AML) and Higher-Risk Myelodysplastic Syndrome (MDS)

Study Design: This is a phase I trial of nivolumab, low dose cyclophosphamide (CTX) and N-803 when given in combination to patients with relapsed/refractory acute myeloid leukemia (AML) and higher-risk myelodysplastic syndrome (MDS) who are not eligible for or decline hematopoietic stem cell transplant. It includes a randomized pilot substudy during stage 1. Arm 1 and Arm 2 previously randomized patients in 1:1 ratio to IV nivolumab 3 mg/kg every 2 weeks and oral cyclophosphamide 50 mg daily or IV nivolumab 3 mg/kg every 2 weeks and oral cyclophosphamide 350 mg weekly. Arms 1 and 2 are now closed.

20 patients enrolled in the study are randomized to either Arm 3 (CTX 50mg PO daily + nivolumab IV and 10 ug/kg SC N-803 every 3 weeks) or Arm 4 (CTX 350 mg PO every 7 days + nivolumab IV and 10 ug/kg SC N-803 every 3 weeks) with 10 subjects in each arm. Nivolumab dosing is 360 mg unless the patient has had a prior allogeneic transplant, in which case the nivolumab dose is 180 mg.

Disease will be assessed by bone marrow biopsy at 21 and 42 days after initiation of study drugs. If the increase in absolute blast count is <25% in peripheral blood or bone marrow from baseline at day 42 or later and there is perceived clinical benefit by the treating physician, the subject will proceed with further treatment with up to five 21-day courses.

Continuous stopping rules are used to monitor excessive toxicity for each arm. If one arm is terminated by the toxicity stopping rule, all subsequent patients will be assigned to the other arm if it is not terminated by the toxicity stopping rule to continue enrollment of up to 20 patients.

All patients receive nivolumab IV and SC N-803 every 3 weeks and oral cyclophosphamide (daily or weekly) as assigned for up to 5

treatment courses with each treatment course equal to 21 days. A final treatment visit occurs 30 days after the last dose of study drug with follow-up for response and survival continuing through 2 years from beginning of treatment. For patients with clinical benefit after the 5 treatment courses, additional therapy with the study drugs or other treatment may be offered.

Primary Objective: To identify the safety, immunological activity, and clinical benefit of combination of nivolumab, N-803 and low-dose oral CTX when given in patients with relapsed/refractory AML and higher-risk MDS

Secondary Objectives:
- To estimate objective response rate (ORR) at 42 days from treatment start. Response is defined as CR + CRi + CRp + PR in AML and CR/PR/hematologic improvement (HI) in MDS
- To estimate progression-free survival (PFS) and overall survival (OS) at 6 months from treatment start

Correlative Objectives:
- To evaluate level of immune activation in blood and bone marrow microenvironment by measuring quantities and activation status of T-cells subsets, natural killer (NK) cells, myeloid derived suppressor cells (MDSC), and dendritic cells (DC) during treatment
- To assess PD1/PD-L1 and TIM-3/galectin-9 interactions through quantified expression of PD-L1 and galectin-9 on leukemic blasts, galectin-9 in serum, PD-1 and TIM-3 on immune cells as biomarkers of response and potential mechanisms of resistance to treatment
- To assess T-cell infiltration through immunohistochemical testing in the bone marrow
- To evaluate immune and leukemia-related gene expression patterns in bone marrow aspirates at baseline and during treatment

Eligible Diseases: Primary (de novo) AML or higher-risk MDS with induction failure: No CR after 2 or more induction attempts with high dose chemotherapy, hypomethylating agents, or other agents; No CR after 1 induction attempt and not eligible for a 2nd induction. Higher risk MDS defined as risk score > 4.5 based on the by revised International Prognostic Scoring System (IPSS) criteria.

Secondary AML (from antecedent hematologic malignancy or treatment-related): Not in CR after 1 or more cycles of chemotherapy

Relapsed AML: Blasts ≥5% in bone marrow or peripheral blood after prior attainment of CR or biopsy-proven extramedullary leukemia in absence of bone marrow involvement; relapse at any time, but currently ≥100 days following allogeneic HCT

Relapsed MDS: morphologic evidence of relapse or increase in blasts to ≥5% in bone marrow or peripheral blood after prior attainment of CR and not eligible for or decline intensive chemotherapy; relapse at any time but currently ≥100 days following allogeneic HCT

Key Inclusion Criteria:

- ≥18 years of age
- ECOG status ≤ 2
- ineligible for or declines allogenic hematopoietic transplantation
- voluntary written consent
- Adequate organ function as defined by ALC > 500 cells/m^3, serum creatinine ≤2 mg/dL, AST and ALT $\leq$ 5x ULN, Bilirubin $\leq$3 x ULN, no O_2 requirements on room air or requiring ≤2L O_2

Key Exclusion Criteria:

- acute promyelocytic leukemia
- pregnant or breastfeeding
- prior allogeneic hematopoietic stem cell transplantation currently within 100 days (note patients with prior alloHSCT receive a reduced dose of nivolumab); they are eligible after that date
- therapeutic immunosuppression within preceding 1 month
- signs or symptoms of graft versus host disease
- active pneumonitis or uncontrolled infection
- active, uncontrolled autoimmune disease
- chemotherapy within the previous 2 weeks
- estimated life expectancy less than 28 days
- Previously treated with N-803 (or “ALT-803”)
- Cellular therapy product within 30 days
- Allogeneic HSCT recipient receiving calcineurin inhibitor within 4 weeks

Accrual Objective: 20 patients randomized (50:50) to 1 of 2 treatment arms

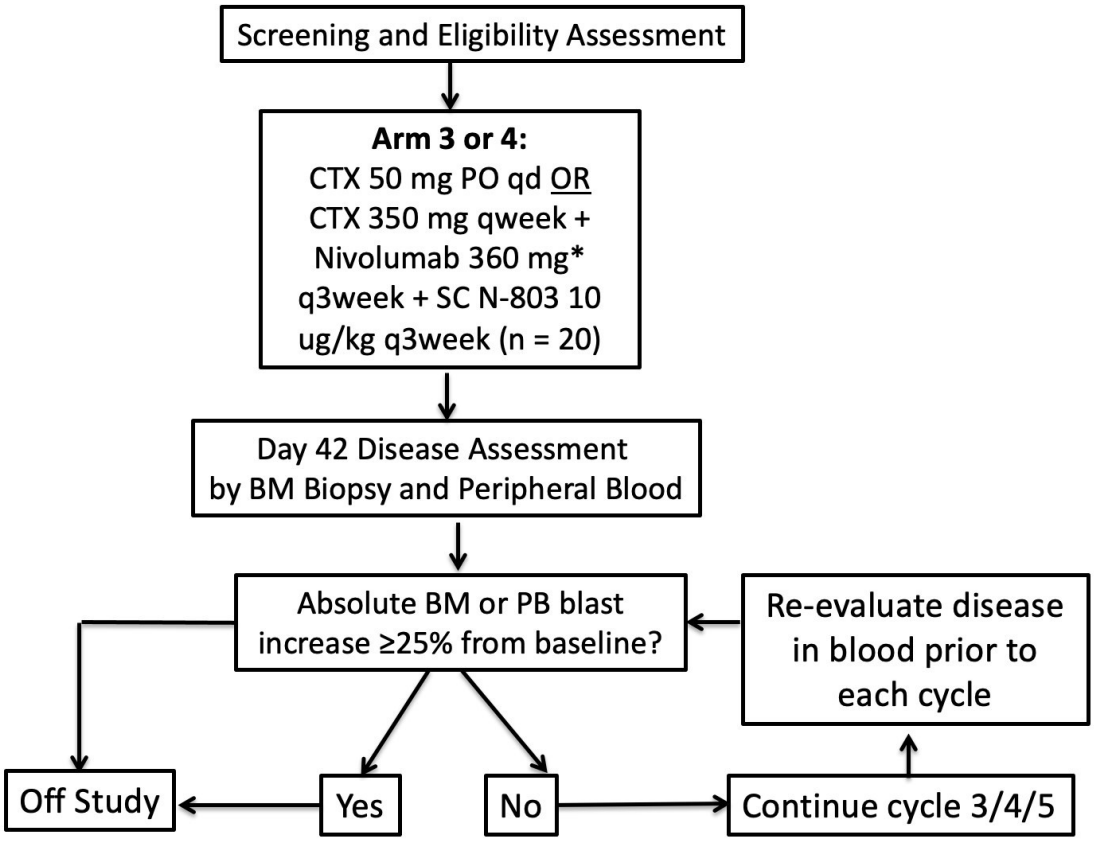
Enrollment: 1-2 patients per month

Study Schema

Arm 1 and Arm 2 previously randomized patients in 1:1 ratio to nivolumab 3 mg/kg every 2 weeks and low dose cyclophosphamide 50 mg daily or 350 mg weekly and enrolled 12 patients. Due to low response rates (no complete responses and only 2 patients with partial response) and 4 patients receiving ≤1 cycle of treatment due to rapid disease progression, arms 1 and 2 are now closed. Arms 3 and 4 are open and will add N-803 to the platform of nivolumab and low dose cyclophosphamide daily or weekly. All patients without prior history of allogeneic stem cell transplantation receive nivolumab 360 mg every 3 weeks, low dose cyclophosphamide (CTX) at assigned dose/schedule (daily versus weekly), and N-803 subcutaneously at 10 ug/kg every 3 weeks for up to 5 treatment courses. Patients with prior allogeneic stem cell transplantation will receive reduced dose of 180 mg nivolumab every 3 weeks.

One treatment course = 3 weeks

Randomized Pilot of 20 patients randomized (1:1) to Arm 3 or Arm 4



*Nivolumab dose is 360 mg unless the patient has had a prior allogeneic hematopoietic stem cell transplant (alloHSCT), in which case, the nivolumab dose is 180 mg.

1 Objectives

1.1 Primary Objective

To identify the safety, immunological activity, and clinical benefit of combination of nivolumab, N-803 and low-dose oral CTX when given in patients with relapsed/refractory AML and higher-risk MDS

1.2 Secondary Objectives

- To estimate the overall response rate (ORR) at 42 days from treatment start. Response is defined as CR + CRi + CRp + PR in AML and CR/PR/hematologic improvement (HI) in MDS
- To estimate progression-free survival (PFS) and overall survival (OS) at 6 months from treatment start

1.3 Correlative Objectives

- To evaluate level of immune activation in blood and bone marrow microenvironment by measuring quantities and activation status of T-cells subsets and natural killer (NK) cells during treatment
- To assess PD1/PD-L1 and TIM-3/galectin-9 interactions through quantified expression of PD-L1 and galectin-9 on leukemic blasts, PD-1 and TIM-3 on immune cells as biomarkers of response and potential mechanisms of resistance to treatment
- To assess T-cell and NK cell infiltration through immunohistochemical testing in the bone marrow
- To evaluate immune-related gene expression patterns in bone marrow aspirates at baseline and during treatment

2 Background and Rationale

2.1 High-risk AML and MDS

Acute myeloid leukemia is a disease with high mortality rate that is predominantly diagnosed in the elderly with median age of diagnosis of 67. Elderly patients are more likely to present with more aggressive disease, as evidenced by higher rates of adverse molecular and cytogenetic features compared to younger patients.[1] Although 50-60% of patients with good performance status may achieve complete remission with cytotoxic chemotherapy (anthracycline and cytarabine in “7+3” regimen), 2-year survival is poor at 15-20% due to high rates of relapse.[2] Salvage cytotoxic chemotherapy can induce objective response in 25 to 40% of patients, but these remissions are not durable, likely due to inability of chemotherapy to kill leukemic stem cells that persist. [3]

Higher-risk MDS, as defined by the revised International Prognostic Scoring System (R-IPSS) which evaluates cytogenetic risk, bone marrow blast count, hemoglobin, platelet count, and absolute neutrophil count, is associated with median overall survival of 0.8 to 1.6 years and 0.7 to 1.4 years for median time to 25% AML evolution. [4] Therapies are limited to hypomethylating agents and AML induction chemotherapy regimens in most cases.

Current approved therapies for AML and high-risk MDS include cytotoxic chemotherapies, molecularly targeted therapies in a fraction of eligible patients with FLT3/*ITD* or IDH2 mutations, and immunotherapy in the form of allogeneic hematopoietic stem cell transplantation (HCT). There is a need for novel treatments that not only increase survival, but also can be well tolerated in this predominantly elderly population.

2.2 Defective Leukemic Immunosurveillance in AML and MDS

Multiple mechanisms of immune evasion have been implicated in active AML and MDS. The tumor microenvironment is permissive towards preventing both innate and adaptive anti-leukemic immunity. Increased proportions of regulatory T-cells and myeloid-derived suppressor cells (MDSCs) have been identified in both AML and MDS patients as poor prognostic markers. [5][6][7] T-cells display aberrant gene expression activation patterns and form defective immune synapses in vitro.[8] Natural killer (NK) cell cytotoxicity is dampened by increased frequency of inhibitory KIR receptors on AML blasts.[9] Other pathways in AML and MDS that may contribute to immune dysfunction include PD-1/PD-L1, TIM3/Galectin-9, and IDO-1.

2.3 PD-1/PD-L1 Pathway in Malignancies

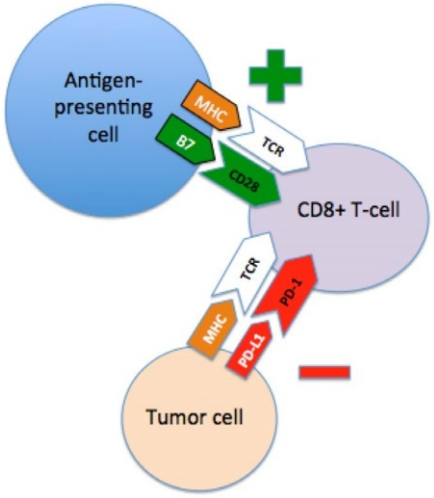


Figure 1. PD-1/PD-L1 pathway

PD-1 is a member of the B7/CD28 family of co-stimulatory receptors that binds its ligands programmed death ligand-1 (PD-L1) and programmed death ligand-2 (PD-L2). PD-1 receptor is most highly expressed on activated mature T-cells, but also at low levels on NK cells, B-cells, and monocytes.[10] PD-1 binding of its ligand PD-L1 along with a co-stimulatory signal of T-cell receptor binding of the major histocompatibility complex (MHC) presenting an antigen results in reduced T-cell receptor signaling, activation, and cytotoxicity as a physiological termination of the immune response (Figure 1). PD-1 overexpression is a phenotype of "exhausted," dysfunctional T-cells in states such as chronic viral infection and

malignancy. PD-L1 is constitutively expressed at a low level in professional and non-professional antigen presenting cells (APCs), but can be overexpressed in malignant cells as a mechanism of immune escape. To exploit this overactive immunosuppressive pathway, PD-1 blockade through nivolumab, a monoclonal antibody to PD-1, has been used to unleash tumor-directed cytotoxicity in multiple types of malignancy.

The safety and efficacy of nivolumab has been established by multiple large trials in solid tumors. In melanoma, the randomized Checkmate-066 trial enrolled newly diagnosed metastatic melanoma patients to nivolumab or dacarbazine (n = 418) and reported higher ORR (34% vs. 9%) and median OS (not reached vs. 10.8 months, HR 0.42, p<0.0001) in the nivolumab arm.[11] Responses were durable with 43 of 63 responding patients achieving ongoing response of 6 months or longer. In patients with metastatic squamous non-small cell lung cancer who had progressed on a platinum-containing regimen, nivolumab was compared to docetaxel (n = 272) in the Checkmate-017 trial and was associated with significantly increased overall survival (median OS 9.2 months vs. 6 months, HR 0.59, p=0.0002).[12] In a similar study of metastatic non-squamous non-small cell lung cancer (n = 582) randomized to nivolumab or docetaxel, overall survival was 12.2 months for nivolumab vs. 9.2 months for docetaxel (HR 0.73, p=0.002).[13] Multiple other studies have solidified the role of PD-1 inhibitors in solid tumors including renal cell carcinoma, [14] squamous cell carcinoma of the head and neck, [15] and hepatocellular carcinoma. [16] Pooled safety analyses in melanoma trials (n=576) reported 71% (95% CI, 67% to 75%) with any-grade treatment-related adverse events (AEs) (most commonly fatigue [25%], pruritus [17%], diarrhea [13%], and rash [13%]), and 10% (95%CI, 8%to 13%) with grade 3 to 4 treatment-related AEs. Median time to onset of AEs ranged from 5 weeks for skin to 15 weeks for renal AEs. [17]

Within hematologic malignancies, PD-1 inhibitors have had efficacy in Hodgkin lymphoma, largely due to genetic alterations of the 9p24.1 locus leading to overexpression of PD-L1 and immune evasion. A phase II study of recurrent Hodgkin lymphoma relapsed post-autologous transplantation enrolled 80 patients and reported ORR of 66% (95% CI 54.8–76.4) with acceptable safety profile. In the post-allogeneic transplantation setting of relapsed Hodgkin lymphoma, a multicenter retrospective review of 31 patients reported ORR of 77%, with 55% rate of acute graft-versus-host disease (9 of 17 with grade III-IV aGVHD or severe chronic GVHD) using standard 3 mg/kg nivolumab dosing. [18] A prospective phase I trial of nivolumab in relapsed hematologic malignancies reported dosing of 1 mg/kg (n = 6) and 0.5 mg/kg (n = 2). In the 1 mg/kg cohort, 1 patient had mild chronic GVHD and 2 patients had immune-related adverse events. [19]

Nivolumab has been shown to be safe and well tolerated up to a dose level of 10 mg/kg, and pharmacokinetic studies have not reported relationship between nivolumab exposure produced by 3 mg/kg and efficacy. This indicates that the safety and efficacy profile of 240 mg nivolumab is similar to that of 3 mg/kg nivolumab. Similarly, a flat dose of 360 mg corresponds to a dose of 4.5 mg/kg q3w, which is similar to the nivolumab doses evaluated in combination with chemotherapy in NCT01454012, ranging from 5-10 mg/kg q3w. The simulated steady state concentration at trough (C^{minss}), peak (C^{maxss}), and average (C^{avgss}) with 360 mg are less than 10 mg/kg every 2 weeks. Thus, these regimens are expected to be safe and tolerable.

2.4 PD-1/PD-L1 Pathway in AML and MDS

In AML, the PD-L1/PD-1 pathway is aberrantly upregulated in an estimated 4257% of AML patients,[20][21] with increased expression of PD-L1 on leukemic blasts at time of relapse [22] and increased expression of PD-1 on cytotoxic Tcells. Aberrant activity of this inhibitory immune checkpoint pathway is associated with defective recognition and cytotoxicity towards leukemic blasts. [23]

The immunosuppressive function of T-regs may be intimately connected to aberrant PD-L1/PD-1 pathways. T-regs were unable to suppress CD8+ proliferation or cytokine secretion in both a PD-1 knockout AML mouse model and PD-1 wild-type AML mouse treated with monoclonal antibody to PD-L1. Treg depletion through IL2-diptheria toxin in combination with PD-1/PD-L1 blockade showed synergistic activity in reducing AML blast burden. [24] Murine models have demonstrated that PD-L1 overexpressing antigen-presenting cells may convert naïve T-cells into a T-regulatory phenotype and aid in their expansion and proliferation, although this has not been tested in an AML model.[25]

Clinical trials of PD-1/PD-L1 blockade in AML are currently in early phase. CT011, a humanized IgG1 monoclonal PD-1 antibody, showed safety and tolerability in a phase 1 trial of advanced hematologic malignancies when given as a single dose of 0.2 to 6.0 mg/kg. [26] Seven of 8 patients with AML who were enrolled in this study had disease progression. One patient had blast reduction from 50% to 5% with two doses of CT-011 5 months apart, with disease control for 61 weeks.

PD-L1 expression has also been widely detected in MDS, but may be more commonly expressed in non-leukemic immune cells than leukemic blasts (12% of CD34+ myeloid progenitor cells, 36% of CD34- myeloid progenitor cells, 26% of lymphocytes).[27] Another study showed increased PD-1 expression in two subtypes of T-regs as well as CD4+ effector memory T-cells in MDS patients compared to healthy controls.[28] PD-L1 expression may increase after failure of

hypomethylating agents (HMA), suggesting PD-L1 upregulation as a mechanism of resistance to HMAs.

Nivolumab has been studied as monotherapy and in combination with azacitadine in a phase II study of MDS patients after failure of hypomethylating agents. Although efficacy was seen with objective response rate of 69% in the combination arm (n = 13), there was no single agent activity noted in the Nivolumab monotherapy arm (n = 15). Pembrolizumab monotherapy was studied in 28 patients with MDS after HMA failure in the phase 1b KEYNOTE-013 study, which showed ORR of 4%, stable disease in 52%, and hematologic improvement in 11%. [29]

2.5 Low Dose Cyclophosphamide

Cyclophosphamide (CTX) is an alkylating agent with differential effects at high and low doses. At maximal tolerated dose, it exerts broad anti-proliferative, cytotoxic effects in a variety of malignancies. At low, continuous dosing ("metronomic"), it has immunomodulatory and anti-angiogenic properties as a monotherapy or in combination with other agents.

Low dose CTX has been reported to decrease number of FOXP3+ regulatory Tcells, although this effect has not been sustained in most studies. [41] In vitro studies have additionally shown an attenuation of T-reg function and proliferative abilities.[42] Other immunomodulating effects of low dose CTX include polarization of CD4+ helper T-cells towards a Th1 profile that secrete type-1 IFNs and IL-2, favorable for conversion into a memory CD8+ T-cell phenotype,[43] and increased Th17+ population that secrete the pro-inflammatory cytokine IL-17.[44] In heavily pre-treated metastatic breast cancer patients, daily oral CTX at 50 mg daily was noted to increase percentage of tumor-specific T-cells and stabilize disease in 7 of 12 patients. Independent of effects on T-regs, studies have shown sustained increase in proliferative capacity of CD8+ effector T-cells. [45] Most studies of low dose CTX are in breast cancer, but efficacy has also been shown in hormone-resistant prostate cancer [46] and metastatic melanoma [47].

The immunomodulatory properties of cyclophosphamide have been exploited by combining it with dendritic cell vaccines to prime the immune system in murine models. Preconditioning with low dose CTX in murine models of colon cancer and melanoma resulted in enhanced numbers of antigen-specific T-cells and increased tumor regression.[48] In addition to enhancing effector T-cells, CTX has been shown to increase number and activation status of dendritic cells in a murine model of colon cancer. [49] A combination of dendritic cell vaccine, low dose CTX, and anti-PD-1 therapy was effective in inducing an antigen-specific immune response leading to long-term tumor control in a murine model of glioma. [50]

Within hematologic malignancies, the use of continuous low dose CTX has been limited to non-Hodgkin lymphoma and multiple myeloma in combination with other agents. CTX and high-dose Celecoxib in a phase II trial had efficacy in a population of relapsed and refractory non-Hodgkin lymphoma patients, with 37% ORR and median PFS of 4.7 months.[51] To date, there are no published studies of low dose CTX for treatment of MDS or AML.

Low dose CTX also has known anti-angiogenic effects, promoting normalization of the bone marrow vasculature through upregulation of thrombospondin-1 (TSP1), a glycoprotein with potent endogenous angiogenesis inhibiting properties. [52] [53] Endothelial cells have been reported to be very sensitive to low doses of CTX. Induction of hypoxia results in disruption of endothelial cell ability to form tubes and leads to apoptosis.[54] In-vitro studies suggest a synergistic mechanism of CTX with other anti-angiogenic agents. Anti-angiogenic effects of CTX have been mainly studied in solid tumor models. In AML, the role of angiogenesis in the vascular niche of leukemic blasts and stem cells has been investigated. Significantly increased microvascular density has been observed in the bone marrow of AML patients compared to healthy controls,[55] and may be a poor prognostic feature.[56] Induction of TSP-1 can directly induce apoptosis in AML cell lines, and is a potential therapeutic mechanism of low dose CTX in this setting. [57] While not proposed for direct testing, this may be a contributing therapeutic effect of the proposed therapy.

2.6 IL-15

Interleukin-15 (IL-15) is a cytokine and growth factor capable of expanding activated T cells and NK cells. By broad consensus, the NCI Immunotherapy Workshop (2007) ranked IL-15 as the #1 agent with “high potential for immunotherapy.”[58] Based on preclinical non-human primate and early phase clinical trial data, including those at the University of Minnesota, IL-15-containing regimens can be designed to prospectively and reproducibly increase T-cell and NK-cell counts.

The NCI Biological Resource Branch has manufactured E. coli-expressed recombinant human IL-15 (rhIL-15); daily administration of rhIL-15 intravenously (IV) or subcutaneously (SC) has been shown to increase the number and activation status of circulating CD8+ T and NK cells, but the cytokine has a very short half-life. Furthermore, high levels of free rhIL-15 decrease circulating IL-15Rα, acting as a negative feedback signal to reduce further IL-15 transpresentation. Trans-presentation appears to be a dominant mechanism for IL- 15 action in vivo in response to immune stimuli.[59] N-803 (Nantcell Inc, Culver City, CA) is a soluble complex consisting of two protein subunits of a human IL-15 variant associated with high affinity to a dimeric human IL-15

receptor α (IL- 15Rα) sushi domain/human IgG1 Fc fusion protein, that physiologically transpresents IL-15.[60][61] The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D)12. The human IL-15Rα sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the human IL-15 receptor α subunit (IL-15Rα) (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the protein sequences are human. N-803 has a prolonged serum half-life in preclinical animal models and has a 4-fold increase in biologic activity greater than wild-type IL-15 (IL-15 wt). [60]

N-803 has been used in a Phase I study of relapsed AML patients post-alloHSCT and found to have good tolerability and some clinical activity. [62] N-803 in combination with Nivolumab was safe and had clinical activity in a study of advanced non-small cell lung cancer patients with previous progression on single agent PD-1 inhibitor.[63] A summary of N-803 by route and dose is found in Section 6.1.4. The University of Minnesota's experience with N-803 was that subcutaneous dosing was generally well tolerated with an injection site related skin rash as the most notable event, often quite widespread; however none were dose limiting. Typically, the rash resolved by 7 days post-injection. Other adverse events included fever, fatigue, and changes in blood pressure.

In phase 1 studies that used weekly dosing of SC N-803, correlative data showed that highest level of immune stimulation, as measured by staining intensity of Ki67 in NK cells, increased concentration of circulating IFN-gamma was highest on day 4, and decreased with subsequent doses. [62] [63] This suggests that prolonged continuous treatment leads to immune anergy and reduced biological responsive. Thus subsequent studies using this agent have been amended to use less frequent dosing schedules for N-803 (NCT02989844). This study will use every 3 week dosing at 10 ug/kg given subcutaneously.

2.7 Areas of Investigation

The somatic mutational profile of AML (Cancer Genome Atlas Project) may be simpler than most solid tumors, with an average of only 13 mutations per gene, though it may be two-fold greater in secondary AML.[64] Cancers with high somatic mutation frequency have shown variable sensitivity to single agent PD1 blockade, from 50-60% clinical benefit with durable response in melanoma to low efficacy in squamous cell carcinoma of the lung, suggesting that other factors contribute to predicting response to checkpoint blockade. Within AML, Wilms Tumor (WT) has been identified as a potential neo-antigen targetable with a WTspecific cytotoxic T-cell response. Subgroups of AML with mutations including FLT3/*ITD* or NPM1 may also have neo-antigen targets and might

benefit from PD-1 blockade. The relative importance of neo-antigen burden compared to factors such as PD-L1 expression on tumor cells and an immune environment that promotes immune activation is unknown.

At cytotoxic doses, CTX may be able to alter the immunosuppressive microenvironment via downregulation of IDO. The effects of low dose continuous cyclophosphamide on IDO regulation have not been well studied. Since upregulation of IDO has been identified as a pathogenic feature of AML and may contribute to tolerogenic immune environment, the interaction of IDO and checkpoint blockade is of interest. [39]

The function and activity of dendritic cells to either induce antigen-directed cytotoxicity or anergy in T-cells in AML and MDS is an important variable in efficacy of PD-1/PD-L1 directed therapy. Dendritic cells have been classified into two groups, myeloid and plasmacytoid, based on expression of CD11c, both which can be derived from a malignant clone.[65] AML-derived DCs, particularly the plasmacytoid variant, have been reported to express tolerogenic cytokines such as IL-10 and secrete IDO, which induces an immunosuppressive Tregulatory response.[66] A study of MDS patients reported reduced numbers of peripheral blood DCs that were all clonally-derived.[67] In newly diagnosed AML patients, a small study showed a range of normal quantitative DC subsets, increased DC subsets, or no detectable DC subsets. Functional studies showed reduced ability to induce proliferative responses in naïve T-cells in DCs from AML patients compared to healthy controls. [68] Further understanding of the role of DCs in tumor evasion in the setting of relapsed/refractory AML and MDS may enhance delivery of effective immunotherapies.

2.8 Rationale

Immunotherapy is a highly promising area of investigation in the treatment of AML and MDS. The ability of alloHCT to eliminate leukemic blasts and leukemiainitiating cells/leukemia stem cells highlights its curative potential. Many patients are not eligible for alloHCT due to pre-existing comorbidities, lack of donor graft availability, older age, and excessive leukemia burden. Immune checkpoint inhibitors of the PD-1/PD-L1 pathway have widespread efficacy across multiple cancer types and favorable tolerability. This research hypothesizes that PD-1 inhibitors in combination with immunomodulatory agents will induce leukemiadirected immune stimulation in AML and higher-risk MDS. Low dose cyclophosphamide is an immunomodulator which has not been reported to cause severe myelosuppression in a population that commonly has neutropenia and increased risk of infection due to active disease. N-803 is a promising immunotherapeutic agent that is safe, has known synergistic activity with PD-1 inhibitors, and is highly potent for increasing frequency and activation of T-cells and NK cells. Immune dysfunction induced by active AML may benefit

from additional stimulation with an agent such as N-803 to induce T-cell cytotoxicity after PD-1 blockade.

3 Study Design

This phase I study consists of a randomized pilot sub-study of two dosage arms of CTX in combination with nivolumab and N-803 in stage I.

Arm 1 (CTX 50 mg PO daily + nivolumab IV) and Arm 2 (CTX 350 mg PO every 7 days+ nivolumab IV) previously enrolled randomized patients in a 1:1 ratio. Due to low response rates (no complete responses and only 2 patients with partial response) and 4 patients receiving ≤1 cycle of treatment due to rapid disease progression, arms 1 and 2 are now closed. In the amended protocol, the first 20 patients enrolled in the study are randomized to either Arm 3 (CTX 50 mg PO daily + nivolumab IV + SC N-803 every 3 weeks) or Arm 4 (CTX 350 mg PO every 7 days+ nivolumab IV + SC N-803 every 3 weeks) with 10 subjects in each arm. Nivolumab is dosed at 360 mg, unless the patient has had a prior allogeneic hematopoietic stem cell transplant (alloHSCT), in which case nivolumab is given at 180 mg IV every 3 weeks. N-803 is dosed at 10 ug/kg every 3 weeks and given subcutaneously.

Continuous stopping rules, defined as selected Grade 3 non-hematologic events, graftversus-host disease, and any Grade 4 or 5 non-hematologic event, will be used to monitor excessive toxicity for each arm.

Efficacy will be assessed with pooled total from both arms 3 and 4 with up to 20 patients available for assessment of OR at 42 days.

All patients receive nivolumab IV over 30 minutes every 3 weeks, N-803 SC every 3 weeks and oral cyclophosphamide as assigned for up to 5 treatment courses with each treatment course equal to 21 days. Nivolumab is dosed at 360 mg, unless the patient has had a prior allogeneic hematopoietic stem cell transplant (alloHSCT), in which case nivolumab is given at 180 mg IV every 3 weeks. N-803 is dosed at 10 ug/kg every 3 weeks and given subcutaneously.

Disease will be assessed by bone marrow biopsy at 21 and 42 days after initiation of study drugs. If the increase in absolute blast count is <25% in peripheral blood or bone marrow from baseline at day 42 or later and there is perceived clinical benefit by the treating physician, the subject will proceed with further cycles of treatment with up to 5 courses of treatment on study.

A final treatment visit occurs 30 days after the last dose of study drug with follow-up for response and survival continuing through 2 years from beginning of treatment. For

patients with clinical benefit after the 5 treatment courses, additional therapy with the study drugs or other treatment may be offered.

4 Patient Selection

Study entry is open to patients 18 years of age and older regardless of gender, race, or ethnic background. While there will be every effort to seek out and include minority patients, the patient population is expected to be no different than that of other relapsed/refractory acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome studies at the University of Minnesota.

4.1 Inclusion Criteria

4.1.1 ≥18 years of age

4.1.2 Meets one of the following disease criteria:

- Primary (de novo) AML or higher-risk MDS with induction failure: No CR after 2 or more induction attempts with chemotherapy, hypomethylating, agents or other agents; no CR after 1 induction attempt and not eligible for a 2nd induction. Higher risk MDS defined as risk score > 4.5 based on the revised IPSS criteria (refer to Appendix II).
- Secondary AML (from antecedent hematologic malignancy or treatment-related): Not in CR after 1 or more cycles of chemotherapy.
- Relapsed AML: Blasts ≥5% in bone marrow or peripheral blood after prior attainment of CR or biopsy-proven extramedullary leukemia in absence of bone marrow involvement; relapse at any time but currently ≥100 days following allogeneic HCT.
- Relapsed MDS: Morphologic evidence of relapse or increase in blasts to ≥5% in bone marrow or peripheral blood after prior hematologic improvement or partial or complete response and not eligible for or decline intensive chemotherapy; relapse at any time but currently ≥100 days following allogeneic HCT.

4.1.3 ECOG Performance Status ≤ 2 – refer to Appendix I

4.1.4 Adequate organ function within 14 days of study registration defined as:

- Absolute Lymphocyte Count: ≥ 500 cells/mm^3
- Hepatic: total bilirubin ≤ 3 x upper limit of institutional normal (ULN); ALT and AST ≤ 5 x ULN
- Renal: Serum creatinine ≤ 2 mg/dL
- Pulmonary: No oxygen requirement on room air or requiring ≤ 2L supplemental O_2

4.1.5 Ability to be off prednisone and other immunosuppressive drugs (>10 mg daily prednisone equivalent) for at least 14 days prior to and while receiving

N-803. Inhaled or topical steroids, and adrenal replacement steroid doses < 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease

4.1.6 Sexually active females of child bearing potential and males with partners of child bearing potential must agree to use effective contraception during therapy and continuing (23 weeks for females, 31 weeks for males) after the last dose of nivolumab

4.1.7 Voluntary written consent

4.2 Exclusion Criteria

4.2.1 Pregnant or breastfeeding –The agents used in this study fall under Pregnancy Category D - Drugs which have caused, are suspected to have caused or may be expected to cause, an increased incidence of human fetal malformations or irreversible damage. Women of childbearing potential must have a negative pregnancy test (urine or serum) within 7 days of study drug administration.

4.2.2 Prior allogeneic hematopoietic stem cell transplantation currently within previous 100 days (note patients with a prior alloHSCT receive nivolumab at the reduced dose of 180 mg); they are eligible after that date

4.2.3 Signs or symptoms of active graft versus host disease

4.2.4 Active pneumonitis or uncontrolled infection

4.2.5 Received chemotherapy drugs within previous 2 weeks

4.2.6 Estimated life expectancy <28 days in the opinion of the enrolling investigator

4.2.7 Previously treated with N-803 (or “ALT-803”)

4.2.8 Received cellular therapy product within 30 days

4.2.9 Allogeneic HSCT recipient receiving calcineurin inhibitor within 4 weeks

5 Screening and Study Enrollment

Written consent must be obtained prior to the performance of any research related tests or procedures. Consent is usually obtained before final eligibility is determined.

5.1 Consent and Study Screening in OnCore

Any patient who has been consented is to be entered in OnCore by the Primary Clinical Research Coordinator (PCRC) or designee. If a patient is consented, but not enrolled, the patient’s record is updated in OnCore as a screen failure and reason for exclusion recorded.

5.2 Study Enrollment and Treatment Assignment

To be eligible for study enrollment, the patient must sign the treatment consent and meet each of the inclusion criteria and none of the exclusion on the eligibility

checklist based on the eligibility assessment documented in the patient's medical record.

The Primary Clinical Research Coordinator (PCRC) or designee will complete the enrollment process in OnCore by assigning the treatment arm and entering the on treatment date.

Up to 20 patients enrolled in the study are randomized via OnCore at the time of study registration to either:
Arm 3: CTX 50 mg PO daily + nivolumab 360 mg (or if prior alloHSCT, 180 mg) IV every 3 weeks + SC N-803 10 ug/kg every 3 weeks or
Arm 4: CTX 350 mg PO every 7 days + nivolumab 360 mg (or if prior alloHSCT, 180 mg) IV every 3 weeks + SC N-803 10 ug/kg every 3 weeks

No additional patients will be enrolled to Arm 1 or 2 (closed). If Arm 3 or Arm 4 is terminated by the toxicity stopping rule, all subsequent patients will be assigned to the other arm if it is not terminated by the toxicity stopping rule to continue enrollment of up to 20 patients.

5.3 Patients Who Do Not Begin Study Treatment

If a patient is enrolled to the study, and is later found not able to begin the first dose of study drug, for whatever reason, the patient will be removed from study and treated at the physician's discretion. The study staff will update OnCore of the patient's non-treatment status (off study). The reason for removal from study prior to starting study treatment will be clearly indicated in OnCore. The patient will be replaced to fulfill study enrollment requirements.

6 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, antimicrobials, etc.).

6.1 Study Drug Administration

Participants will receive one of the two following treatment plans based on assignment at enrollment:

Arm 3: CTX 50 mg PO daily + nivolumab 360 mg (or if prior alloHSCT, 180 mg) IV every 3 weeks + SC N-803 10 ug/kg every 3 weeks or
Arm 4: CTX 350 mg PO every 7 days + nivolumab 360 mg (or if prior alloHSCT, 180 mg) IV every 3 weeks + SC N-803 10 ug/kg every 3 weeks

Each treatment course consists of 3 weeks with up to 5 treatment courses permitted. A window of ±3 days is permitted for the nivolumab infusion to accommodate scheduling difficulties (i.e. holidays, inclement weather).

To begin a new treatment course (Day 1) the following criteria must be met:

- Cyclophosphamide
  - No grade 3-4 oral mucositis or grade 3-4 cystitis ○ No signs or symptoms of new infection
- Nivolumab ○ No contraindication for continuation based on immune-related adverse events (see Section 7.1)
  - No signs or symptoms of new infection ○ No active GVHD
- N-803 ○ Previous injection site reaction is not showing signs of resolving (improving) based on measurement or intensity ○ No hypotension (systolic blood pressure <90 mmHg) not resolving with intravenous fluids
  - No contraindication for continuation based on immune-related adverse events (see Section 7.1)
  - No signs or symptoms of new infection ○ No active GVHD

Patients who have delay of nivolumab and/or N-803 greater than 3 weeks for toxicities that are at least possibly related despite supportive treatment should be considered to have study treatment discontinued, unless the treating physician considers that the patient may benefit from continued study treatment after resolution of AEs.
If nivolumab is delayed, N-803 and/or cyclophosphamide should continue if no contraindication is present.
If N-803 is delayed, nivolumab and/or cyclophosphamide should continue if no contraindication present.
If cyclophosphamide is delayed, nivolumab and/or N-803 should continue if no contraindication present.
If nivolumab and N-803 are both permanently discontinued, cyclophosphamide should be discontinued and patient should be taken off study.

6.1.1 Nivolumab (All Participants)

Nivolumab will be given at a fixed dose of 360 mg (or if prior alloHSCT, 180 mg) IV over 30 minutes every 21 days on Days 1 of each cycle for up to five 21-day courses.

Nivolumab will be administered in the outpatient setting; however, inpatient administration is permitted as medically appropriate if a patient is hospitalized. No premedication is required. Monitoring will be according to institutional guidelines with interruption or slowing of rate of infusion with grade 1 to 2 infusional toxicities and discontinuation of nivolumab with grade 3 to 4 infusional toxicities.

No dose modifications are permitted for nivolumab but dose may be withheld based on occurrence of significant immune-related AE Grade 2 or higher based on NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03, as described in Section 7.1.

6.1.2 Oral Cyclophosphamide (CTX) Administered Daily (Arm 3)

The patient should be instructed to take CTX once a day, every day, by mouth at approximately the same time each day. The tablet must be swallowed whole, with a full glassful of water. Cyclophosphamide tablets are best taken early in the day about an hour before a meal. However, to avoid queasiness, it may be taken with food. The patient should be encouraged to drink plenty of liquids.

If a dose is missed and it is more than 12 hours before the next dose is due, the patient should take the missed dose and retain the previous schedule. If the next dose is due in less than 12 hours, the patient must skip the missed dose, recording the dose as missed on the daily drug log.

6.1.3 Oral Cyclophosphamide (CTX) Administered Weekly (Arm 4)

The patient should be instructed to take CTX once weekly at approximately the same time each week. The tablet must be swallowed whole, with a full glassful of water. Cyclophosphamide tablets are best taken early in the day about an hour before a meal. However, to avoid queasiness, it may be taken with food. The patient should be encouraged to drink plenty of liquids.

If a dose is missed, it may be taken if less than 48 hours from missed dose. If greater than 48 hours from missed dose, the patient must skip the missed dose and resume at next scheduled dose if clinically appropriate.

6.1.4 N-803 (All Participants)

N-803 at 10 ug/kg is administered subcutaneously on D1 of a 21-day cycle for a maximum of 5 doses, or until stopping rules are met or patient refusal. A window of +/- 1 week if permitted for each N-803 injection in the event of scheduling issues (i.e. holiday, bad weather, or other scheduling issues). N-803 will be administered in the outpatient setting; however, inpatient administration is permitted as medically appropriate if a patient is hospitalized.

N-803 dosing is calculated using a weight obtained within 3 days prior to the first dose. For patients > 100 kilograms weight, the N-803 dose is calculated using a weight capped at 100 kg. The patient's weight is re-checked prior to each dose (within 7 days) and the dose re-calculated if ≥ 10% change from the weight used for the previous dose calculation. Rounding of a dose is permitted per institutional policy.

If the total subcutaneous dose is greater than 1.5 mL volume, the dose will be divided by the pharmacist into 2-3 subcutaneous injections as needed. Injections are given in the abdominal area. The injection site should be rotated per institutional guidelines and each injection site separated by at least 1 inch.

Pre-medication

Use of pre-medications is at the discretion of the treating physician on an individual patient basis. Acetaminophen may be given prior to the injection to reduce the intensity of the fever that often occurs a few hours later.

Required post N-803 dose monitoring

Patients will be observed for a minimum of 2 hours after the 1st dose of N-803 for immediate adverse events. Vital signs (heart rate, blood pressure, respiration, temperature, and oxygen saturation) will be documented prior to the N-803 injection and then at 30, 60 and 120 minutes with a ± 20 minute window for each time point.

If the 1st dose of N-803 is well tolerated, subsequent doses may be administered with a 30-minute post dosing observation period. Vital signs (heart rate, blood pressure, respiration, temperature, and oxygen saturation) will be documented prior to each N-803 injection and then at 30 minutes ± 10 minutes.

6.1.5 Dose Reduction

A one-time dose decrease to 6 mcg/kg is allowed for recurrent N-803 related constitutional symptoms (e.g. fever, fatigue, muscle aches) interfering with activities of daily living (ADL) despite pre-medication. Patients unable to tolerate the reduced dose will be discontinued from further N-803. Re-escalation of the dose is not permitted.

6.2 GVHD Management Guidance in recipients of allogeneic HSCT

New or worsening skin rash outside of injection site area and other GVHD manifestations should be managed per institutional guidelines. Refer to Section 7.3 for management of skin rash/injection site reaction within the vicinity of the injection.

GVHD will be graded using the University of Minnesota aGVHD Grading Scale (Appendix IV).

N-803 and Nivolumab will be permanently discontinued with diagnosis of new onset of GVHD.

6.3 Supportive Care

Concurrent Hydroxyurea use will be permitted for high blast count leukemia for up to 1 month after enrollment in study.

Steroids or other immune modulatory medications should be avoided unless required for treating a grade 3-4 Nivolumab or N-803-related adverse event.

Supportive Care will be provided per University of Minnesota institutional guidelines.

6.4 Duration of Study Treatment

Patients will receive five 21-day treatment courses unless any one of the following occurs:

- Unacceptable toxicity
- Progression as defined by increase in absolute blast count of ≥25% in peripheral blood or bone marrow by day 42 or later
- A different anti-cancer treatment is indicated
- Patient is non-compliant or refuses treatment
- In the opinion of the treating investigator, continuation of study therapy is not of benefit to the patient

A final treatment visit occurs 30 days after the last dose of study drug (maximum of 5 courses).

Any patient unable to receive at least 1 cycle of study therapy will be replaced.

If follow-up ends before Day 42 for a reason other than death or progression, the patient will be considered unevaluable and will be replaced per Section 14.

6.5 Duration of Study Participation

Follow-up for disease status and survival for study end points occurs at 6 months by clinic visit, review of the medical record or contact with a local medical doctor unless the patient is:

- Unevaluable (e.g. unable to receive at least 1 cycle of study therapy)
- enters hospice care
- refuses follow-up

After 6 months, follow-up for survival only by record review will continue until 2 years from study enrollment.

The date and cause of death should be entered into the follow-up tab in OnCore upon knowledge.

7 Management of Selected Toxicity

The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used to grade adverse events. A copy of the CTCAE v 4.03 can be downloaded: (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-0614_QuickReference_8.5x11.pdf).

7.1 Nivolumab and N-803

Immune-Related Adverse Events Management

Immune-related adverse events (irAEs) from nivolumab and N-803 will be managed depending on severity (NCI CTCAE 4.03 grade) as outlined below.

Grade 1 to 2: Treat symptomatically or with moderate dose steroids, more frequent monitoring.

Grade 1 to 2 (persistent): Manage similar to Grade 3 to 4 irAE

Grade 3 to 4: Treat with high dose corticosteroids

Any Grade 4 irAEs require treatment discontinuation with nivolumab and N-803 except for single laboratory values out of normal range that are unlikely related to study treatment as assessed by the Investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management

Any Grade 3 irAEs require withholding nivolumab and N-803 except for any of the following: Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management, transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1 and single laboratory values out of normal range (excluding Grade ≥ 3 liver function test increase) that are unlikely related to study treatment according to the Investigator, do not have any clinical correlate, and resolve to Grade ≤ 1 within 7 days with adequate medical management

Any Grade 2 irAEs should be managed as follows:

If a Grade 2 irAE resolves to Grade ≤ 1 by the next planned cycle, treatment may continue.

If a Grade 2 irAE does not resolve to Grade ≤ 1 by the next planned cycle, treatment should be withheld at next cycle. If at the end of the following cycle the event has not resolved to ≤ Grade 1, the subject should permanently discontinue treatment with nivolumab and N-803 (except for hormone insufficiencies, that can be managed by replacement therapy).

Upon the recurrence of the same Grade 2 irAE (except for hormone insufficiencies that can be managed by replacement therapy) in the same subject, treatment with nivolumab and N-803 should be permanently discontinued. Treatment of gastrointestinal, dermatological, pulmonary, hepatic, renal, cardiac, neurological and endocrine irAEs should follow guidelines set forth in the table below.

Gastrointestinal irAEs		
Severity of Diarrhea/Colitis	**Management**	**Follow-up**
Grade 1 Diarrhea: < 4 stools/day over baseline Colitis: asymptomatic	Continue nivolumab and N-803 therapy Symptomatic treatment (for example, loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2 or 3/4
Grade 2 Diarrhea: 4 to 6 stools per day over baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Withhold nivolumab and N-803 Symptomatic treatment	If improves to Grade ≤1: Resume nivolumab and N-803 therapy If persists > 5-7 days or recur • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol If worsens or persists > 3-5 with oral steroids, then treat as Grade 3 to 4

Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over Baseline; incontinence; IV fluids ≥ 24 hrs.; interfering with ADL Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: lifethreatening, perforation	Permanently discontinue nivolumab and N-803 for Grade 4 Withhold nivolumab and N803 for Grade 3 1.0 to 2.0 mg/kg/day methylprednisolone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider colonoscopy if needed	If improves, continue steroids until Grade < 1, then taper over at least 1 month If worsens, persists > 3 to 5 days, or recurs after improvement, add infliximab 5 mg/kg (if no contraindication), Note: Infliximab should not be used in cases of perforation or sepsis Grade 3: If improves to Grade ≤1: Resume nivolumab and N803 therapy

Dermatological irAEs		
Grade of Rash	**Management**	**Follow-up**
Grade 1 to 2 Covering ≤ 30% body surface area	Symptomatic therapy (for example, antihistamines, topical steroids) Hold nivolumab and N803, may resume if resolving after 1 week	If persists > 1 to 2 weeks or recurs: Consider skin biopsy Delay nivolumab and N-803. Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume nivolumab. If worsens. treat as Grade 3 or 4

Grade 3 to 4 Covering > 30% Grade 4: body surface area; life threatening consequences	Withhold nivolumab and N-803 for Grade 3 Permanently discontinue nivolumab and N-803 for Grade 4 or recurrent Grade 3 Consider skin biopsy, Dermatology consult 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent	If improves to ≤Grade 1, taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections Resume nivolumab and N-803 therapy
Pulmonary irAEs		
Grade of Pneumonitis	**Management**	**Follow-up**
Grade 1 Radiographic changes only	Consider delay of nivolumab and N-803 therapy Monitor for symptoms every 2 to 3 days Consider pulmonary and Infectious disease consults	If improves or resolves, continue planned therapy. Re-image at least every 3 weeks If worsens, treat as Grade 2 or 34
Grade 2 Mild to moderate new symptoms	Delay nivolumab and N-803 therapy Pulmonary and Infectious Disease consults Monitor symptoms daily, consider hospitalization 1.0 mg/kg/day methylprednisolone IV or oral equivalent Consider bronchoscopy, lung biopsy	Re-image every 1-3 days When symptoms improve to Grade ≤ 1, taper steroids over at least 1 month and then resume nivolumab and consider prophylactic antibiotics If not improving after 2 weeks or worsening, treat as Grade 3 to 4

Grade 3 to 4 Severe new symptoms; New / worsening hypoxia; life-threatening	Permanently discontinue nivolumab and N-803 Hospitalize Pulmonary and Infectious Disease consults 2 to 4 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	When symptoms improve to Grade ≤ 1, taper steroids over at least 6 weeks If not improving after 48 hours or worsening:, add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)
Hepatic irAEs		
Grade of Liver Test Elevation	**Management**	**Follow-up**
Grade 1 Grade 1 AST or ALT > ULN to 3.0 x ULN and / or total bilirubin > ULN to 1.5 x ULN	Continue nivolumab and N-803	Continue liver function monitoring If worsens, treat as Grade 2 or 3 to 4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and / or total bilirubin > 1.5 to ≤ 3 x ULN	Withhold nivolumab and N-803 Increase frequency of monitoring to every 3 days	If returns Grade ≤ 1, resume routine monitoring, resume nivolumab and N-803 If elevations persist > 5-7 days or worsens, 0.5 to 1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to Grade ≤ 1, taper steroids over at least 1 month, consider prophylactic antibiotics for
		opportunistic infections, resume nivolumab and N-803

Grade 3 to 4 AST or ALT > 5 x ULN and / or total bilirubin > 3 x ULN	Permanently discontinue nivolumab and N-803 Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections, Consult gastroenterologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If returns to Grade ≤ 1, 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 If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines

Renal irAEs		
Grade of Creatinine Elevation	**Management**	**Follow-up**
Grade 1 Creatinine > ULN and > than baseline but ≤ 1.5x baseline	Continue nivolumab and N-803 Monitor creatinine weekly	If returns to baseline: •Resume routine creatinine monitoring per protocol If worsens: •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	Delay nivolumab and N-803 Monitor creatinine every 2-3 days 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent Consider renal biopsy with nephrology consult	If returns to Grade 1: •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume nivolumab and N-803 therapy and routine creatinine monitoring per protocol If elevations persist > 7 days or worsen: •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	Permanently discontinue nivolumab and N-803 Monitor creatinine daily 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent Consult nephrologist Consider renal biopsy	If returns to Grade 1 : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

Neurological irAEs		
Grade of Neurological Toxicity	**Management**	**Follow-up**
Grade 1 Asymptomatic or mild symptoms; Intervention not indicated	Continue nivolumab and N-803	If returns to baseline: •Resume routine monitoring per protocol If worsens: •Treat as Grade 2 or 3/4

Grade 2 Moderate symptoms; Limiting instrumental ADL	Delay nivolumab and N-803 Treat symptoms per local guidelines Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent	If returns to baseline: • resume nivolumab and N-803 therapy when returned to baseline If worsen: •Treat as Grade 3-4
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	Permanently discontinue nivolumab and N-803 Obtain neurology consult Treat symptoms per local guidelines 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: Taper steroids over at least 1 month If worsens or atypical presentation: Consider IVIG or other immunosuppressive therapies per local guidelines

Cardiac irAEs		
Myocarditis	**Management**	**Follow-up**

Grade 2 Symptoms with mild to moderate activity or exertion	Withhold nivolumab and N-803 Hospitalize. Urgent Cardiology consult to establish etiology and rule-out immune-mediated myocarditis, consider myocardial biopsy; management o Troponin and BNP o ECG +/- continuous cardiac monitoring o Echocardiogram o Cardiac MRI Prompt initiation of 2 mg/kg/day methylprednisolone IV or equivalent	If symptoms improve and immune-mediated etiology is ruled out, resume nivolumab and N-803. If worsens, intensify treatment according to grade • Upon recovery, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms • Repeat cardiac MRI for post treatment assessment and cardiology follow-up • Retreatment may be considered after recovery and completion of steroid taper
Grade 3 or Grade 4	Permanently discontinue nivolumab and N-803 Hospitalize to intensive cardiac monitoring Cardiac evaluation to include: • Troponin and BNP monitoring • ECG +/- continuous cardiac monitoring • Echocardiogram • Cardiac MRI • Myocardial biopsy if feasible • Immediate initiation of 2 mg/kg/day methylprednisolone IV or 1 g IV bolus • Consider adding a second	Once improving, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms If no improvement or worsening, consider additional immunosuppressants (e.g. azathioprine, cyclosporine A) Repeat cardiac MRI for post treatment assessments and cardiology follow-up

	immunosuppressive agent Additionally, for Grade 4: • Hospitalize/transfer to institution with expertise in intensive cardiac monitoring • Consider ATG as second agent given its immediate effect	

Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue nivolumab and N-803 If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathy	Evaluate endocrine function Consider pituitary scan Withhold nivolumab and N-803 1 mg/day prednisone equivalents Initiate appropriate hormone therapy as indicated Consider Endocrinology consult No abnormal lab/pituitary MRI scan but symptoms persist: Repeat labs in 1-3 weeks / MRI in 1 month	If improves (with or without hormone replacement): Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections Resume nivolumab and N803 Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component

Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness	Delay or Permanently discontinue nivolumab and N803 Rule out sepsis Stress dose of IV steroids with mineralocorticoid activity IV fluids Consult endocrinologist If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy	

Nivolumab may induce graft versus host disease in patients who have had a prior allogeneic hematopoietic stem cell transplant. To reduce this risk, patients who have undergone a prior alloHSCT will receive a lower dose of nivolumab (180 mg instead of 360 mg).

Complications of allogeneic hematopoietic stem cell transplantation after nivolumab have been seen including severe or refractory graft versus host disease, hyper-acute GVHD, steroid requiring febrile syndrome without an identified infectious cause, encephalitis, and hepatic veno-occlusive disease. The consent contains language instructing the participant to inform the transplant team of previous nivolumab treatment if he/she undergoes a HSCT at any time in the future.

7.2 Oral Cyclophosphamide

Management of toxicities from oral cyclophosphamide will be per the package insert and standard institutional guidelines.

For Grade 2 or greater GI toxicities assessed as likely attributable to CTX, drug may be held for up to 2 weeks and restarted after improvement of symptoms.

For neutropenic fever with ANC <500 cells/mm^3, drug may be held for up to 2 weeks and restarted as assessed as clinically appropriate by the treating physician.

7.3 N-803

Hypotension (systolic blood pressure < 90 mm Hg)
N-803 dosing should be held for hypotension (defined as systolic blood pressure less than 90 mm Hg) if in the presence of any clinically significant symptoms (in the opinion of the treating physician), until the systolic blood pressure reading is stable. If mild dehydration is suspected, an IV fluid bolus may be used per standard of care.

Based on current experience, localized skin rashes are common with subcutaneous administration. If a rash occurs and the rash area surrounding the N-803 injection site is > 6 cm and symptomatic (painful and/or itchy), it may be treated (at the discretion of the treating physician) with topical 0.05% clobetasol propionate (i.e. 0.05% Cormax) or 0.1% triamcinolone (i.e., Kenalog) cream. Diphenhydramine may be administered pre- (25-50 mg TID orally) and postdosing (25-50 mg TID orally x 2 days) of N-803 at the discretion of the treating physician. Diphenhydramine should be eliminated if not tolerated.

8 Schedule of Tests and Procedures

Scheduled evaluations through during treatment may be performed ±3 days from the targeted date. The end of treatment visit may be performed ±7 days of the targeted date. Follow-up for disease status and survival for study end points occurs at 6 months by clinic visit, review of the medical record or contact with a local medical doctor. Followup for survival only will continue until 2 years from study enrollment. Collection of research samples are linked with the standard of care testing as no visit is done solely for research. Targeted days may be altered as clinically appropriate.

8.1 Required Clinical Care Evaluations

Evaluations/Tests and Procedures	Baseline (within 14 days of study registration)	During each treatment cycle (up to 5 cycles)			Final Treatment Visit (30 days post treatment)	Follow-up from C1D1
		Day 1[1]	Day 8	Day 15		
Consent	X					
Medical History	X					
Physical Exam	X	X			X	
Weight	X	X		X		
Brief Provider Assessment[1]		X	X	X		
ECOG PS	X					
Adverse Event Assessment including targeted toxicities		X	X	X	X	
CBC, diff, plt	X	X	X	X	X	
Complete metabolic panel (CMP)	X	X	X	X	X	
Pregnancy test for WOCBP[2]	X					
Bone Marrow Biopsy and Aspirate	X[4]	Prior to cycle 2 and 3				
Response assessment in BM		Prior to cycle 2 and 3				
Response assessment in peripheral blood		Prior to cycle 2, 3, 4, 5				
CT or PET-CT scan (extramedullary disease only)	X	Prior to cycle 3 and 5				
Survival (PFS and OS) by record review						X (through 2 years)
Nivolumab IV (charge to research)		X				
Cyclophosphamide PO (charge to research)[3]		X	X	X		
Cyclophosphamide drug diary and reconciliation		X			X	
N-803 SC (charge to research)		X				
N-803 Skin Rash Diary	X	X			X	

1 – include assessment for infection and bleeding

2 – women of child bearing potential must have a negative pregnancy test (serum or urine) within 7 days of each cycle day 1 3

-- cyclophosphamide administration per patient's assigned regimen (daily vs weekly)

8.2 Research Related Evaluations

	Screen	Weekly	Cycle 1			Cycle 2			Cycle 3 D1	Cycle 4 D1	Cycle 5 D1	Final Treatment Visit
			D1	D8	D15	D1	D8	D15				
Assessment of immune related AEs		X										X[6]
TSH (to FV lab)	X					X						X
Bone Marrow Aspirate (20 mL heparinized sample syringe or divided into 4 green top tubes, 2 x 2 mL tubes (4 mL) in Qiagen Paxgene syringe) at time of SOC BM bx[5]	X					X			X		X	
Peripheral Blood (40 mL heparinized serum divided into 3 heparin green top tubes and 1 red top tube)	X		X	X	X	X	X	X	X	X	X	X

4 – Bone Marrow Biopsies may be omitted if not medically safe to perform. If so, peripheral blood counts will be used for disease assessment. Baseline bone marrow biopsy may be within 21 days of study registration.

5 If additional bone marrow biopsies performed as standard of care, research aspirates may be obtained

6 – BMS requires SAE reporting for 3 months after last dose of nivolumab per Section 11.4.

All research samples are submitted to TTL unless otherwise noted.

Note: if a patient is not abiding by the required clinical care calendar (Section 8.1), the collection schedule of the toxicity data and research related samples may be altered or discontinued on an individual patient basis, as appropriate.

Research related testing on the bone marrow aspirate includes analysis of immune checkpoint receptor expression on T-cell and NK cell subsets by flow cytometry and bone marrow infiltration by immunohistochemistry. AML blast immune checkpoint ligand expression will be evaluated by flow cytometry. Gene expression analysis of genes will be performed by Nanostring Ncounter to evaluate T-cell immune activation

Research related testing on the peripheral blood includes analysis of T-cell and NK cell subsets, and myeloid blast immune checkpoint ligand expression by flow cytometry.

9 Disease Assessment Criteria

See Appendix II and Appendix III.

10 Drug Formulation, Availability and Preparation

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment. Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with un-preserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination. The total administered dose of study drug may be rounded up or down within a range of 5% of the actual calculated dose. Drug dosages need not be changed unless the calculated dose changes by at least 10%.

10.1 Nivolumab

Nivolumab (Opdivo) is a humanized IgG4 monoclonal antibody to PD-1. Its mechanism of action is to block an inhibitory signal that decreases cytotoxicity towards malignant cells. Nivolumab was initially FDA-approved in 2014 for treatment of unresectable or metastatic melanoma, and since has received approvals for treatment of metastatic non-small cell lung cancer, metastatic renal cell carcinoma, metastatic squamous cell carcinoma of the head and neck, relapsed classical Hodgkin lymphoma, and metastatic urothelial carcinoma. FDA-approved dosing is 240 mg every 2 weeks or 480 mg every 4 weeks for Hodgkin Lymphoma and squamous cell carcinoma of the head and neck. For NSCLC, renal cell carcinoma, and urothelial carcinoma, FDA-approved dosing is 240 mg every 2 weeks or 480 mg every 4 weeks. There have not been any observed differences in safety and efficacy between nivolumab dose of 3 mg/kg and 240 mg every 2 weeks in patients with melanoma, NSCLC, and RCC.

Availability

Cartons containing 5 vials of 100 mg, 10 mg/mL Nivolumab will be provided for the study.

Storage and Stability

Nivolumab must be dispensed only from official study sites and to eligible patients under the supervision of the site investigator. Nivolumab should be stored in a

secure area according to local regulations. It is the responsibility of the site investigator to ensure that study drug is only dispensed to patients on this study. Store Nivolumab under refrigeration at 2°C to 8°C (36°F to 46°F). Protect nivolumab from light by storing in the original package until time of use. Do not freeze.

The product does not contain a preservative. After preparation, store the nivolumab infusion either:

- at room temperature for no more than 8 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or
- under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation.

Administration

Refer to the current Investigator's Brochure for the most up to date information.

Nivolumab is to be administered as an intravenous infusion over 30 minutes according to institutional practice.

Toxicities

Refer to the current Investigator's Brochure for the most up to date information.

The most common (>10%) adverse events reported in patients treated with nivolumab are: fatigue, rash, pruritus, diarrhea, nausea, hypothyroidism, loss of appetite, constipation, musculoskeletal pain, peripheral neuropathy, and pyrexia. Events reported in 5-10% of subjects included pneumonia, vomiting, abdominal pain, hyperthyroidism, headache, dyspnea, ventricular arrhythmia, nephritis, cough, dry mouth, colitis, autoimmune hypothyroidism, thyroiditis upper respiratory tract infection and peripheral edema. Laboratory abnormalities reported in >10% of patients include hypertransaminasaemia (including ALT increased, AST increased, Transaminases increased), increased alkaline phosphatase, increased lipase, increased amylase, hyponatremia, and hyperkalemia.

As of the most recent Nivolumab Investigator Brochure, (v19) Fulminant type 1 diabetes mellitus is included as a SAR based on a comprehensive evaluation. Fulminant type 1 diabetes mellitus is characterized by rapid-onset diabetic ketoacidosis, low glycated hemoglobin (HbA1c) value, undetectable serum Cpeptide, and negative islet-related autoantibodies. Fulminant type 1 diabetes mellitus is generally believed to be a sub-type of non-autoimmune diabetes, but some reports show evidence of involvement of autoimmune mechanism in the

onset of fulminant type 1 diabetes. In cases observed in nivolumab clinical trials, most patients received insulin therapy and were able to control the event of diabetes mellitus. It has been established that nivolumab and other check point inhibitors may cause immune-related endocrinopathies, including diabetes. Clinical experts do not alter treatment or management strategies for onset of type 1 diabetes in patients on immunotherapy and do not recommend discontinuation of therapy in response to diabetes-related event.

10.2 Low Dose Cyclophosphamide

Cyclophosphamide is a synthetic alkylating agent chemically related to the nitrogen mustards. CTX received initial approval by the FDA in 1959. Leukemias are among the approved indications. Dosing can be oral or intravenous. CTX is activated by cytochrome P450, mainly CYP 2B6 and 3A4, to its active metabolite, phosphoramide mustard which forms cross links in DNA. The active dosing for malignant diseases is generally 1 mg/kg to 5 mg/kg PO per day for cytotoxic effect. For immunomodulatory effect, CTX has been given most commonly at 50 mg PO daily.[69]

Refer to the FDA-approved package insert for oral cyclophosphamide for product information, extensive instructions, and a comprehensive list of adverse events.

Availability

Oral cyclophosphamide in 25 mg and 50 mg tablets will be provided for the study.

Storage and Stability

Store at room temperature at or below 77 degrees F (25 degrees C) away from light and moisture. Brief storage not exceeding 86 degrees F (30 degrees C) is permitted.

Unopened vials of cyclophosphamide are stable until the date indicated on the package when stored at or below 25°C (77°F).

Administration

Oral, cannot be crushed

10.3 N-803 Formulation, Supply, and Potential Toxicity

N-803 (previously known as ALT-803), a recombinant human superagonist IL-15 complex, is the working name of the drug under investigation. Its active ingredient is N-803 and its pharmacologic class is an anti-cancer and anti-viral immunotherapeutic.

N-803 has been referred to as IL-15N72D:IL-15RαSu/IgG1 Fc complex in various preclinical study reports, publications, and other related documents.

10.3.1 Formulation and Composition

The biological drug product, N-803, is formulated in a phosphate buffered saline (PBS) solution. The solution appears as a clear and colorless liquid. The drug substance is produced by a recombinant mammalian cell line and is manufactured using a protein free media. The vialed quantitative composition of N-803 is listed in the table below.

Quantitative Composition of N-803

Component[a]	Concentration	Amount/Vial
N-803	1.0 mg/mL	0.6 mg
N-803	2.0 mg/mL	1.2 mg
PBS[b]	QS	0.6 mL[c]

a – N-803 is available in 2 different concentrations. The volume of PBS used is the same for both. b- PBS Formulation: Sodium Chloride (USP) 8.18 g/L; Sodium Phosphate Dibasic (USP) 2.68 g/L; Potassium Phosphate Monobasic (NF) 1.36 g/L pH 7.4. c- Fill volume is 0.6 mL, extractable volume is 0.5mL.

10.3.2 Structural Formula

N-803 is a soluble complex consisting of 2 protein subunits of a human IL-15 variant associated with high affinity to a dimeric IL-15R sushi domain/human IgG1 Fc fusion protein. The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D).6. The human IL-15R sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the IL-15R subunit (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the protein sequences are human. Based on the amino acid sequence of the subunits, calculated molecular weight of the complex comprising 2 IL15N72D polypeptides and a disulfide linked homodimeric IL-15RαSu/IgG1 Fc protein is 92.4 kDa. Each IL-15N72D polypeptide has a calculated molecular weight of approximately 12.8 kDa and the IL-15RαSu/IgG1 Fc fusion protein has a calculated molecular weight of approximately 33.4 kDa. Both the IL-15N72D and IL-15RαSu/IgG1 Fc proteins are glycosylated resulting in an apparent molecular weight of N-803 as approximately 114 kDa by size exclusion chromatography. The isoelectric point (pI) determined for N-803 range from approximately 5.6 to 6.5. Thus, the fusion protein is negatively charged at pH 7. The calculated molar extinction coefficient at A280 for N-803 is 116,540 M-1, or 1.26 OD280 for a 1 mg/mL solution of N-803, or one OD280 is equivalent to 0.79 mg/mL solution of N-803.

10.3.3 Storage and Handling

N-803 is supplied in a 2-mL single-dose/single-use vial containing 0.6 mL of N803 (extractable volume is 0.5 mL) at a concentration of 1 mg/mL or 2 mg/mL. Vials are packaged in cartons and shipped to the clinical site. Study medication must be maintained at a temperature between 2°C and 8°C.

10.3.4 Study Drug Preparation and Administration

N-803 dose calculation will be based on actual body weight; however for patients > 100 kilograms weight, the N-803 dose will be calculated using a weight capped at 100 kg. Refer to Section 6.1.4 for additional details.

The calculated amount of N-803 will be drawn into a syringe for subcutaneous injection. The current IDS stock concentration is 1 mg/ml. Doses will be drawn directly into the syringe for injection. If the total subcutaneous dose is greater than 1.5 mL, the dose will be divided into 2-3 subcutaneous injections as needed.

10.3.5 Agent Inventory Records

The investigator, or a responsible party designated by the investigator (e.g. institutional investigational pharmacy), must maintain a record of the inventory and disposition of study product using the Study Agent Drug Accountability Record.

10.3.6 Toxicity

The most common side effects seen in studies with subcutaneous (under the skin) injections have been, fever, chills, hypoalbuminemia, and injection site reaction, and skin rash, which at times has been widespread. These localized skins reactions are common (occurring in more than 50% of patients).

In June 2020 an SAE was filed on a study participant who developed atrial fibrillation (a-fib) with no prior history resulting in a hospitalization. We identified a previous case of a patient who developed a-fib while on treatment from a different clinical trial that used N-803 weekly to treat relapse. In both cases of afib, there were other potential explanations that could have triggered the arrhythmia. While we cannot directly attribute causality between N-803 and a-fib, it is at least possible that they are related, directly or indirectly. A-fib may be indirectly triggered in susceptible patients by fevers and/or use of diuretics for fluid retention that can be observed with some N803 regimens. Based on this information, we decided to add the development of a-fib as a possible rare side effect from N-803 in the consent form.

Very common	Common	Rare

• injection site reaction (skin rash), which may be large (> 2 inches), itchy, and/or painful • fever • chills • anemia • change in blood pressure • nausea • swelling of hands or feet • temporary changes in routine lab results including decreased albumin and decreased lymphocytes	• flu-like symptoms, including headache, muscle, or joint pain • fatigue • decreased appetite • diarrhea, vomiting • abdominal pain • itchy skin and/or skin irritation • shortness of breath • high blood sugar (hyperglycemia) • changes in electrolytes on routine lab work	• inflammatory reaction • infection including upper respiratory infection • atrial fibrillation (a-fib) – may be asymptomatic, but when symptoms do appear they may include irregular and often rapid heartbeat, shortness of breath, and fatigue

Tuberculosis is a potential risk of N-803. One instance of tuberculosis has been reported in a participant receiving N-803 in combination with BCG (a live, attenuated strain of Mycobacterium bovis) administered into the bladder by a urinary catheter. A causal relationship between N-803, in combination with BCG, and tuberculosis infection cannot be definitely ruled out.

Anti-N-803 antibodies have been detected in subjects receiving N-803. The impact of anti-N-803 antibody formation on clinical efficacy and safety of N-803 is unknown.

Previous editions of the N-803 Investigator's Brochure prior to 2019 relied heavily on clinical experience with the related cytokine therapeutic Proleukin® Interleukin-2 to anticipate potential risks associated with (N-803 administration. This approach was based on the fact that N-803 and IL-2 are both γ chain cytokines and thus could reasonably be predicted to have similar immunostimulatory properties. However, the substantial accumulated information on N-803 effects in humans presented the most recent IB indicates that many side effects of IL-2 are not observed in patients treated with N-803 at the doses being used clinically. For this reason, side effects observed in subjects treated with IL-2 but not N-803, such as capillary leak syndrome, pulmonary dysfunction, acidosis, and gastritis, have been removed from the IB and are reflected in this protocol and its consent form.

Refer to the current Investigator Brochure (version 7) for additional information.

10.4 N-803 in Combination with Nivolumab

The combination of N-803 + nivolumab has been associated with AEs of injection site reaction, flu-like symptoms, fever, fatigue, nausea, pain, chills, dizziness, hypotension, cough, dyspnea, anorexia, constipation, and vomiting.

11 Adverse Event Monitoring, Documentation and Reporting

Toxicity and adverse events will be classified and graded according to NCI's Common Terminology Criteria for Adverse Events version 4.03 (CTCAE) and reported on the schedule below. A copy of the CTCAE v4.03 can be downloaded from the CTEP home page (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf)

The following definitions of adverse events (AEs) and serious adverse events (SAEs) will determine whether the event requires expedited reporting via the OnCore SAE Report Form in addition to routine documentation in the OnCore AE case report form (CRF).

SAE Email Address: Worldwide.Safety@BMS.com
SAE Facsimile Number: +1 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/. The website will instruct you where to send the SAE forms.

The reporting timeframes for SAEs and other reportable events are located in Section 11.3.

Note: throughout this section the generic term “study drug” refers to the study related treatment (nivolumab, N-803, and cyclophosphamide). Adverse event monitoring will begin with the 1st dose of study drug.

11.1 Adverse Event Terminology

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Serious Adverse Event: An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

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

Unexpected Event: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Attribution of Event:

- 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.

The following definitions are from the Masonic Cancer Center's Standard Operating Procedure (SOP) Deviation Reporting:

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

11.2 Adverse Event Documentation

Adverse event monitoring and documentation begins with the 1st dose of the study drug through the final treatment visit. For the purposes of this study, adverse event documentation will focus on immune related adverse events (irAEs). Most commonly affected are the skin (rash/vitiligo/pruritis); the liver (hepatitis/rise in liver enzymes); the bowel (diarrhea/colitis), and the endocrine system (hypophysitis, thyroiditis, adrenal insufficiency). More rarely, uveitis, conjunctivitis, neuropathy, myopathy, and nephritis may occur. In addition, any unexpected grade 3 or greater toxicity at least possibility related to study treatment will be documented.

Patients self-administering oral cyclophosphamide will complete a drug diary to document administration and side effects.

At visits through the End of Treatment visit a targeted toxicity worksheet (Appendix V) will be completed to document select expected toxicities associated with N-803 as follows:

- Prior to each planned N-803 injection
- At the end of dose monitoring period (as described in Section 6.1.4) with ±20 minute window for 2 hour monitoring or ±10 minute window for 30 minute monitoring
- At the End of Treatment visit

Events that count toward an early stopping rule:

The following events may or may not meet the definition of a reportable event; however they will be reported to the University of Minnesota Primary Clinical Research Coordinator or designee as counting toward an early study stopping rule (refer to Section 14). These will be monitored throughout stage 1 of the study by treatment arm.

- Selected Grade 3 non-hematologic events ○ diarrhea ○ colitis ○ pneumonitis ○ transaminitis ○ myocarditis ○ adrenal insufficiency ○ rash that does resolve to grade ≤2 in 1 week
  ○ cystitis
- Any new onset of graft versus host disease
- Any Grade 4 or 5 non-hematologic adverse event
- Any adverse events requiring discontinuation of any study drug

11.3 Required Reporting FDA, IRB, and Masonic Cancer Center's SAE Coordinator

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/fax numbers
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm. refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xral0sh	Within 5 business days of event discovery	Report Form	irb@umn.edu
	Protocol or Subject Deviations	Per current IRB reporting requirements		
FDA	Unexpected and fatal or life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Days	UMCC SAE	Submit as an amendment to IND with copy to BMS
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 Calendar-Days		
	All other events per CFR 312.33	At time of IND annual report	Summary format	Submit as part of the IND annual report
BMS	Refer to Section 11.4			
Cancer Center SAE Coordinator	Any event that counts toward a stopping rule	Upon reporting	Event Form	n/a

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports or other relevant information.

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

11.4 Additional BMS Requirements for Nivolumab

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE (AE that is not an SAE) information should begin at initiation of study drug. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

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

The following laboratory abnormalities should be documented and reported appropriately:

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

Other Safety Considerations

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

Serious Adverse Events (SAEs)

BMS requires monitoring for Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing.

12 Study Data Collection and Monitoring

12.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore® (Online Enterprise Research Management Environment).

The Oncore database resides on dedicated secure and PHI compliant hardware consisting of 3 physical servers: dev, DR, and production. The dev server is located in the University of Minnesota (UMN) datacenter (WBOB) and houses six database instances (test, train, sandbox, mcc reports, oncdm, and vendor) that are backed up locally because the data is refreshed from Oncore production data. The production server is located in the UMN datacenter (WBOB). All the data servers are managed by the Academic Health Center – Information Systems (AHC-IS) virtual servers which utilize clustered infrastructure to provide real-time failover of virtual servers. This real-time clustering is physically limited to the UMN data center. All relevant AHC IS procedures related for PHI compliant servers (as required by the Center of Excellence for HIPAA Data) apply to Oncore databases.

The integrated data will be stored in PHI compliant servers managed by AHC IS with access given to those authorized users in the Clinical and Translation Science Institute Informatics team (CTSI BPIC and MCC CISS). The data will be integrated and extracted to researchers through the CTSI Informatics team and will be delivered through secure and compliant mechanisms (e.g. AHC IE data shelter, BOX, sftp, etc). If data de-identification is needed, then compliant AHC IE data de-identification tools will be used. The informatics team will grant the IRB approved study team members access to data.

Additional immune monitoring data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-directed correlative research samples is stored in their Laboratory Information Management System (LIMS). The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center

Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

12.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Primary Clinical Research Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

12.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp

For the purposes of data and safety monitoring, this study is classified as high risk (investigator initiated). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress at least quarterly.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in Section 11.3 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the Sponsor-Investigator will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

12.4 Monitoring

The IND sponsor/investigator will permit study-related monitoring, audits, and inspections by the Masonic Cancer Center or their designee, IRB, government regulatory bodies, and University of Minnesota compliance groups. All study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.) will be made available. The S/I will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

12.5 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

13 Endpoints

Primary endpoint:

Secondary endpoints:

Overall response rate (ORR) at 42 days from start of treatment defined as:

- ORR = [# CR/PR/HI for MDS] + [# CR + CRi + CRp + PR for AML] at day 42 divided by number of evaluable patients enrolled in study (Appendix II – R-IPSS Criteria for MDS, Appendix III – Modified Response Criteria for AML)

Progression free survival (PFS) at 6 months

- PFS is defined as the time (in months) from treatment start date to the date of progression or death, whichever occurs first. Patients who do not progress nor die will be censored on their last tumor assessment date. Patients who have no post-treatment tumor assessment, will be censored on their treatment start date.

6-month PFS rate will be estimated using the Kaplan-Meier method.

Overall survival (OS) at 6 months

- OS is defined as the time (in months) from treatment start date to death date. Patients who do not die will be censored on their last known alive date. 6-month OS rate will be estimated using the Kaplan-Meier method.

14 Statistical Considerations

14.1 Study Design

This is a phase I trial which includes a randomized pilot sub-study of two dosing arms of cyclophosphamide in combination with Nivolumab and N-803. The primary objective is safety and the secondary objective is efficacy, as defined by overall response rate. The study will consist of relapsed/refractory AML and higher-risk MDS.

The primary endpoint of the trial is to determine the appropriate dosing arm for phase 2 study based on safety and toxicity profile. The secondary endpoints include ORR at day 42, OS at six months and PFS at six months. Exploratory endpoints include correlation of immune checkpoint ligands PD-L1 and TIM-3 on leukemic cells with response to treatment, and evaluation of subsets of T-cell, NK cell, MDSCs, and DCs in bone marrow and peripheral blood during treatment.

By September 2019, there were 12 patients enrolled on Arm 1 and Arm 2 who received treatment with combination of nivolumab and cyclophosphamide daily or weekly. The previous treatment Arms 1 and 2 are closed due to no patients obtaining complete response and 4 patients receiving <1 cycle of treatment due

to disease progression. These 12 subjects in Arms 1 and 2 will be analyzed separately for safety and efficacy from Arms 3 and 4.

Arms 3 and 4 will enroll 20 patients with addition of N-803 to treatment plan. Patients will be randomized to Arm 3 (CTX 50mg daily + nivolumab 360 mg q3week + SC N-803 10 ug/kg q3week) and Arm 4 (CTX 350 mg PO q7 days+ nivolumab 360 mg q3week + SC N-803 10 ug/kg q3week) with 10 subjects in each arm. Continuous stopping rule will be used to monitor excessive toxicity for each arm. If one arm is terminated by the toxicity stopping rule, all subsequent patients will be assigned to the other arm if it is not terminated by the toxicity stopping rule to continue enrollment of up to 20 patients. If both arms succeed, then for future studies, selection of one of the two products will be based on evaluation of tolerability, clinical benefit as assessed by hematologic improvement and blast reduction, and immunological parameters. Immune stimulation will be assessed as decrease in T-regulatory cells and MDSCs, and increase in cytotoxic T-cells and differentiated NK cells.

1. A randomized comparison of two CTX treatments (CTX 50mg daily + nivolumab 360 mg q3week + N-803 10 ug/kg SC q3week vs. CTX 350 mg PO q7 days+ nivolumab 360 mg q3week + N-803 10 ug/kg SC q3week) using 10 patients in each Arm.
2. 20 patients available for assessment of day 42 overall response for the entire study population.

14.2 Data Analysis Plan

Only subjects enrolled after addition of N-803 to treatment protocol will be included in the data analysis. The endpoints of OR and toxicity rate will be estimated by simple proportion with 95% confidence intervals. All toxicity and safety assessments will be presented in the data listings and summarized with descriptive statistics.

The endpoints of overall survival (OS) and progression-free survival (PFS) at six months will be estimated by Kaplan-Meier curves.[70] Other correlative endpoints will be described with simple descriptive statistics such as medians, ranges, interquartile ranges, proportions, charts and plots. Logistic regression will be the primary method to compare the PD-L1 and Galectin-9 expression with response.

Groups that will be considered for comparison include: AML vs. MDS, high vs. low blast count at time of study enrollment, high, intermediate, or low disease risk

by cytogenetic and molecular subtype, high vs. low total peripheral lymphocyte count, high vs. low immune cell subsets in blood and bone marrow at time of study enrollment, younger vs. older age, number of prior treatments, high vs. low PD-L1 expression, and prior transplant vs. no transplant.

P-values for subgroup analyses will be adjusted for multiplicity.

All analyses will be conducted using SAS software 9.4 (SAS Institute Inc., Cary, NC). Results will be deemed statistically significant at the 0.05 significance level unless otherwise specified.

14.3 Sample Size Justification

This phase I study for the secondary endpoint of overall response uses pooled efficacy analysis. Based on this design, patients from both arms will be analyzed for efficacy analysis. Up to 20 patients will be used which is sufficient to maintain an overall type I error of 5% while providing 80% statistical power. The maximum number of the patients will be 20 and the minimum number of patients will be 18 to maintain statistical power of 77% if patients become unevaluable or withdraw from study.

Sample Size	Power
18	77%
20	80%
24	85%

This is based on our null hypothesis of ≤10% ORR versus the alternative hypothesis of ORR ≥32%.

The accrual will start after amendment to add N-803 to treatment protocol is implemented. The 12 patients enrolled by September 2019 treated without N803 will be analyzed separately. A patient will be considered unevaluable for the primary endpoint and replaced if unable to complete ≥1 cycle of nivolumab and cyclophosphamide. If patient receives ≥1 cycle of nivolumab and cyclophosphamide and follow up is not discontinued prior to 42 days for reasons other than progression or death, they will be considered evaluable.

ORR will be analyzed using the evaluable patient population.
PFS and OS will be analyzed using the all treated patient population.

14.4 Early Study Stopping Rule for Safety

Continuous stopping rules are in place to stop the study in case there are excessive complications. In the case that the boundaries are crossed, the Principal Investigator will be notified who will report it to the Masonic Cancer Center Data Safety Monitoring Committee and Bristol Myers Squibb. The protocol will be reviewed to determine if the protocol should be terminated. Stopping rules were developed using Pocock stopping boundaries.[71] The stopping rules will be applied separately for each platform.

Early stopping rule for each randomization study arm for subjects enrolled after amendment to add N-803 to treatment protocol is implemented.

The goal is to construct a boundary based on selected Grade 3 non-hematologic events that result in the permanent discontinuation of study drugs, new onset of GVHD, and grade 4-5 non-hematologic toxicities (CTCAE v4.03) such that the probability of early stopping is at most 10% if the toxicity rate is equal to 15% and our sample size is 10. With these stipulations, the study will be stopped if there are events in 2 out of 2, 3 out of 6, 4 at any time within a 60 day follow-up time period. If the actual probability of failure is 40% or 50%, the probability of reaching the boundary will be 66% or 85%. The stopping rule will be applied to Arm 3 and 4 separately.

15 Ethical and Regulatory Considerations

15.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

15.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

15.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved Consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

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Appendix I – ECOG Performance Status

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group.*Am J Clin Oncol.* 1982;5:649-655.

Appendix II – Revised International Prognostic Scoring System
(R-IPSS) Criteria for MDS

Prognostic Variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	-	Good	-	Intermediate	Poor	Very Poor
BM blast %	≤ 2%	-	> 2%-< 5%	-	5%-10%	> 10%	-
Hemoglobin	≥ 10	-	8 to 10	<8	-	-	-
Platelets	≥ 100	50-<100	< 50	-	-	-	-
ANC	≥ 0.8	< 0.8	-	-	-	-	-

Cytogenetic Prognostic subgroups	
Very Good	-Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

Risk category	**Risk score**
Very low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very high	> 6

Appendix III – Response Criteria For MDS and AML

Response to treatment will be defined per modified criteria as outlined below.[72][73]

Complete Remission
For subjects to be classified as being in CR, they must have bone marrow regenerating normal hematopoietic cells and achieve a morphologic leukemia-free state and must have an ANC > 1 x 10^9/L and platelet count ≥ 100 x 10^9/L and normal marrow differential with
< 5% blasts, and they will be RBC and platelet transfusion independent (defined as 1 week without RBC transfusion and 1 week without platelet transfusion). There should be no evidence of extramedullary leukemia.

Complete Remission with Incomplete Platelet Recovery (CRp)
For subjects to be classified as being in CRp, they must achieve CR except for incomplete platelet recovery (<100 x 10^9/L).

Complete Remission with Incomplete Hematologic Recovery (CRi)
For subjects to be classified as being in CRi, they must fulfill all the criteria for CR except for incomplete hematological recovery with residual neutropenia <1 x 10^9/L with or without complete platelet recovery. RBC and platelet transfusion independence is not required.

Partial Remission (PR)
For subjects to be classified as being in PR, they must have ≥50% bone marrow blast reduction or decrease to 5 to 25%.

Hematologic Improvement (HR)
For subjects to be classified as having HI, they must have pre-treatment hemoglobin of <11 g/dL, platelets of <100 x 10^9/L, and absolute neutrophil count < 1000 cells/mm^3. They should have improvement in hemoglobin by ≥1.5 g/dL, platelets by ≥30 x 10^9/L, and neutrophil count by 100% or 500 cells/mm^3. Responses should be sustained for at least 4 weeks.

Appendix IV – GVHD Grading Scales

Acute GVHD:

Consensus Clinical Stage and Grade of Acute GVHD (Glucksberg *et al*, 1974; Thomas *et al*, 1975, Przepiorka *et al*, 1995)

Stage	Skin	Liver	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dl	Diarrhea 500 – 1000 mL/day or 280 – 555 mL/m^2	No protracted nausea and vomiting
2	Maculopapular rash 25-50% body surface	Bilirubin 3.1 – 6.0 mg/dl	Diarrhea 1000 – 1500 mL/day or 556 – 833 mL/m^2	Persistent nausea, vomiting or anorexia
3	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dl	Diarrhea >1500 mL/day or >833 mL/m^2	
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dl	Severe abdominal pain, with or without ileus, or stool with frank blood or melena	

University Of Minnesota Acute GVHD Grading

Acute Grade	GVHD Skin Stage	Liver Stage	Lower GI Stage	Upper GI Stage
I	1-2	0	0	0
II	3	1	1	1
III	-	2-4	2-3	
IV	4	-	4	

- Each column identifies minimum criteria for organ grade.
- Each grade is based on maximum stage for each individual organ involved

e.g. Grade II = skin stage 3 and/or liver stage 1 and/or gut stage 1 and/or UGI stage 1

Late Acute and Chronic GVHD:

Late acute and chronic GVHD will be assessed using the National Institutes of Health (NIH) Consensus Criteria.

Patient ID: _____

~~Date of late acute or chronic GVHD diagnosis (mm/dd/yyyy)~~ / / **Onset of chronic GVHD was:**

- ○ Progressive (acute GVHD progressed directly to chronic GVHD)
- ○ Interrupted (acute GVHD resolved, then chronic GVHD developed)
- ○ De novo (acute GVHD never developed)
- ○

Chronic GVHD flare (symptoms reactivated within 30 days of drug tapering or discontinuation)

Karnofsky Performance status:

- ○ 100 Normal, no complaints; no evidence of disease
- ○ 90 Able to carry on normal activity, minor signs or symptoms of disease
- ○ 80 Normal activity with effort; some signs or symptoms of disease
- ○ 70 Cares for self; unable to carry on normal activity or do active work
- ○ 60 Requires occasional assistance, but is able to care for most of his/her needs
- ○ 50 Requires considerable assistance and frequent medical care
- ○ 40 Disabled; requires special care and assistance
- ○ 30 Severely disabled; hospitalization is indicated. Death not imminent
- ○ 20 Very Sick, hospitalization necessary, active supportive treatment necessary
- ○ 10 Moribund, fatal processes, progressing rapidly
- ○ 0 Dead

Diagnosis was based on:

- ○ Histologic evidence / biopsy proven
- ○ Clinical evidence
- ○ Both
- ○ Unknown

Maximum grade of chronic GVHD

- ○ Limited
- ○ Extensive

Overall severity of chronic GVHD

- ○ Mild
- ○ Moderate
- ○ Severe

Organ/System Involvement (check if yes)

- ○ Sclerosis of skin
- ○ Other skin or hair involvement (rash, ulcers, pruritus or itching, dyspigmentation, alopecia, lichenoid skin changes, etc)
- ○ Eyes (xerophthalmia (dry eyes), abnormal Schirmer's test, abnormal slit lamp, corneal erosion / conjunctivitis, etc)
- ○ Mouth (lichenoid changes, mucositis / ulcers, erythema, etc)
- ○ Bronchiolitis obliterans
- ○ Other lung involvement

Organ/System Involvement (check if yes)

- ○ Gastrointestinal tract (esophageal involvement, chronic nausea / vomiting, chronic diarrhea, malabsorption, abdominal pain / cramps, etc)
- ○ Liver
- ○ Genitourinary tract (vaginitis / stricture, etc)
- ○ Musculoskeletal (arthritis, contractures, myositis, myasthenia, etc)
- ○ Thrombocytopenia (< 100 x 10^9/L)

- ○ Eosinophilia
- ○ Autoantibodies
- ○ Other hematologic involvement
- ○ Serositis
- ○ Weight loss
- ○ Other organ involvement from chronic GVHD Specify

other organ:

Ref: Jagasia MH, Greinix HT, Arora M. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: The 2014 Diagnosis and Staging Working Group Report. Biol Blood Marrow Transplant. 2015 March; 21(3): 389–

Appendix V - N-803 Targeted Toxicity Worksheet

MT2017-33 CTCAE v4 Refer to Section 11.2 for time points

Patient Initials: _____ Date of Assessment: _____ Assessment Time point: _____

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
N-803 Injection site reaction	None	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated
Dyspnea	None or no change	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	……	Decreased O_2 saturation with exercise (e.g., pulse oximeter < 88%) intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or $PaO_2 \leq 55$ mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)
Fever	None	38.0 - 39.0◦ C (100.4 - 102.2◦ F)	> 39.0 - 40.0◦C (102.3 - 104.0◦F)	> 40.0 ◦ C (>104.0◦ F) for ≤ 24 hrs	> 40.0 ◦ C (>104.0◦ F) for > 24 hrs
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	……
Hypertension	None	Pre-hypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 99 mm Hg); medical intervention indicated; recurrent or persistent ≥ 24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated.	Stage 2 hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated.	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated.
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Edema	None	Localized to dependent areas, no disability or functional impairment	Moderate localized edema and intervention indicated; limiting instrumental ADL	Severe localized edema and intervention indicated; limiting self care ADL	……
Pneumonitis	None	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g. intubation or tracheotomy)
Headache	None	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	……
Confusion (Altered Mental Status)	None	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Rash (outside of the injection site reaction)	None	Covering < 10% body surface area (BSA)	Covering 10-30% body surface area (BSA)	>30% body surface area (BSA)	Generalized exfoliative, ulcerative, or bullous dermatitis
Gait Disturbance	None	Mild change in gait (eg, wide-based, limping or hobbling)	Moderate change in gait (eg, widebased, limping or hobbling); assistance device indicated; limiting instrumental ADL	Disabling; limiting self care ADL	……

Person Completing Form: _____ ADL = activities of daily living

Appendix VI – N-803 Injection Site Reactions Diary

The Diary is to be completed by the patient as a self-assessment in association with each dose of N-803.

A new diary must be started for each N-803 injection.

If the injection reaction has not resolved by Day 6 post-injection, continue to collect information on page 2 based on the days from that injection.

For inpatients, the diary is completed by study personnel.

Patient Number*		**Date of Study Drug Injection***	_____/_____/_____	*To be completed by the site.

Please answer all questions below <u>daily</u> for 7 days, beginning with day of treatment. Be sure to bring back this completed diary to your next clinic visit.

	Instructions	**Day of Study Drug Injection** ___/___/_____	**Day 1** **Post Injection** ___/___/_____	**Day 2** **Post Injection** ___/___/_____	**Day 3** **Post Injection** ___/___/_____	**Day 4** **Post Injection** ___/___/_____	**Day 5** **Post Injection** __/__/_____	**Day 6** **Post Injection** ___/___/_____
1. Is there redness at the injection site?	Check: Yes or No If yes, measure longest diameter in cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm
2. Is there firmness or swelling at the injection site?	Check: Yes or No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No
3. Have you experienced any pain or itching at the injection site?	Check the pain and/or itch box if present And tell us if the pain and/or itching is mild, moderate or severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe
4. Have you taken or applied any medication for injection site pain or itching?	Check: Yes or No Provide name of medication(s)	☐Yes ☐No Name(s):	☐Yes ☐No Name(s):	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:

5. Have you experienced any chills?	Check: Yes or No If yes, circle the worse severity of the chills: mild, moderate or severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe
6. Record your daily temperature upon waking (do not drink anything 5 minutes before taking your temperature)	Check: Yes or No If your temperature is 101°F for more than 24 hours notify your study doctor or research staff.	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM

Grading Injection Site Pain or Itching
Mild – Noticeable, does not interfere with activity
Moderate – Interferes with activity, limiting activities of daily living
Severe – Severely limiting self-care activities of daily living, incapacitating

Grading Chills
Mild – Mild sensitive of cold, shivering, chattering of teeth
Moderate – Moderate tremor of entire body, medication taken
Severe – Prolonged or severe, does not respond to medication

Page 2 – Please continue to complete for Injection Site Reaction if ongoing after page 1 has been filled out.

Patient Number*		**Date of Study Drug Injection***	_____/_____/_____	*To be completed by the site.

Complete daily for 7 days or until the injection site has resolved (returned to normal) . Please bring back this completed diary to your next clinic visit.

	Instructions	**Day 7** **Post Injection** ___/___/_____	**Day 8** **Post Injection** ___/___/_____	**Day 9** **Post Injection** ___/___/_____	**Day 10** **Post Injection** ___/___/_____	**Day 11** **Post Injection** ___/___/_____	**Day 12** **Post Injection** __/__/_____	**Day 13** **Post Injection** ___/___/_____
1. Is there redness at the injection site?	Check: Yes or No If yes, measure longest diameter in cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm
2. Is there firmness or swelling at the injection site?	Check: Yes or No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No
3. Have you experienced any pain or itching at the injection site?	Check the pain and/or itch box if present And tell us if the pain and/or itching is mild, moderate or severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe
4. Have you taken or applied any medication for injection site pain or itching?	Check: Yes or No Provide name of medication(s)	☐Yes ☐No Name(s):	☐Yes ☐No Name(s):	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:

Grading Injection Site Pain or Itching
Mild – Noticeable, does not interfere with activity
Moderate – Interferes with activity, limiting activities of daily living
Severe – Severely limiting self-care activities of daily living, incapacitating