A Phase 1 Trial of CD25/Treg-depleted DLI Plus Ipilimumab for Myeloid Disease Relapse After Matched-HCT

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TITLE: A phase 1 trial of CD25/Treg-depleted DLI plus Ipilimumab for myeloid disease relapse after matched-HCT

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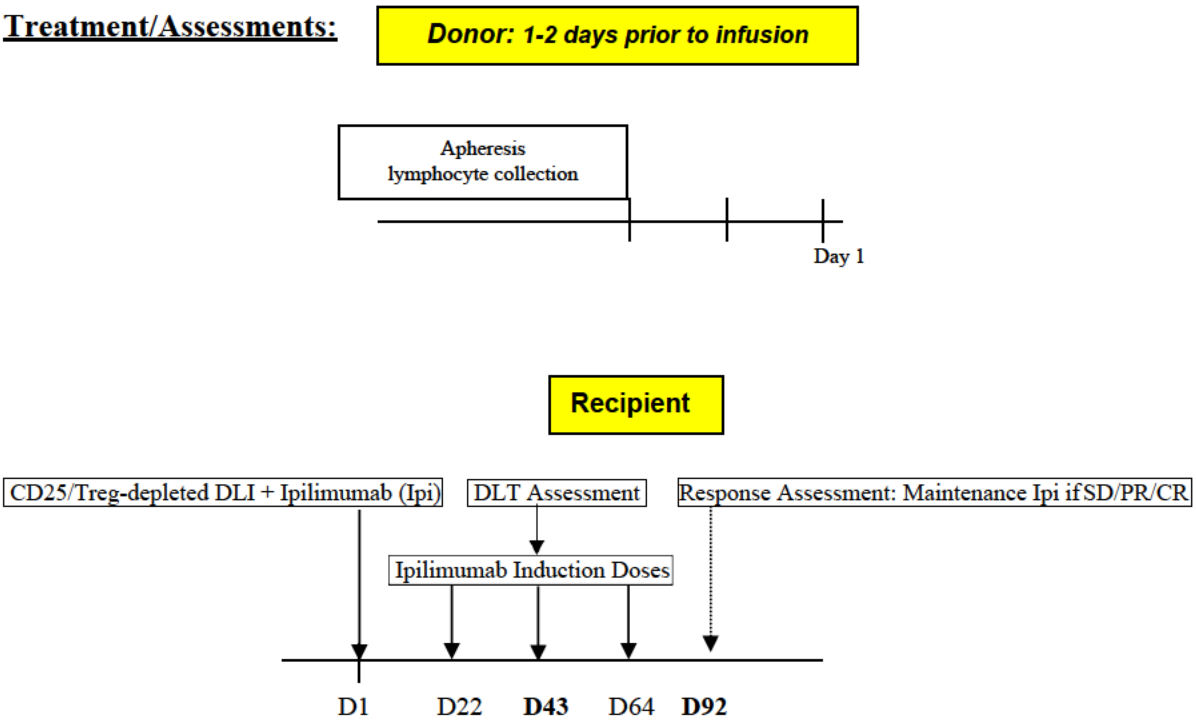
SCHEMA

Patient Eligibility:

- AML, MDS and MPN patients ≥18 years and relapsed ≥2 months after matched-HCT
- Adequate organ function and performance status
- No active GVHD, not on any systemic immune-suppression
- No uncontrolled active infection
- >20% T cell donor chimerism in the 4 weeks prior to cell infusion
- <50% BM involvement (% cellularity) in the 4 weeks prior to cell infusion

Treatment/Assessments:



Trial design: Phase 1A:

Level +2: CD25/Treg-depleted DLI $3x10^7$ $CD3^+$ cells/kg + Ipi 10 mg/kg (n up to 5)

↑ (≤1/5 DLT: MTD)

Level +1: CD25/Treg-depleted DLI $3x10^7$ $CD3^+$ cells/kg + Ipi 3 mg/kg (n up to 5)

↑ (≤1/5 DLT: dose-escalate)

Level 0: CD25/Treg-depleted DLI $3x10^7$ $CD3^+$ cells/kg + Ipi 1 mg/kg (n up to 5) (Starting Dose)

↓ (≥2/5 DLT: dose-de-escalate)

Level -1: CD25/Treg-depleted DLI $1x10^7$ $CD3^+$ cells/kg + Ipi 1 mg/kg (n up to 5)

Phase 1B: Additional 10 pts treated at MTD CD25/Treg-depleted DLI + Ipilimumab

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1. OBJECTIVES

1.1 Study Design:

This is an open-label, single-center Phase 1 study of CD25/Treg-depleted donor lymphocyte infusion (DLI) plus Ipilimumab in patients with AML, MDS, MPN who relapse after 8/8 HLA-matched hematopoietic cell transplantation (matched-HCT). Four dose levels combinations of CD25/Treg-depleted DLI plus Ipilimumab will be considered, starting at $3x10^7$ $CD3^+$ cells/kg and 1 mg/kg Ipilimumab. Cells will be infused on Day 1 and Ipilimumab will be administered every 3 weeks for 4 doses (days 1, 22, 43, and 64). Maintenance Ipilimumab every 3 months for 4 doses will be administered at weeks 24, 36, 48 and 60, to patients without disease progression at the day 92 (end of week 13) response assessment.

1.2 Primary Objectives

To determine the safety (MTD) of CD25/Treg-depleted DLI plus Ipilimumab in patients with myeloid relapse after matched-HCT.

1.3 Secondary Objectives

1. To determine complete remission (CR/CRi) rate at day 43 (6 weeks).
2. To determine the rate of progression-free survival (PFS) and overall survival (OS)at ~3 months (day 92) and ~1 year (week 60) post cell infusion.
3. To determine the ~3 month (day 92) incidence and severity of acute GVHD rates after cell infusion.
4. To determine the ~1 year (week 60) incidence and severity of chronic GVHD rates after cell infusion.

1.4 Correlative Objectives

1. To assess the immunologic impact of infusions of CD25/Treg-depleted DLI plus Ipilimumab. These include immunophenotypic analysis of T-cell subsets, B and NK cells; phenotypic and functional readouts of effector T (Teff) and regulatory T (Treg) cell activation status; changes in plasma cytokines and chemokines; tumor microenvironment; and tumor mutation profile.
2. To correlate clinical response with changes in circulating Teff vs. Treg cell counts and activation; the persistence of adoptively transferred Teff cells; changes in cytokine and chemokine levels; tumor infiltration by activated $CD8^+$ T cells; and tumor mutation profile, blast phenotype and tumor microenvironment.
3. In an exploratory analysis, to perform tumor neoantigen discovery to identify novel T cell targets and correlate tumor mutational load with response.

2. BACKGROUND

2.1 Introduction

For many patients with myeloid malignancies (e.g. acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN)), allogeneic hematopoietic cell transplantation (HCT) offers the only opportunity for cure. Unfortunately, myeloid disease relapse after transplant is associated with dismal outcomes, and remains a major obstacle to HCT success[1]. Currently, up to 40% of patients with AML/MDS undergoing HLA-matched related or unrelated HCT (matched-HCT) relapse[2,3]. HCT relapses are usually treated by withdrawal of IS, chemotherapy for interim disease control, and/or donor lymphocyte infusion (DLI) to invoke potentially curative graft-versus-tumor (GVT) immune responses. However, AML/MDS remissions after DLI are infrequent and short-lived, with poor patient survivals typically measured in months[4,5]. Clinical graft-versus-host-disease (GVHD) toxicity is common after DLI. Innovative approaches to enhance anti-tumor immune responses are urgently needed.

Immune escape (i.e. tumor evasion of the donor immune system) contributes to myeloid disease relapse after allogeneic HCT. In HLA-matched HCT, presentation of host hematopoietic and/or tumor epitopes within the concordant HLA context can invoke T cell receptor (TCR)-mediated recognition and elimination by donor-derived effector T (Teff) cells and induce curative GVT responses, a process that may be antagonized by suppression of co-stimulatory immune pathways, inhibitory regulatory T (Treg) cells, or by activation of inhibitory immune checkpoint pathways[6-8].

We therefore hypothesized that Treg cells in the DLI product could suppress GVT, and selective depletion of $CD25^{hi}$ cells (including constitutive $CD25^{hi}$ Treg cells) from the DLI product could boost its clinical efficacy, if severe clinical GVHD was avoided. In a phase 1 study we manufactured CD25/Treg-depleted DLI (via ClinMACS CD25 reagent system, Miltenyi Biotec GmbH), documenting its safety and promising efficacy at a dose of $3x10^7$ $CD3^+$ T cells/kg, highlighting complete durable remissions in relapsed AML/MDS[9].

We additionally hypothesized that blockade of the inhibitory CTLA-4 immune checkpoint with Ipilimumab Ipi may induce a GVT effect in patients in relapse after HCT. In a phase 1 trial of Ipilimumab monotherapy for patients with relapsed hematologic malignancies including AML/MDS after HCT, we documented the feasibility, safety and promising early efficacy of CTLA-4 blockade, including some complete durable remissions in patients with myeloid disease[10]. Response was associated with increased tumor infiltration of perforin-expressing Teff cells, and fewer circulating Treg cells.

We now propose to leverage our prior experience and combine CD25/Treg-depleted DLIwith CTLA-4 blockade via Ipilimumab to maximally augment Teff activity while avoiding infusion of suppressive Treg cells. We hypothesize that such combination therapy will enhance the efficacy of DLI in patients with myeloid disease relapse after matched-HCT.

2.2 IND Agents

2.2.1 CD25/Treg-depleted DLI for matched HCT relapse

Tregs are $CD4^+CD25^{hi}Foxp3^+$ lymphocytes that comprise approximately 5-10% of the circulating $CD4^+$ T cell population, that act to dominantly suppress autoreactive lymphocytes, and control innate and adaptive immune responses[11-18]. In murine studies, transfer of $CD4^+$ T cells depleted of Tregs induces autoimmunity, while transfer of $CD4^+$ Tregs is protective[19,20]. Patients with systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis exhibit deficiencies in Treg number and function[21-23]. The relationship between Tregs and autoimmunity prompted interest in their potential role in GVHD. Adoptive transfer of donor Treg at HCT can prevent or mitigate GVHD in animal models[24-26]. Several groups examined the PB of patients at various stages after HCT and found that the number of circulating Tregs inversely correlated with GVHD[27-30]. We previously reported that in-vivo Treg enhancement with daily subcutaneous (SC) low-dose interleukin-2 (IL-2) therapy can induce clinical responses in steroid-refractory chronic GVHD[31,32]. Tregs can also potentially suppress the GVT response. In vitro Tregs suppress proliferation of $CD4^+$ and $CD8^+$ Teff cells after polyclonal or antigenic stimulation[17]. In vivo Tregs diminish immune responses to solid and liquid tumors, including AML[33-36]. We therefore hypothesized that selective depletion of Tregs (by depletion of $CD25^{hi}$ cells) from the DLI product could boost its clinical efficacy, if severe clinical GVHD was avoided.

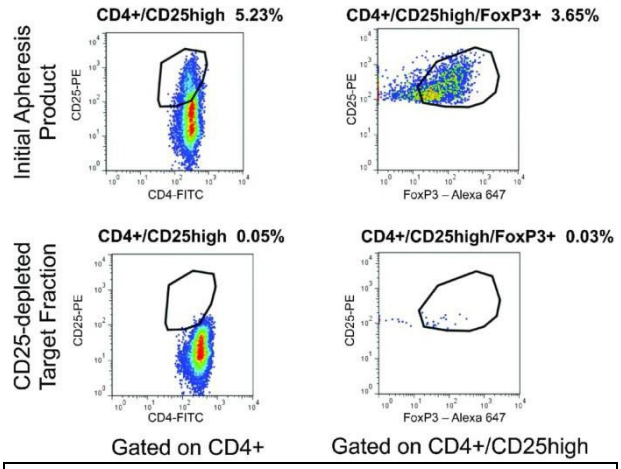

Fig 1: Flow Cytometric Analysis of CD4+CD25+FoxP3+ Cells Confirms Treg Depletion. The initial apheresis product (top plots) and CD25-depleted target fraction (bottom plots) indicate reduction in CD25hi cells (left) and FoxP3+ cells (right)

In a phase 1 study for patients with disease relapse after matched-HCT, we evaluated the feasibility of manufacturing Treg depleted DLI products as well as the safety and potential efficacy of these cells. Treg depleted DLI products were obtained by depleted $CD25^{hi}$ cells from donor apheresis products using antibody-conjugated magnetic beads (Miltenyi Biotech – CliniMacs)[9]. Sixteen of 21 subjects received prior cytoreductive therapy, but only four were in complete remission at time of infusion. Two DLI dose levels were administered: $1x10^7$ (n=6) and $3x10^7$ $CD3^+$ cells/kg (n=15). A median 2.3 log-depletion of $CD4^+CD25^{hi}FOXP3^+$ Treg cells was achieved in the released product (**Fig. 1**).

Infusion of CD25/Treg-depleted products was not associated with acute toxicity. Seven subjects (33%) developed clinically significant GVHD by one year, including one fatal case. At Dose Level 1, five treated patients demonstrated progression, while one patient had stable disease. At Dose Level 2 however, nine subjects (60%) achieved responses (8 CR, 1 PR), including seven who had active disease at time of DLI. One-year survival was 53%. When compared to unmodified DLI in contemporaneous patients meeting study eligibility (n=14), CD25/Treg-depleted DLI at dose-level 2 was associated with an improved response rate (60% vs. 14%, p=0.02) and 1-year event-free survival (EFS, 27% vs. 0%, p=0.0075, **Fig. 2**), with a reduction in

relapse incidence (67% vs. 100%, p=0.002), and no increase in non-relapse mortality (NRM). Importantly four of 8 (50%) subjects with AML relapse remained in remission at 1 year.

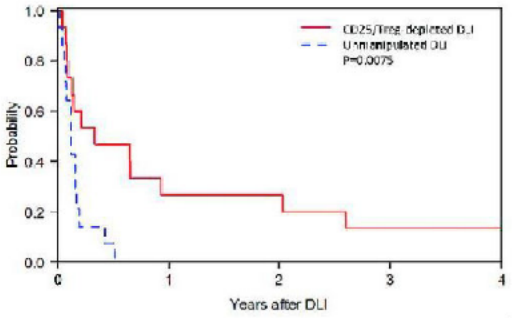

Fig 2: Clinical Outcomes after CD25/Treg-depleted DLI (dose-level 2). 1-year EFS benefit for Treg-depleted DLI (27%) vs. Unmanipulated DLI (0%).

In correlative analyses, while significant shifts within the naïve and memory T compartments of circulating $CD4^+$ T cells were observed at 1 and 2 months after CD25/Treg-depleted DLI, no differences were observed between responder and non-responder populations, perhaps due to limited sample size. While these results are promising, strategies to further enhance curative GVT responses by optimizing Teff activation (e.g., via CTLA-4 blockade as indicated below) are attractive.

2.2.2 Ipilimumab for post HCT relapse

The engagement of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1) receptors by their ligands B7-1/2 and PD-L1/2 respectively inhibits Teff function. Tumor cells often express these inhibitory ligands, thereby selectively blocking antitumor immunity. In murine models, PD-1 blockade led to an increase in GVHD, whereas selective blockade of CTLA-4 to treat late relapses after transplantation augmented GVT effects without accelerating GVHD[37,38]. We hypothesized that CTLA-4 blockade with Ipilimumab may induce a GVT effect in patients in relapse after HCT.

In a phase 1 study of Ipilimumab dosed at 3 and 10 mg/kg, we evaluated its safety and potential efficacy in 28 patients with relapsed hematologic malignancies post-transplant (12 AML patients, including 3 with leukemia cutis and 1 with myeloid sarcoma; and 2 patients with MDS)[10]. At the 10 mg/kg dose-level, Ipilimumab dose-limiting toxicity (DLT) included two cases of chronic GVHD of the liver and one case of grade II acute GVHD of the gut, all of which resolved with steroids but precluded further Ipilimumab administration. One patient died 42 days after initial dose of Ipilimumab, after grade 3 colitis and grade 4 pneumonitis developed. In the 3 mg/kg cohort, no responses were noted, but one patient with extramedullary AML of the breast had a tumor flare after two doses of Ipilimumab, and then a 27% decrease in the size of the mass at the end of Ipilimumab induction, maintained for 12 additional weeks. In the 10 mg/kg cohort, 7 of 22 patients (32%) had a response; 5 patients (23%) with a complete response (CR), including all 3 patients with leukemia cutis (**Fig. 3**), 1 with myeloid sarcoma involving lymph nodes, and 1 with MDS developing into AML. Four patients who had a response continued to have a durable remission for more than 1 year. In

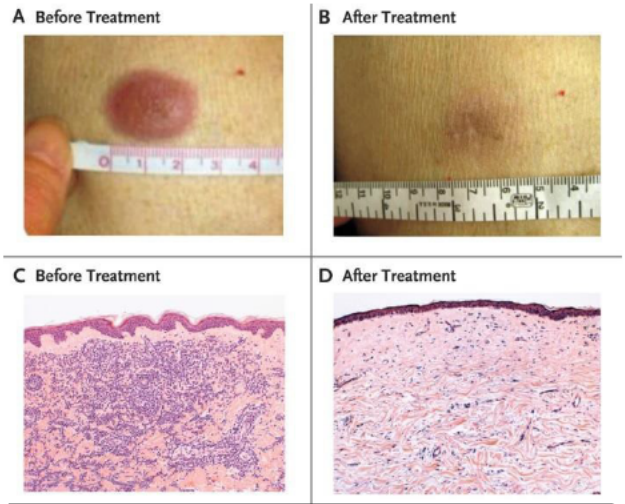

Fig 3: Clinical and Histopathological Responses to Ipilimumab in a Patient with Leukemia Cutis.

unpublished data, 5 mg/kg Ipilimumab dose appears less effective than 10 mg/kg, with similar toxicity. While promising, these data indicate a need for further enhancement of GVT beyond that obtained with Ipilimumab alone.

Exploratory immunohistochemical and gene-expression data indicated that in leukemia cutis, Ipilimumab therapy drove infiltration of cytotoxic $CD8^+$ T cells to the site of disease both in responders and non-responders, but $CD8^+$ T-cell perforin-1 up-regulation was more apparent in two responders compared to non-responders. PB analysis showed decreased circulating $CD4^+$ Tregs and increased $CD4^+$ Teff cells in patients who had a response. Moreover, in responders the proportion of activated $CD4^+$ Treg cells was less than in non-responders. These observations suggest that CTLA-4 blockade may be effective after allogeneic HCT by inducing an intratumoral GVT response, but that responses might be blunted by Tregs. Strategies to avoid infusing Tregs while de-repressing Teff cells might therefore help to further enhance therapeutic GVT responses.

2.3 Rationale

Based on the initial safety and promising efficacy observed in our phase 1 trials of CD25/Treg-depleted DLI and Ipilimumab individually, we hypothesize that combination therapy will be safe in patients with AML, MDS and MPN who relapse after matched-HCT and provide an enhanced curative effect for these patients with otherwise extremely poor prognosis.

2.4 Correlative Studies Background

Clinical trial administration of adoptively transferred CD25/Treg-depleted DLI plus Ipilimumab provides a unique opportunity to study their biology within patients. Peripheral blood (PB), bone marrow (BM) and accessible extramedullary disease site biopsy (e.g. leukemia cutis, if applicable) samples obtained before (at baseline), during, and after therapy (e.g. landmark time points like Day +30, Day +100 etc.,) and at the time of relapse (if any) will be obtained to assess the immunologic effects of treatment.

Viable cells as well as plasma will be isolated and cryopreserved at each time point using validated lab SOPs. Since all patients will have active relapse at the initiation of therapy, baseline samples will have tumor cells as well as normal donor immune cells. We will conduct detailed immunologic and tumor assessments, characterizing immune cells pre- and post-treatment, evaluating their functional activity, and assessing the tumor microenvironment to identify biologic predictors of response. We hypothesize that infusion of DLI preferentially depleted of Treg cells in combination with Ipilimumab will result in enhanced GVT mediated by donor Teff cells. To examine this hypothesis, correlative studies will use multiple orthogonal approaches to monitor changes in T cell activation, T cell specificity and T cell function in responders and non-responders. *Specifically, we will seek to correlate clinical response with 1) changes in circulating Teff vs. Treg cell counts and activation status* via flow cytometry and mass cytometric analysis*;*
2) the persistence of adoptively transferred Teff cells via TCR sequencing*;*
3) changes in cytokine and chemokine levels; 4) tumor infiltration by activated $CD8^+$ T cells; and 5) tumor mutation profile, blast phenotype and tumor microenvironment via unbiased single-cell expression profiling of the immune microenvironment (e.g. $CD8^+$ T, $CD4^+$ Teff, $CD4^+$ Treg, NK, B, DC, Macrophage, MDSC, blast cells) using specialized microfluidic platforms (e.g.

inDrop and 10x Genomics) we have optimized for use in cryopreserved BM, PB, and other single cell suspensions. In an exploratory analysis, we will perform tumor neoantigen discovery to identify novel T cell targets and correlate tumor mutational load with response.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Histologically or cytologically or molecularly confirmed relapse of AML, MDS or MPN including ***CMML, myelofibrosis or MDS/MPN***. Disease relapse includes patients with myeloid blasts by flow and/or morphology in marrow or extramedullary disease sites; or persistence and/or recurrence of disease defining mutations.

3.1.2 Relapse at ≥2 months after any 8/8 or better HLA-matched HCT

3.1.3 Available original stem cell donor.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of Ipilimumab in participants <18 years of age, children are excluded from this study.

3.1.5 ECOG performance status ≤2 (Karnofsky performance status ≥60, see **Appendix A**).

3.1.6 Recipient donor T cell chimerism ≥20% within 4 weeks prior to cell infusion.

3.1.7 <50% bone marrow involvement within 4 weeks prior to cell infusion.

3.1.8 No systemic corticosteroid therapy for GVHD (≤5 mg of prednisone or equivalent doses of other systemic steroids for non-GVHD, non-autoimmune indications for at least 4 weeks prior to cell infusion).

3.1.9 No other systemic medications/treatments (e.g. ECP) for GVHD for at least 4 weeks prior to cell infusion.

3.1.10 Ability to understand and willingness to sign written informed consents.

3.1.11 Adequate organ function as defined below:

- Total bilirubin: ≤1.5 x institutional upper limit of normal (ULN) (except Gilbert's or disease-related hemolysis, then < 3 x ULN)
- AST(SGOT)/ALT(SGPT): ≤3 x institutional ULN
- creatinine clearance: ≤1.5 x institutional ULN
- O2 saturation: ≥90% on room air
- LVEF >40%

3.1.12 The effects of CD25/Treg-depleted DLI and Ipilimumab on the developing human fetus are unknown. For this reason and because immunomodulatory agents are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study or within 23 weeks after the last dose of study drug, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and for at least 31 weeks after completion of Ipilimumab administration.

3.1.13 Negative pregnancy test for females of childbearing potential only

3.2 Exclusion Criteria

3.1.14 Extramedullary relapse involving immuno-privileged sites (e.g. CNS, testes, eyes). Other sites of extramedullary relapse (e.g. leukemia cutis, granulocytic sarcoma) are acceptable.

3.1.15 Participants who had cytotoxic chemotherapy or other investigational agents within 4 weeks prior to cell infusion (6 weeks for nitrosoureas or mitomycin C), or immunotherapy within 8 weeks prior, or those who have not recovered from adverse events due to agents administered more than 4 weeks prior. Use of hydroxyurea, HMAs (e.g. azacytidine, decitabine) and/or approved novel agents (e.g. venetoclax, FLT3 inhibitors, IDH1/2 inhibitors etc.) to control counts within 4 weeks prior to cell infusion is permitted.

3.1.16 Prior history of DLI

3.1.17 Prior history of treatment with anti-CTLA-4 or anti-PD-1 pathway therapy, or CD137 agonist therapy.

3.1.18 Prior history of severe (grade 3 or 4) acute GVHD, or ongoing active GVHD requiring systemic treatment.

3.1.19 Organ transplant (allograft) recipient.

3.1.20 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Ipilimumab or other agents used in study.

3.1.21 Autoimmune disease: Patients with a history of inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, are excluded from this study, as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [e.g., Wegener's Granulomatosis]) and motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome and Myasthenia Gravis). Patients with Hashimoto's thyroiditis are eligible to go on study.

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

3.1.23 Pregnant women are excluded from this study because of the unknown teratogenic risk. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother, breastfeeding should be discontinued if the mother is treated on this study.

3.1.24 HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with *agents used in this study.* Inaddition, these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.1.25 Individuals with active uncontrolled hepatitis B or C are ineligible as they are at high risk of lethal treatment-related hepatotoxicity after HCT.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT

OVERALL TREATMENT PLAN

This is a Phase 1 study of CD25/Treg-depleted DLI plus Ipilimumab for eligible patients with relapsed myeloid malignancies after 8/8 HLA-matched donor bone marrow or peripheral blood stem cell transplantation. Donor $CD25^{hi}$ cells will be depleted *ex-vivo* from peripheral blood mononuclear cells prior to infusion, and Ipilimumab will be administered after DLI. The study will follow a Phase 1 design in which matched-HCT recipients will receive escalating or de-escalating doses of CD25/Treg depleted donor lymphocytes and Ipilimumab. Patients will receive maintenance Ipilimumab per their assigned dose level.

5.1 Treatment Regimen

5.1.1 Patient preparation:

Prior to enrollment in the study, all patients will undergo a BM aspirate and biopsy (and/or biopsy of accessible extra-medullary disease sites, e.g. leukemia cutis) to confirm histologic or cytologic relapse. Patients may receive combination chemotherapy to control relapsed disease and/or re-induce remission if clinically indicated prior to DLI. Patients must have all cytotoxic or cytostatic agents (except hydroxyurea) discontinued at least 4 weeks prior to DLI (6 weeks for nitrosoureas or mitomycin C). Hydroxyurea may be instituted before or after the CD25/Treg-depleted donor lymphocyte infusion, if needed to temporarily control peripheral blood counts, after approval by the Study PI.

5.1.2 Donor Leukapheresis.

Prior stem cell donors will undergo apheresis to obtain donor lymphocytes. When possible, this

will be performed via peripheral intravenous access. Donor evaluation, consents and apheresis may be performed at other centers (including international sites), and will require completion of appropriate consents and medical evaluations, per each donor center's standard operating procedures (SOPs). Apheresis for this study does not entail any added risk to the donor compared with unmodified DLI.

We anticipate that sufficient numbers of CD3+ donor lymphocytes will be collected from a single apheresis. After apheresis, an aliquot of the product will undergo analysis to determine the dose of CD3+ cells. Dosing will be determined by the CD3+ cells/kg as defined in **Table 2**. An aliquot will also be removed for microbiologic/sterility testing. If the collected product does not contain sufficient CD3+ cells (i.e. at least 1.5X the number of CD3+ cells/kg planned for infusion) the unmanipulated product will be administered off-study, dosed per the providers preference, and another patient will be recruited instead. The product, if adequate, will be processed per CMCF standard operating procedure and infused within the stated product expiration time.

5.1.3 Preparation and Infusion of CD25/Treg-depleted cells.

CD25/Treg depletion: All cell processing will be performed at the DFCI cGMP CMCF (Connell O'Reilly Cell Manipulation Core Facility). Upon receipt of sufficient donor leukapheresis product from the donor center, the product will be selectively depleted of $CD25^{hi}$ Treg cells using the CliniMACS® CD25 Reagent System according to manufacturer's instructions. A detailed protocol for the preparation and use of the CliniMACS® CD25 reagent is provided to the clinical site in the CliniMACS® User Manual. Samples of the donor leukocyte preparation will be removed prior to processing and after completion of the $CD25^{hi}$ cell depletion. These samples will be used to determine cell viability, and enumerate the CD3+, CD4+, CD8+, CD14+, CD20+, CD25+, CD45+, CD56+, CD127+, and FOXP3+ cells (in combination as necessary, e.g. $CD4+CD25^{hi}FOXP3+$ cells) before and/or after processing. Gram stain, endotoxin and sterility testing will also be performed on the donor leukocyte preparation. The $CD25^{hi}$ depleted product will be denoted as 'TC-T Reg ($CD25^{hi}$) reduced cells'. **Release criteria are indicated in Table 1**. Product will be infused even if the final CD3+ cell dose/kg is below target at the maximum number of cells available after requisite QC samples obtained.

If the total product collected contains <1.5-fold excess of CD3+ cells/kg per planned infusion dose, the product will NOT undergo $CD25^{hi}$ depletion, and the unmanipulated product will be given to the patient, per institutional standards of care. The patient will not be assessable for the study objectives, and will be replaced by another patient.

If the total product collected contains ≥1.5-fold excess of CD3+ cells/kg per planned infusion dose, then the product will be $CD25^{hi}$ depleted with the CliniMACS CD25 device according to manufacturer instructions. After depletion, the volume of processed product infused will be adjusted to the targeted CD3+ cell dose per the dose level schema, while maintaining ≤0.5% $CD4+CD25^{hi}$ cells. There will be no add back of the depleted fraction enriched for $CD4+CD25^{hi}$ cells.

If the composition of the product after processing cannot meet both targets above, then the volume infused will be adjusted to best target their CD3+ dose per dose-level cohort. The patient will be unevaluable for DLT assessment.

Table 1: Product Release Criteria

Test Performed	Test Method	Release Criteria
Viable CD3+ cell dose*	Flow Cytometry	Dose Level +2: 3 x 10^7/kg Dose Level +1: 3 x 10^7/kg Dose Level 0: 3 x 10^7/kg Dose Level -1: 1 x 10^7/kg
CD4+ $CD25^{hi}$ cells	Flow cytometry	≤0.5% of viable lymphocytes
Viability	Trypan blue	≥70%
Endotoxin	Charles River Endosafe® -PTS™ System	< 5.0 EU/Kg/hr
Gram stain	BWH Microbiology laboratory	No organisms

* Acceptable range: 0.8-1 x 10^7 viable CD3+ cells/kg for DL -1; 2.5-3 x 10^7 viable CD3+ cells/kg for DL 0, +1, +2

Donor Lymphocyte Infusion: The donor lymphocyte product will not be administered until it has passed the release criteria for infection control monitoring, as defined by the DFCI CMGTL and the FDA (**Table 1**). If release criteria (except CD3+ cell dose and CD4+$CD25^{hi}$ cell fraction) are not met, the DLI product will NOT be infused, and the patient will be unevaluable for DLT assessment and will be replaced.

Once release criteria have been met, the DLI product will be infused into the recipient through a central or peripheral venous catheter, per the cell infusion SOP. The donor lymphocytes will be infused over 5-15 minutes. Acetaminophen 650mg PO and diphenhydramine 25mg IV or PO will be given as premedication prior to the infusion. Patients will be monitored for infusional reactions for 1 hour after the end of the infusion. Excess product, if any, will be cryopreserved per standard operating procedure for future use if necessary.

CD25/Treg-depleted DLI Dose Schema: Patients will receive a defined dose of $CD25^{hi}$ Treg depleted DLI. 5 patients will be enrolled, initially at dose level 0, and subsequent cohorts will be dose adjusted per the CD3+ dose escalation/de-escalation schema detailed below.

Table 2: CD25/Treg-depleted DLI Dose Escalation/De-escalation Schema Per Cohort

Cohort	***Viable CD3+ Dose* (#cells/kg**)***
Dose Level +2	**3 x 10^7**
Dose Level +1	**3 x 10^7**
Dose Level 0	***3 x 10^7***
Dose Level -1	**1 x 10^7**

** Recipient's body weight in Kg

** Acceptable range: 0.8-1 x 10^7 viable CD3+ cells/kg for DL -1; 2.5-3 x 10^7 viableD3+ cells/kg for DL 0, +1, and +2

5.1.4 Ipilimumab administration.

Ipilimumab will be administered intravenously over 90 ± 10 minutes after the DLI product observation period is completed, and dosing will continue in 3-week intervals for 4 cycles, unless unacceptable toxicity or symptomatic disease progression occurs (see Appendices E-F for disease progression criteria). MTD will be assessed at day 43 by DLT criteria as defined in **Section 5.4**. At their day 92 evaluation, patients without evidence of progressive disease or unacceptable toxicity can receive maintenance Ipilimumab **per their assigned dose** level on a q 3 monthly basis starting from week 24, for 4 doses.

5.1.5 Dose-escalation/De-escalation schema: Four dose-level combinations of CD25/Treg-depleted DLI plus Ipilimumab will be evaluated to determine MTD.

Table 4: CD25/Treg-depleted DLI plus Ipilimumab Dose Escalation/De-escalation Schema Per Cohort

Cohort	***Viable CD3+ Dose (#cells/kg*)***	***Ipilimumab Dose***
Dose Level +2	**3 x 10^7**	**10 mg/kg**
Dose Level +1	**3 x 10^7**	**3 mg/kg**
Dose Level 0	***3 x 10^7***	***1 mg/kg***
Dose Level -1	**1 x 10^7**	**1 mg/kg**

** Recipient's body weight in Kg

** Acceptable range: 0.8-1 x 10^7 viable CD3+ cells/kg for DL -1; 2.5-3 x 10^7 viableD3+ cells/kg for DL 0, +1, and +2

To ensure at least 3 patients will be evaluable within a group, a cohort of 5 eligible patients will enter at each dose level. If there are fewer than 3 evaluable patients in a cohort, additional patients will be added at that dose-level. Patients may be enrolled in the next cohort when 3 or more evaluable patients from the previous cohort have completed their day 43 assessment. Patients will be considered unevaluable for the determination of MTD if they require removal from the study due to alternative treatments for progressive disease (except hydroxyurea, with PI approval) or other reasons not related to an adverse event within 6 weeks after initial dosing. Additional patients may be enrolled at a specific dose cohort to substitute for patients within the cohort who are considered unevaluable for MTD. **There will be at least one-week interval between consecutive enrollments at the same dose level during phase 1A.**

Dose escalation or de-escalation is as follows: If ≤1 DLTs are observed within a cohort of up to 5 evaluable patients at dose level 0, then a dose escalation will take place. If ≤1 DLTs are observed at dose-level -1 or dose-level +2, this dose will be the MTD. If ≥2 DLTs are observed in a cohort of up to 5 evaluable patients, then the MTD is considered exceeded. If this is dose level 0, dose de-escalation will take place, and up to 5 patients will be enrolled at dose level -1. If ≥2 DLTs are observed at dose level +2, then dose level+1 will be the MTD. If ≥2 DLTs are observed at dose level -1, accrual will stop.

5.1.7 Further Therapy for Patients with Progressive Disease: Treatment with hydroxyurea to temporarily control counts, with PI approval, will be acceptable on study. Patients who require additional therapy due to progressive disease will come off study to be treated at the discretion of the treating physician. Patients who receive off-study therapy (e.g. chemotherapy) will be followed for 1 year following DLI infusion for survival outcomes. Phase 1a patients who are removed from study without evidence of toxicity prior to their week 6 assessment (e.g. for progressive disease) will be considered unevaluable for MTD assessment, and will be replaced as necessary. **There will be at least one-week interval between consecutive enrollments at the same dose level during phase 1A.**

5.1.7 Phase 1b: In phase Ib, an additional 10 patients will be enrolled at the MTD to better define toxicity and describe preliminary efficacy at this dose level. No patients will be replaced in this phase.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

In order to start on therapy, subjects must meet all of the inclusion criteria outlined in Section 3.1, and must not have any of the exclusion criteria outlined in Section 3.2.

5.2.2 Other Cycles

To proceed with subsequent cycles, subjects must not have:

- grade 2 or higher acute GVHD (see **Appendix C** for GVHD grading) requiring systemic immunosuppression
- extensive (or severe) chronic GVHD requiring systemic immunosuppression
- ongoing non-hematologic toxicity of grade 2 or higher unrelated to underlying malignancy or GVHD

Subjects will be allowed to proceed to subsequent cycles if they have ongoing:

- immune-related adverse events not defined as GVHD (e.g. diarrhea, pruritus, endocrinopathy, and rash) that have responded to corticosteroids and improve to grade 1 or less

5.3 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 6**. Dose modifications are not allowed in this study per **Section 6.1.3**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.3.1 Ipilimumab

Unit: Ipilimumab 5 mg/mL, in 10-mL or 40-mLvials

Route: Intravenous infusion

Appearance: Clear, colorless solution

Preparation, Handling, and Storage of Study Drug:

Please refer to **Section 8.1** for detailed information in this regard.

Drug administration and dosage schedule:

Induction Phase:

Ipilimumab is administered by IV infusion every 3 weeks for a total of four doses (Cycles 1-4). Infusions should be given over 90 +/- 10 minutes (not bolus or IV push).

Maintenance Phase:

Ipilimumab is administered by IV infusion every 12 weeks (3 months), beginning at Cycle 5, 24 weeks after the first dose of Ipilimumab, for up to 4 doses. Patients who show clinical benefit may be eligible to continue to receive Ipilimumab every 12 weeks beyond Cycle 8 with prior approval from the Study PI.

Dose delays are allowed as per the dosing criteria described later in this Section.

Dose Levels:

There will be three Ipilimumab dose levels in this study in separate cohorts:

Dose levels -1, 0: 1mg/kg

Dose level +1: 3 mg/kg

Dose level +2: 10 mg/kg

5.4 Definition of Dose-Limiting Toxicity (DLT)

Toxicities are to be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), Version 5.0 (**Appendix D**). DLT observation period is 42 days (6 weeks) after cell infusion plus Ipilimumab on day 1.

Definition of DLTs:

1. Development of solid organ/non-hematologic toxicity (exclusive of those attributable to

GVHD or Immune-related AEs) of persistent CTCAE grade 3 (i.e. without improvement to Grade 1 or less within 3 weeks) or grade 4 or higher (any duration) within 6 weeks of cell infusion and considered possibly, probably, or definitely related to cells and/or Ipilimumab. Non-clinically significant metabolic adverse events (abnormalities in serum sodium, potassium, chloride, magnesium, calcium, phosphorous, blood urea nitrogen,

and glucose that last less than 48 hours that respond to appropriate medical therapy) will not be considered DLTs regardless of CTCAE grade. Infections are expected in the context of allotransplantation and relapse, and will not considered DLTs.

2. Severe cytopenias (ANC < 500/µl and/or Plts < 20,000/µl) not related to the underlying disease or infections (e.g CMV) or other etiologies (e.g. emptying of relapsed bone marrow), occurring within 6 weeks after cell infusion, that persist despite 1 week of growth factor support (e.g., G-CSF or GM-CSF). Patients would be evaluated with a BM biopsy to assess for underlying disease vs. infection (e.g., CMV) vs. loss of relapsed host hematopoiesis. *If cytopenias are persistent, not explained by these or other transplant- or disease-associated causes, and possibly, probably or definitely related to cells and/or Ipilimumab, then the pancytopenia will be deemed a DLT.*
3. Acute GVHD: grade 3 or greater acute GVHD that does not improve to grade 2 or lower within 14 days of treatment.
4. Grade 3 or higher non-neurologic immune-related adverse events (e.g. diarrhea, pruritus, rash, endocrinopathies) that respond to corticosteroids and improve to grade 1 or less within 3 weeks will ***NOT*** count as DLTs. Biopsy of affected organs will be encouraged to help determine whether the immune-related condition is due to direct effects of Ipilimumab versus GVHD
5. CTCAE grade 3 and 4 infusion reactions related to the study DLI and/or the initial infusion of Ipilimumab.
6. Any grade 5 toxicity not due to underlying malignancy.

Management and dose modifications associated with the above adverse events are outlined in **Section 6** (Expected Toxicities and Dosing Delays).

Dose escalation will proceed within each cohort according to the scheme outlined in **Section 5.1.5**.

5.5 General Concomitant Medication and Supportive Care Guidelines

Antiviral, antifungal and antibacterial prophylaxis and monitoring should follow institutional practice as per the BMT-SOP. These typically include: daily acyclovir (or equivalent) for HSV prophylaxis, bactrim (or equivalent) for PCP prophylaxis, IV gammaglobulin for hypo-gammaglobulinemia, and azole (with mold activity) use for fungal prophylaxis as well as monitoring of beta-glucan and galactomannan levels. Growth factor support (e.g. GCSF, erythropoietin) and transfusion support is allowed, and can be used at the discretion of the investigators.

5.6 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression, and tolerance. In the absence of treatment delays due to adverse events,

treatment (including induction followed by maintenance therapy) may continue for 60 weeks or until one of the following criteria applies:

- Disease progression (as defined in **Appendices E-F**). As T-cell infiltration of the tumor site (e.g. granulocytic sarcoma, skin) can look like progressive disease, please discuss the results of clinical or radiographic assessments (e.g. leukemia cutis site inflammation; granulocytic sarcoma scans) showing progressive disease with the study PI before removing a patient from the study for disease progression. Patients with radiographic progression who are deriving clinical benefit will be allowed to remain on treatment until the next schedule efficacy assessment.
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s), requiring temporary or permanent stopping of Ipilimumab defined in **Section 6.1.3**
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant
- unacceptable for further treatment in the opinion of the treating investigator

5.7 Criteria for Taking a Participant Off Protocol Therapy

Participants will be removed from study when any of the criteria listed in Section 5.7 apply. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

Patients will be removed from the study for any of the following reasons:

- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- Dana Farber Cancer Institute decides to close the study

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator.

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5.8 Duration of Follow Up

Participants removed from treatment for unacceptable AEs will be tracked for 30 days from last study treatment to document AE outcome (resolution, improvement, persistence or progression). For all patients, data on survival, disease status (remission/progression/relapse), and alternative therapies (if any) will be tracked for 1 year after completion of last study treatment.

Participants will also be asked to allow for long-term follow up so that survival, relapse and late toxicities, should they occur beyond 1 year, may also be identified. Long-term follow up will be at DFCI if participants live locally or with their local oncology providers if they live remotely. For participants living remotely, phone calls to their local oncology providers may be made on a 6-month basis.

5.9 End of Study Definition.

The end of study is defined as any one of the following (whichever occurs first):

- The date of the 1-year follow-up
- The date of death
- Lost to follow-up
- Patient withdraws consent

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications during the study therapy will be made using the following recommendations. Toxicity assessments will be done using the CTEP Version 5.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All CTCAE grade 3 and higher events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting in addition to routine reporting.

6.1.1 Adverse Event List for CD25/Treg-depleted DLI

Unselected DLI for treatment of malignant disease relapse has been associated with Teff-mediated GVHD flare, and with myelosuppression primarily in cases of significant leukemia/lymphoma involving the bone marrow (owing to replacement of host hematopoiesis). However, infusion of the donor CD25/Treg-depleted cells was found to be safe in our phase I clinical trial. All patients will be closely monitored for any adverse effects including GVHD and marrow suppression.

- GVHD
  The severity of acute GVHD is measured according to the extent of involvement of each of the three organs: skin, liver, and GI tract. The current grading system for acute GVHD ranges from grade 0 (no GVHD) to grade IV (severe, life threatening), and is shown in Appendix 1. Details on the management of acute GVHD will be discussed in **Section 6.1.7**

- Pancytopenia
  Myelosuppression can be seen after DLI and may be pronounced in patients with active disease in the bone marrow at the time of DLI. The management of pancytopenia is outlined in **Section 6.1.8**

6.1.2 Adverse Event List for Ipilimumab

Ipilimumab can result in severe and fatal immune-mediated adverse events probably due to T-cell activation and proliferation. A listing is provided in **Section 7.2.1.2**. These can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune thyroiditis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, and adrenal insufficiency), ocular manifestations (e.g., uveitis, iritis, conjunctivitis, blepharitis, and episcleritis), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome. The majority of these reactions manifested early during treatment; however, a minority occurred weeks to months after discontinuation of Ipilimumab especially with the initiation of additional treatments.

6.1.3 Ipilimumab Dose Modifications/delays

Intra-patient dose modifications of Ipilimumab are not allowed in this study as lower doses have not been found to be less likely to lead to resolution of immune-related AEs that were seen at higher doses.

Decisions to delay an Ipilimumab dose will be made based on specified safety criteria. Treatment with Ipilimumab will be delayed or discontinued if the subject experiences at least one adverse event, specified below, considered by the

investigator to meet the criteria as outlined. The investigator should contact the Protocol Chair for any adverse event that will prompt a delayed dose or discontinuation of Ipilimumab. The following criteria will be used to determine dose delays, restarting doses, or discontinuing Ipilimumab:

It is necessary to delay study drug dosing for the following related adverse event(s):

• Any ≥ Grade 2 non-skin related adverse event (including irAEs), except for asymptomatic laboratory abnormalities
• Any ≥ Grade 3 laboratory abnormality
• Any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued dosing
• Any ≥ Grade 3 skin related adverse event regardless of causality

Restart Ipilimumab dosing if/when the adverse event(s) resolve(s) to ≤ Grade 1 severity or return to baseline within 3 weeks of initiating the dose delay (no more than 6 weeks since the most recent prior dose administration).

The maximum amount of time that induction Ipilimumab can be delayed due to adverse events before patients must be taken off treatment is 3 weeks, (i.e. 6 weeks between doses, respectively). Patients on maintenance Ipilimumab dosing must be taken off treatment if dosing is delayed for more than 4 weeks due to toxicity.

In the event of a dose delay:

• Certain study assessments, including bone marrow biopsy, T-cell chimerism, tumor measurement (CT or PET/CT), and/or correlative study samples associated with day 1 of the delayed cycle may be assessed on the original planned cycle day 1 (first day of dose delay) only (do not need to be re-assessed on actual cycle day 1, the day of cells and Ipilimumab infusion)
• In the event of a dose delay of the first dose (cycle 1, day 1) baseline bone marrow biopsy, radiographic assessment, T-cell chimerism do not need to be reassessed, even if those tests fall outside the required baseline time frames.
• If Ipilimumab dosing must be delayed for > 3 weeks (more than 6 weeks since the prior dose administration) for any of the above reasons, patients must come off treatment, except after approval by the study chair for individual cases with ongoing clinical benefit.

6.1.4 Ipilimumab Discontinuation

Criteria that Require Permanent Discontinuation of Ipilimumab:

Subjects MUST discontinue study treatment for any of the following reasons that are not related to a related adverse event:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Pregnancy
- Termination of the study
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

Subjects must permanently discontinue Ipilimumab if they experience any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued dosing.

The following neurological adverse events require permanent discontinuation of Ipilimumab and defines unacceptable neurotoxicity:

- Any neurologic motor toxicity ≥ Grade 3 regardless of causality
- Any ≥ Grade 3 treatment-related sensory neurologic toxicity

Ipilimumab need NOT be discontinued in the following situations:

- Potentially reversible inflammation (< Grade 4), attributable to a local antitumor reaction and a potential therapeutic response
- Subjects with the following conditions where in the investigator's opinion continuing study drug administration is justified
- Ocular toxicity that has responded to topical therapy
- Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy.

Note: Ipilimumab may not be restarted while the subject is being treated with systemic corticosteroids except for subjects on stable doses of hormone replacement therapy such as hydrocortisone for adrenal insufficiency.

6.1.5 Concomitant Treatments

Prohibited and/or Restricted Treatments

Subjects in this study may not have anti-tumor therapy (except hydroxyurea, with PI approval) for up to 4 weeks before and after dosing with Ipilimumab. Subjects must have not had immune suppression within 4 weeks of starting Ipilimumab. Patients started on systemic anti-neoplastic chemotherapy or immune suppression while on treatment will be taken off the study. The administration of live vaccinations while on treatment is prohibited. Short courses of corticosteroids for disease symptom control may be permitted after discussion with the Study Chair.

6.1.6 Study Procedures by Visit and Treatment Cycle

Results of all safety laboratory tests (that is, all chemistry and all hematology results) must be obtained and reviewed before day 1 Ipilimumab administration, as applicable. All laboratory results must be within the established range before Ipilimumab is administered. All induction period baseline laboratory samples must be collected within a window of up to 7 days before administration of Ipilimumab.

6.1.7 Suggested Guidelines for the Management of GVHD

Biopsy of affected organs (e.g. liver, gut) should be considered to distinguish GVHD vs. immune-related toxicity when clinically appropriate (e.g. prior to systemic immune suppressive therapy). Clinically significant acute GVHD (Grade ≥ 2) may be treated with systemic steroids equivalent to 1-2 mg/kg/d of prednisone equivalent given daily until the disease is under control, then tapered 10-20% per week thereafter at the treating physician's discretion. Cyclosporine, tacrolimus, or other medications may be added as adjunctive therapy at the discretion of the treating SCT physician.

6.1.8 Suggested Guidelines for the Management of Pancytopenia

Pancytopenia is a common and expected condition for patients after chemotherapy and conventional DLI. As such, we will institute the following plan of action to minimize the duration of pancytopenia to reduce the risk of infection:

If any patient has persistent severe cytopenia post cell infusion with ANC <500/µl and/or Plts <20,000/µl persisting for >2 weeks even after 1 week of growth factor support, they would be evaluated with a BM biopsy to assess for diseaseprogression vs. GVHD vs. infection (e.g., CMV) vs. loss of chimerism.

Patients with pancytopenia will be also evaluated for infections (e.g. CMV) or graft loss/disease progression which can also cause pancytopenia in post HCT setting. If the pancytopenia is deemed on bone marrow biopsy to be secondary to disease progression and graft loss (e.g. increased blasts, loss of donor chimerism), disease response (emptying of a diseased marrow, and fall of host chimerism) or if an alternate cause is found (e.g. CMV infection), it will not be considered a DLT.

A hematopoietic cell infusion (CD34 selected 'stem cell boost') from the original donor can be considered in cases where there is no evidence of persistent disease and persistent donor chimerism.

6.1.9 Immune-Related Adverse Events (irAEs) Reactions and Immune- mediated Adverse Reactions: Definition, Monitoring, and Treatment

For the purposes of this study, an immune-related adverse reaction is defined as an adverse reaction of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic,

infectious, metabolic, toxin or other etiologic causes prior to labeling an event an irAEs. Serologic, immunologic, and histologic (biopsy) data should be used to support the diagnosis of an immune-related toxicity. Suspected immune-related adverse reactions must be documented on an AE or SAE form. Another term for an irAE is an immune-mediate adverse reaction, as it is termed in the Ipilimumab US Prescribing Information. Both terms may be used in this protocol document. Patients should be informed of and carefully monitored for evidence of clinically significant systemic immune-mediated adverse reactions (e.g., systemic lupus erythematosus-like diseases) or organ-specific immune-mediated adverse reaction (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an immune-mediated adverse reaction is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary.

It is unknown if systemic corticosteroid therapy has an attenuating effect on Ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from Ipilimumab. If utilized, corticosteroid therapy should be individualized for each patient.

Recommended guidelines for specific immune-mediated adverse reactions are included in **Section 6.1.13** below, in **Appendix G**, and the Ipilimumab package insert. These recommendations should be utilized as clinically appropriate for the treatment of individual patients. Please contact the study PI directly for any questions.

6.1.10 Other Guidance

The following guidance is provided for the management of Ipilimumab treatment related events. These recommendations, treatment algorithms in **Appendix G**, and further information in the IB, should be considered in the context of appropriate medical treatment for each patient. Subjects with ongoing adverse effects due to Ipilimumab at the conclusion of the study will be provided additional medical care as needed by the investigators.

6.1.11 Treatment of infusion reactions associated with Ipilimumab

Since Ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of Ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. Such reactions were however not seen in our prior phase 1 trial for relapse after HCT. No prophylactic pre-medication should be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations.

For mild symptoms (*e.g.*, localized cutaneous reactions such as mild pruritus, flushing, rash):

- Decrease the rate of infusion until recovery from symptoms, remain at bedside

and monitor patient.
- Complete the Ipilimumab infusion at the initial planned rate.
- Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring
- Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses of Ipilimumab

For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mmHg):

- Interrupt Ipilimumab.
- Administer diphenhydramine 50 mg IV.
- Monitor patient closely until resolution of symptoms.
- Corticosteroids may abrogate any beneficial immunologic effect, but may be administered after approval by Study PI
- Resume Ipilimumab infusion after recovery of symptoms
- At the discretion of the treating physician, Ipilimumab infusion may be resumed at *one half the initial infusion rate, then increased incrementally to the initial infusion rate.*
- If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional Ipilimumab should be administered that day
- The next dose of Ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
- At the discretion of the treating physician, additional oral or IV antihistamine may be administered prior to dosing with Ipilimumab

For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure < 80 mm Hg, or angioedema):

- Immediately discontinue infusion of Ipilimumab, and disconnect infusion tubing from the subject.
- Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed
- Patients should be monitored until the investigator is comfortable that the symptoms will not recur
- No further Ipilimumab will be administered without approval from study PI

In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

6.1.12 Treatment of Ipilimumab-Related Isolated Drug Fever

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the fever is related to the Ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at approximately 6 and 12 hours after Ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further Ipilimumab.

6.1.13 Monitoring and Management of Immune-mediated Adverse Reactions

We anticipate that Ipilimumab immune-mediate adverse reactions and GVHD will be difficult to distinguish clinically. No increase in GVHD was seen in the low dose, single-administration study of Ipilimumab in patients with hematologic malignancies post alloSCT. Therefore, for the present study, will need to use the experience with Ipilimumab in studies of solid tumor patients to guide us in distinguishing immune-related AEs from GVHD. Below is an overview of the approach to immune-related AEs utilized on prior Ipilimumab studies, which we will follow for the present study. In the present study, we will also encourage biopsy of the affected organ so that more can be learned about how to distinguish immunerelated AEs from GVHD pathologically.

Immune-mediated Enterocolitis
The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia, with or without fever. However inflammation may occur in any part of the GI tract including esophagitis and gastritis. Fatalities due to GI perforation have been reported in clinical trials of Ipilimumab. Patients should be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of Ipilimumab therapy should be evaluated to exclude infectious or alternate etiologies.

In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation with biopsy to establish etiology and for persistent or severe symptoms. Withhold Ipilimumab dosing for any patients with enterocolitis pending evaluation; administer anti-diarrheal treatment and, if persistent evaluate with colonoscopy and biopsy (to distinguish GVHD vs. immune-mediated inflammation) and initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

Permanently discontinue Ipilimumab in patients with severe enterocolitis and evaluate with colonoscopy and biopsy (to distinguish GVHD vs. immune-mediated inflammation), and initiate systemic corticosteroids at a dose of 1 to 2

mg/kg/day of prednisone or equivalent. Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In clinical trials, rapid corticosteroid tapering has resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Immune-mediated Hepatitis and Pancreatitis

Hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Fatal hepatic failure has been reported in clinical trials of Ipilimumab. Serum transaminase and bilirubin and lipase levels must be evaluated before each dose of Ipilimumab as early laboratory changes may be indicative of emerging immune-related hepatitis/ pancreatitis and elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms. Increase in LFT or total bilirubin should be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or other medications, and monitored until resolution. Liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages), and biopsy should be considered in patients with grade 2 and higher hepatotoxicity (to distinguish GVHD vs. immune-related hepatotoxicity).

Monitor liver function tests (hepatic transaminase and bilirubin levels, lipase) and assess patients for signs and symptoms of hepatotoxicity/ pancreatitis before each dose of Ipilimumab. Withhold Ipilimumab in patients with grade 2 hepatotoxicity and consider liver biopsy (to distinguish GVHD vs. immune-related hepatotoxicity).

Permanently discontinue Ipilimumab in patients with grade 3–5 hepatotoxicity/ pancreatitis, consider liver biopsy (to distinguish GVHD vs. immune-related hepatotoxicity), and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When liver function tests show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month.

Immune-mediated Dermatitis

Skin immune-related AEs presented mostly frequently as a rash and/or pruritus. Some subjects reported vitiligo associated with Ipilimumab administration. Fatal toxic epidermal necrolysis has been reported in clinical trials of Ipilimumab.

Monitor patients for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

For mild to moderate dermatitis, such as grade 2 localized rash and pruritus, treat symptomatically. For persistent grade 2, grade 3, or greater, topical steroids may be administered. Administer topical or systemic corticosteroids as indicated if there is no improvement of symptoms within 1 week.

Withhold Ipilimumab dosing in patients with moderate to severe signs and symptoms.

Permanently discontinue Ipilimumab in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month.

Immune-related Neurological Events

Fatal Guillain-Barré syndrome has been reported in clinical trials of Ipilimumab. Patients may present with muscle weakness and myasthenia gravis, cranial nerve palsy (nVII Bells palsy), and aseptic meningitis, encephalopathy.

Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 days should be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, nerve entrapment, and medications should be excluded as causes.

Withhold Ipilimumab dosing in patients with any evidence of neuropathy pending evaluation.

Permanently discontinue Ipilimumab in patients with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies.

Immune-mediated Endocrinopathies

Ipilimumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thryroiditis with positive antibodies) and adrenal glands, hypophysitis, hypopituitarism, and resulting thyroid and adrenal insufficiency, low ADH, prolactin, FSH, LH. Hyperthyroid with Graves' disease and positive antibody has been reported.

Patients may present with subtle and nonspecific symptoms. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, and electrolyte disturbances including hyponatremia and hypotension. Adrenal crisis as a cause of the patient's symptoms should be excluded. Most of the subjects symptomatically improved with hormone replacement therapy. Long-term hormone replacement therapy with HC and levothyroxine will typically be required for subjects developing hypophysitis/hypopituitarism after treatment with Ipilimumab. Some patients have regained partial function following steroid treatment.

Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated and drug withheld pending evaluation. Patients may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

Monitor thyroid function tests and clinical chemistries at the start of treatment and recheck if signs/symptoms of thyroid dysfunction arise. Withhold Ipilimumab dosing in symptomatic patients. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent, and initiate appropriate hormone replacement therapy.

Other Immune-mediated Adverse Reactions, Including Ocular Manifestations
Ocular inflammation, manifested as grade 2 or grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few subjects (<1%) and occasionally occurred in the absence of clinically apparent GI symptoms. Other presumed immune-related AEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, immune-related (aseptic) meningitis, immune-related nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which were individually reported for <1% of subjects.

Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis. Permanently discontinue Ipilimumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Immune-related Cardiac Toxicities

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

Overall, immune-related AEs commonly started within 3 to 10 weeks from first dose, were successfully managed in most instances by omitting doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as detailed above. Immune-related AEs generally resolved within days to weeks in the majority of subjects.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Definitions

7.1.1 Adverse Event

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

7.1.2 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

7.1.2.1 Expected Adverse Event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

7.1.2.2 Unexpected Adverse Event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

7.1.2.3 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting. However, they still must be reported through the routine reporting mechanism (see **Section 7.4**):

Adverse Event

- Infection: CTCAE gr 3 or less
- Immune-related events (e.g. diarrhea, pruritus, rash, endocrinopathies) that respond to corticosteroids and improve to grade 1 or less with steroids: CTCAE gr 3 or less

7.2 Expected Toxicities

7.2.1 Adverse Events Lists

7.2.1.1 Adverse Event List for CD25/Treg-depleted Cells.

Unselected donor lymphocyte infusion for treatment of malignant disease relapse has been associated with Teff-mediated GVHD flare, and with myelosuppression with depletion of host hematopoiesis primarily in cases of significant disease relapse involving the bone marrow. However, infusion of the CD25/Treg-depleted cells was found to be safe in our phase 1 clinical trial. These patients will be closely monitored for any adverse effects including GVHD and marrow suppression. Biopsy of affected organs (e.g. gut, liver) is recommended in appropriate patients to distinguish GVHD from immune-related inflammation, as per section 6.1.13.

7.2.1.2 Adverse Event List(s) for Ipilimumab

A list of reported and/or potential adverse events (AE) associated with Ipilimumab is provided in the investigators brochure (IB), and is briefly summarized below**.** In addition to the comprehensive list, a subset is identified with ***bold and italicized*** text. This subset of AEs (indicated with *) is a list of events that are protocol-specific exceptions to expedited reporting (except as noted below).

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

The following side effects have been reported in clinical trials in patients receiving 3 mg/kg Ipilimumab:

Very common (may affect more than 1 in 10 people)
- anorexia
- ***diarrhea* (gr 3)***, vomiting, or ***nausea* (gr 3)***
- ***skin rash* (gr 3)***, ***pruritis* (gr 3)***
- ***fatigue* (gr 3)***, reaction at site of injection, ***fever* (gr 2)***

Common (may affect up to 1 in 10 people)
- tumor pain
- hypothyroidism, hypopituitarism
- dehydration
- confusion
- neuropathy (causing pain, weakness and cramps), dizziness, headache,
- blurred vision, pain in the eye
- low blood pressure, temporary redness of the face and neck, feeling of intense heat with sweating and rapid heart beat

 shortness of breath, cough
 bleeding in the stomach or intestine, inflammation of the intestines ***(colitis)* (gr 3)***, constipation, heartburn, stomach pain
 abnormal function of the liver
 inflammation of the inner surface lining of a particular organ
 inflammation and redness of the skin, skin color change in patches (vitiligo), hives, hair loss or thinning, excessive sweating at night, dry skin
 pain in muscles and joints, muscle spasms
 shivering, lack of energy, swelling, pain
 flu-like illness
 weight loss

Uncommon (may affect up to 1 in 100 people)
 sepsis, septic shock, meningitis, gastritis, colitis, urinary tract infection, infection of the respiratory tract
 paraneoplastic syndromes such as hypercalcemia, hypercholesterolemia and hypoglycemia
 allergic reaction
 adrenal insufficiency, hyperthyroidism, defect of the glands producing sex hormones
 decreased function of the adrenal glands caused by an underactive hypothalamus
 tumor lysis syndrome
 changes in mental health, depression, lowered sex drive
 Guillain-Barré syndrome, fainting, inflammation of the nerves within the brain, hydrocephalus, ataxia, shaking, brief involuntary muscle contraction, difficulty in speaking
 inflammation of the eye which causes redness or pain, bleeding in the eye, iritis, reduced vision, foreign body sensation in the eyes, swollen runny eyes, swelling of the eye, inflammation of the eyelids
 irregular or abnormal heart beat
 inflammation of the blood vessels, disease of the blood vessels, restriction in the blood supply to the extremities, low blood pressure when standing up
 extreme difficulty in breathing, pleural effusion, pneumonitis, hay fever
 bowel perforation, inflammation of the membrane of the stomach wall and small intestine, pancreatitis, peptic ulcer, esophagitis, intestinal obstruction
 liver failure, inflammation of the liver, enlarged liver, jaundice
 toxic epidermal necrolysis
 myositis, arthralgia
 inflammation of the thyroid gland, the kidney, or the central nervous system
 multi organ inflammation
 muscle weakness
 kidney function failure, kidney disease
 absence of menstrual periods
 multi organ dysfunction, reaction related to infusion of the medicine
 change in hair color

Rare (may affect up to 1 in 1,000 people)

 inflammatory disease of blood vessels (most commonly cranial arteries)
 inflammation of the anus and the rectal wall (marked by bloody stools and a frequent urge to defecate)
 psoriasis
 erythema multiforme
 a type of severe skin reaction characterized by rash accompanied by one or more of the following features: fever, swelling of the face or lymph glands, increase of eosinophils, effects on liver, kidneys or lungs (DRESS).

In addition, the following uncommon (may affect up to 1 in 100 people) side effects have been reported in patients who received doses other than 3mg/kg of Ipilimumab in clinical trials:

 meningism: neck stiffness, intolerance of bright light and headache, flu-like discomfort
 inflammation of the heart muscle, weakness of the heart muscle, fluid around the heart
 inflammation of the liver or the pancreas, nodules of inflammatory cells in various organs of your body
 infection within the abdomen
 erythema nodosum
 overactive pituitary gland
 decreased function of the parathyroid gland
 inflammation of the eye, eye muscle inflammation
 decreased hearing
 poor blood circulation which makes toes and fingers numb or pale
 damage to the tissues of the hands and feet resulting in redness, swelling and blisters

Ipilimumab may cause changes in the results of laboratory tests. These include:

 a variation in the number of red blood cells, white blood cells or platelets
 an abnormal variation of hormones and liver enzyme levels in the blood
 abnormal liver function test
 abnormal levels of calcium, sodium, phosphate or potassium in the blood
 hematuria, proteinuria
 an abnormally high alkalinity of the blood and other body tissues
 kidneys unable to remove acids from blood normally
 presence of autoantibodies

Please refer to the Ipilimumab IB for a comprehensive list of adverse events.

7.3 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate

treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next Section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

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

7.4 Adverse Event Reporting

7.4.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI. Additionally, BMS must be notified within 24 hours.

BMS Contact:

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

7.4.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 90 days of the last dose of treatment on the local institutional SAE form. Per BMS guidelines, Ipilimumab follow up for AE/SAEs is 90 days from the last dose of treatment.

7.4.3 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4.4 Protocol-Specific Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

Events not considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

7.5 Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.6 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.7 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in **Section 6**.

8.1 Ipilimumab

Bristol-Myers Squibb Inc. (BMS) supplies distributes Ipilimumab at no cost for this study.

8.1.1.1 Description

Chemical Name or Amino Acid Sequence: 4 polypeptide chains, 2 identical heavy chains with 447 amino acids and 2 identical light chains consisting of 215 amino acids.
Other Names: Anti-CTLA-4 monoclonal antibody, MDX-010
Classification: Human monoclonal antibody
M.W.: 147,991 Daltons
Mode of Action: Ipilimumab is specific for the CTLA-4 antigen expressed on a subset of activated T-cells. CTLA-4 interaction with the B7 molecule, one of its ligands expressed on professional antigen presenting cells, can down-regulate T-cell response. Ipilimumab is thought to act by blocking the interaction of CTLA-4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA-4/B7 creates the interaction.

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

8.1.2 Form

Ipilimumab will be provided in open-label containers. Container labels will contain the batch number, contents, storage conditions, and Investigational New Drug caution.

Ipilimumab is available as a 200 mg /40 mL or 50 mg /10 mL (5 mg/mL) single-use vial. The vial is a clear, colorless, sterile, isotonic aqueous solution (pH of 7) that may contain particles. Each vial is a Type I flint glass vial with gray butyl stoppers and sealed with aluminum seals.

	Process C	
Component	**50 mg/ vial[a]**	**50 mg/ vial[a]**
Ipilimumab	53.5 mg	213 mg
Sodium Chloride, USP	62.6 mg	249 mg
TRIS-hydrochloride	33.7 mg	134.3 mg
Diethylenetriamine pentacetic acid	0.42 mg	1.67 mg
Mannitol, USP	107 mg	426 mg
Polysorbate 80 (plant-derived)	1.18 mg	4.69 mg
Sodium Hydroxide	QS to pH 7	
Hydrochloric acid	QS to pH 7	
Water for Injection	QS: 10.7 mL	QS: 42.6 mL

Nitrogen[c]	Processing agent

[a]Includes 0.7 overfill; [b]Includes 2.6 mL overfill.
[c]Nitrogen is used to transfer the bulk solution through the pre-filled and sterilizing filters into the aseptic area.

8.1.3 Storage and Stability

Storage: Store intact vials refrigerated at (2-8° C), protected from light. Do not freeze.
Stability: Shelf-life surveillance of the intact vials is ongoing. Solution as described above is stable up to 24 hours refrigerated at (2-8° C) or at room temperature/ room light.

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

8.1.4 Compatibility

Ipilimumab will be administered individually on this study, and thus issues of compatibility with other agents are not anticipated.

8.1.5 Handling

The investigational product must be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. The investigator must ensure that the investigational product is stored in accordance with the environmental conditions (temperature, light, and humidity) determined by the sponsor. If concerns regarding the quality or appearance of the investigational product arise, the investigational product must not be dispensed and BMS must be contacted immediately, via:

Brittney McLaughlin
Project Manager, Syneos Health
Providing services to Bristol-Myers Squibb
3401 Princeton Pike
Lawrenceville, NJ 08648
[redacted]
syneoshealth.com

Ipilimumab (BMS-734016) Injection (5 mg/ml), must be stored refrigerated (2ºC - 8ºC) with protection from light. In preparation of infusion, Ipilimumab may be stored in IV infusion bags (PVC, non-PVC/non-DEHP) or glass infusion containers at room temperature or refrigerated (2ºC - 8ºC) for up to 24 hours. Drug must be

completely delivered to the subject within 24 hours of preparation. This includes any time in transit plus the total time for the infusion.

As with all injectable drugs, care should be taken when handling and preparing Ipilimumab. Whenever possible, it should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents, applying aseptic technique. Gloves are required. If Ipilimumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused Ipilimumab solution should be disposed of at the site following procedures for the disposal of anticancer drugs.

8.1.6 Availability

Ipilimumab is an investigational agent supplied by BMS.

8.1.7 Preparation and Administration

The following instructions are suggested guidelines for preparation of Ipilimumab which may be prepared per institutional standards and package insert. The supplies needed for Ipilimumab preparation and administration include calibrated syringes and infusion containers. Ipilimumab is to be administered as an IV infusion over 90 +/-10 minutes through an IV line containing a sterile, nonpyrogenic, low-protein-binding in-line filter per institutional standard practice. Flush IV line with NS or D5W after each dose per institutional standard practice. The total dose for each subject's first Ipilimumab infusion (Cycle 1, Day 1) must be calculated using the most recent subject weight (obtained within 3 days of the dosing visit, and prior to the infusion). The same total dose may be given in the subsequent infusions if the most recent subject weight (obtained within 3 days of dosing visit, and prior to the infusion) is within 5% of the subject weight prior to the first infusion. If the most recent subject weight (obtained within 3 days of the dosing visit, and prior to the infusion) at a subsequent dosing visit is **not** within 5% of the subject weight prior to the first infusion, the total dose must be recalculated based on the most recent subject weight for that dosing visit.

1) As Ipilimumab is stored at refrigerated temperatures (2 - 8ºC), allow the appropriate number of vials of Ipilimumab to stand at room temperature for approximately 5 minutes.

2) Aseptically withdraw the required volume of Ipilimumab solution into a syringe. Insert the needle at an angle into the Ipilimumab vial by placing the needle bevel side down against the glass, with the tip touching the neck of the vial. The initial solution concentration is 5 mg/mL. [Note: A sufficient excess of Ipilimumab is incorporated into each vial to account for withdrawal losses].

3) Ensure that the Ipilimumab solution is clear, colorless, and essentially free from particulate matter on visual inspection. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall, and so on.

4) Preparation of ipilimumab will follow package insert and/or institutional standard practice.

5) Mix by GENTLY inverting several times. DO NOT shake.

6) Visually inspect the final solution. If the initial diluted solution or final dilution for infusion is not clear, or the contents appear to contain precipitate, the solution should be discarded.

7) Do not draw into each vial more than once. Any partial vials should be safely discarded and should not be stored for reuse.

8) Ipilimumab should be administered under the supervision of a physician experienced in the use of intravenous agents. Ipilimumab is administered as an intravenous infusion only.

9) Ipilimumab is administered over 90 +/- 10 minutes, and hydration is allowed but not required per protocol. Patients should be observed closely for infusion reactions during treatment, with vital signs checked at least every 30 minutes, including after an observation period of 30 minutes after completing the infusion. A detailed description of common infusion reactions and their management is outlined in **Section 6.1.11**

8.1.8 Ordering

Ipilimumab will be provided by BMS. Investigational supplies of study drug will be provided to DFCI research pharmacy as per standard procedures and agreements with BMS.

BMS protocol#: CA184-559
BMS vendor contact for drug orders is
distribution.allentown@thermofisher.com

8.1.9 Accountability

The investigator, or a responsible party designated by the investigator, should maintain

a careful record of the inventory and disposition of the agent using a drug accountability form.

8.1.10 Destruction and Return

Expired ipilimumab supplies will be destroyed on site per SOP. At the end of the study, unused supplies of ipilimumab will be destroyed on site per SOP. Destruction will be documented in the computerized institutional Drug Accountability Record Form.

9. CORRELATIVE STUDIES

9.1 Laboratory Correlative Study Sample Collection

All laboratory tests are mandatory and will be processed at the Cell Manipulation Core Facility at DFCI. Please refer to the study operations manual for details regarding processing and shipping study specimens.

Samples will be obtained pre-dose at screening or Cycle 1 Day 1 (C1D1), per the schema in the required data table (**Table 6**).

9.1.1 Peripheral Blood Correlatives

Each time point will require a PASQ3P (three - 10mL EDTA tubes) for correlative analysis. These research tubes will preferably be drawn at the same time as clinical labs drawn at the below time points, though can be drawn at an alternative time for scheduling convenience. There are two exceptions:

a) **Cycle 1 Day 1 (C1D1):** Order a PASQ6P – six – 10mL EDTA tubes. Each lab involved requires a baseline sample. <u>This needs to be collected prior to the patient receiving any study treatment.</u>

b) **Cycle 3 Day 1, Cycle 4 Day 29, Cycle 5 Day 1, Cycle 6 Day 1, Cycle 8 Day 1, Off Rx:** For every time point when a BM aspirate is collected, instead of collecting a PASQ3P (three-10mL EDTA tubes) order a PASQ4P (four-10 mL EDTA tubes).

9.1.2 Bone Marrow Aspirate and Biopsy

Bone marrow aspirate and biopsy samples will be obtained per the schema in the required data table (**Tables 6, 7**). Two-10 mL research bone marrow aspirates and one bone marrow core biopsy should be collected for correlatives at each time point a biopsy is noted in the study calendar below.

9.1.3 Skin/Extra-Medullary Disease Biopsies

Skin/other sites of extra-medullary disease will also be biopsied per the required data table schema, if deemed safe and clinically feasible. Tissue should be sent in a sterile, leak proof container that contains DMEM at room temperature to the TIGL. Sample should be delivered within 4 hours and be at least 3 mm in diameter.

Archival tissue from patients on study may be utilized for correlatives studies as necessary.

9.2 Planned Correlative Studies

The goals of the correlative analyses include:

To assess the immunologic impact of infusions of CD25/Treg-depleted DLI plus Ipilimumab. These include immunophenotypic analysis of T-cell subsets, B and NK cells; phenotypic and functional readouts of effector T (Teff) and regulatory T (Treg) cell activation status; changes in plasma cytokines and chemokines; tumor microenvironment; and tumor mutation profile. We also seek to correlate clinical response with changes in circulating Teff vs. Treg cell counts and activation; the persistence of adoptively transferred Teff cells; changes in cytokine and chemokine levels; tumor infiltration by activated $CD8^+$ T cells; and tumor mutation profile, blast phenotype and tumor microenvironment. In an exploratory analysis, we will perform tumor neoantigen discovery to identify novel T cell targets and correlate tumor mutational load with response.

The specific studies include:

- Assessment of donor cell chimerism (molecular testing and cell based assays).
- Immune cell frequencies and numbers (flowcytometry, mass cytometry, immune histochemistry).
- CD4 Teff and Treg, CD8 T, and NK cell frequency, number, phenotype, proliferation (flow or mass cytometry).
- Teff cell function assays and phenotypic readouts in vivo and in vitro.
- Teff TCR sequencing
- Teff, Treg, CD8 T, NK cell gene expression / transcriptome profiling.
- Next generation sequencing of measurable residual disease.
- AML/MDS/MPN immune-evasion assessments (flow or mass cytometry, genome sequencing of baseline and relapse samples, including non-malignant tissue).
- Serum cytokine, chemokine assessments

Samples will be collected at time points mentioned above (**Tables 6, 7**).

10. STUDY CALENDAR

Baseline disease assessments (e.g. BM, Scans), cardiac/pulmonary evaluations, and Labs/IDM must be done ≤4 weeks prior to the start of therapy. Baseline other labs (e.g. CBC, chemistries) should be repeated within 1 week prior to start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

There will be at least one-week interval between consecutive enrollments at the same dose level during phase 1A. All assessments must be performed prior to administration of any study medication. All study assessments should be performed within ± 3 days of the protocol-specified date during induction and ± 7 days during maintenance, unless otherwise noted. Ipilimumab should be administered within ± 3 days of the protocol-specified date during induction (**Table 6**) and ± 7 days during maintenance (**Table 7**), unless otherwise noted. Note that in the calendar below, entry to maintenance Ipilimumab during weeks 24-60 are only for patients with stable disease or better at time of Cycle 4 day 29 (D92) response assessment time point.

Table 6: Induction Phase									
	Screening	C1 D1 (D1)	C1 D8 (D8)	C1 D15 (D15)	C2 D1 (D22)	C2 D8 (D29)	C3 D1 (D43) **(DLT Endpoint)**	C4 D1 (D64)	**C4 D29 (D92) (Response Endpoint)**
CD25/Treg depleted DLI		X							
Ipilimumab*		X			X		X	X	
Informed Consent	X								
EKG[1]	X								
Cardiac Evaluation[1,2]	X								
PFT	X								X
β-HCG Test[3]	X								
Complete History	X								
Interval History		X	X	X	X	X	X	X	X
Physical exam (Ht, Wt, VS[4], PS)	X[4]	X	X	X	X	X	X	X	X
CBC and differential	X	X	X	X	X	X	X	X	X
Serum Chemistry[5]	X	X	X	X	X	X	X	X	X
TSH	X								X
Chimerism testing	X						X		X
PB Correlative studies[6]	X	X[11]	X	X	X	X	X[12]	X	X[12]
BM aspirate and core[7]	X						X		X
aGvHD assessment[8]	X	X	X	X	X	X	X	X	X
cGVHD assessment[9]	X						X		X
Viral Panel[10]	X								

Table 7: Maintenance Phase (For patients with SD/PR/CR at D92 Response Assessment)												Off Rx**
	C4 D57	C5 D1 (Wk 24)	C5 D29	C5 D57	C6 D1 (Wk 36)	C6 D29	C6 D57	C7 D1 (Wk 48)	C7 D29	C7 D57	C8D1 (Wk 60) and beyond	
Ipilimumab		X			X			X			X	
PFT		X			X			X			X	X
Complete History												X
Interval History	X	X	X	X	X	X	X	X	X	X	X	
Physical exam[4] (Ht, Wt, VS, PS)	X	X	X	X	X	X	X	X	X	X	X	X
CBC and differential	X	X	X	X	X	X	X	X	X	X	X	X
Serum Chemistry[5]	X	X	X	X	X	X	X	X	X	X	X	X
TSH											X	X
Chimerism testing		X										X
PB Correlative studies[6]	X	X[12]			X[12]			X			X[12]	X[12]
BM aspirate and core[7]		X			X						X	X
aGvHD assessment[8]	X	X	X	X	X	X		X	X	X	X	X
cGVHD assessment[9]		X			X			X			X	X

* – Patients will be dosed at start of week 1, 4, 7, and 10 (induction) and return for an end of induction response evaluation on start of week 14. Maintenance dosing will then occur at weeks 24, 36, 48 and 60. Patients who experience clinical benefit may continue to receive Ipilimumab q12 weeks till week 60 unless there is unacceptable toxicity, disease progression, or withdrawal of consent.
**– End of study off-treatment visit will be 3 months after last dose of maintenance Ipilimumab. Note: for IND/IDE trials, follow up visits or other contact are required in order to identify SAEs during the 30 days following the end of study treatment
1 – A baseline EKG and cardiac ECHO (or MUGA) is required for all patients, and anytime thereafter as clinicallyindicated.
2 – For those with evidence of CHF, myocardial infarction, myocarditis or cardiomyopathy at any time during the study, a detailed cardiac evaluation including labs (CPK, Troponin), cardiology consult, with EKG and ECHO (or MUGA) at minimum is required, as clinically indicated. Please refer to **Section 6.1.13** for guidance on cardiac toxicity management for Ipilimumab.
3 – For females of childbearing potential only
4 – 0_2 Saturation on room air required only at screening; Weight on day closest prior to infusion will be used for study dosing calculations, height only needs to be collected at screening.
5 – Includes: comprehensive metabolic panel, LFTs, magnesium, phosphorus, uric acid, LDH, lipase, eGFR
6 – See **Section 9.1.1.**
7 – See **Section 9.1.2 and 9.1.3.** If there is no bone marrow involvement at baseline and/or extra-medullary disease is documented, the other sites of disease relapse will be assessed as clinically appropriate (**section 9.1.3** e.g. measurement of lesions of leukemia cutis including skin biopsy, PET/CT assessments for other extramedullary sites (e.g. granulocytic sarcoma)
8 – See **Appendix C** for aGVHD assessment
9 – Per NIH consensus criteria for cGVHDassessment
10 – Viral Panel includes HIV, Hep B and C
11 – Exception under 9.1.1 a). Draw a PASQ6P
12 – Exception under 9.1.1 b). Draw a PASQ4P

Supplemental Scheduling Table			
Induction Phase	**Total Days**	**Maintenance Phase**	**Total Days**
Cycle 1 Day 1 (Ipi)	1	Cycle 4 Day 57	120
Cycle 1 Day 8	8	Cycle 5 Day 1 (Ipi)	169
Cycle 1 Day 15	15	Cycle 6 Day 1 (Ipi)	253
Cycle 2 Day 1 (Ipi)	22	Cycle 7 Day 1 (Ipi)	337
Cycle 2 Day 8	29	Cycle 8 Day 1 (Ipi)	421
Cycle 3 Day 1 (Ipi)	43	Off Treatment	
Cycle 4 Day 1 (Ipi)	64		
Cycle 4 Day 29	92		

*Induction Phase +/- 3 days for appointment window

**Maintenance Phase +/- 7 days for appointment window

***Patients who do not complete the entirety of the Induction and Maintenance (8 cycles) phases are not required to complete the Off-Treatment visit outlined in the Study Calendar. However, any corresponding data that can be gathered through Stand of Care appointments should be collected. Toxicities will be recorded until 3 months after the last dose of Ipilimumab.

11. MEASUREMENT OF EFFECT / CLINICAL RESPONSE

Evaluable for toxicity- Participants who receive cell infusion at the specified dose plus Ipilimumab will be evaluated for toxicity. Evaluable patients are those who experience a DLT before day 43, or are assessable for DLT at day 43. Phase 1a patients who are unevaluable for DLT may be replaced.

Evaluable for response- Only those participants who have measurable disease present at baseline, have received cell infusion at the specified dose and least two cycles of Ipilimumab therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the disease-specific definitions stated in **Appendices E-F**. Patients unevaluable for response will not be replaced.

Response Criteria will be disease-specific, and are provided in detail in the following appendices:

- **Appendix E**: Acute Myelogenous Leukemia
- **Appendix F:** Myelodysplastic Syndrome / Myeloproliferative Neoplasm

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

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

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

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression or death whichever occurs first.

Overall Survival (OS) is defined as the duration of time from start of treatment to time of death.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

Note: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Collaborative Agreements Language

Not applicable

13. STATISTICAL CONSIDERATIONS

13.1 Study Design

This is an open-label, single center phase 1 study with the primary objective of establishing the safety and exploring the efficacy of infusing CD25/Treg-depleted cells plus Ipilimumab in AML, MDS and MPN patients who relapse after 8/8 HLA-matched donor hematopoietic cell transplant.

13.2 Primary endpoints

The primary endpoint of this study is to determine the maximum tolerated dose (MTD) of CD25/Tre-depleted DLI followed by Ipilimumab in AML, MDS and MPN patients relapsed after HLA-matched donor stem cell transplantation.

13.3 Secondary endpoints

- ORR (To determine complete remission (CR/CRi) rate at 6 weeks (day 43) after the cell infusion plus Ipilimumab
- To determine the rate of progression-free survival (PFS) and overall survival (OS) at ~3 months (day 92) post cell infusion plus Ipilimumab
- To determine the rate of progression-free survival (PFS) and overall survival (OS) at ~ 1-year (60 weeks) post cell infusion plus Ipilimumab
- To determine the incidence and severity of acute GVHD rates after cell infusion plus Ipilimumab
- To determine the incidence and severity of chronic GVHD rates after cell infusion plus Ipilimumab

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13.4 Determining MTD

Four escalating/de-escalating doses of the combination therapy will be considered to determine the MTD: CD25/Treg depleted DLI $3x10^7$ $CD3^+$ cells/kg + Ipilimumab 10 mg/kg (Dose level +2), CD25/Treg depleted DLI $3x10^7$ $CD3^+$ cells/kg + Ipilimumab 3 mg/kg (Dose level +1), CD25/Treg depleted DLI $3x10^7$ CD3+ cells/kg + Ipilimumab 1 mg/kg (Dose level 0, starting level), and CD25/Treg depleted DLI $1x10^7$ $CD3^+$ cells/kg + Ipilimumab 1 mg/kg (Dose level -1). Considering the DLT observation period of 6 weeks and the accrual rate, the study will enroll 5 eligible patients at each dose level to minimize delays in accrual. Patients will be unevaluable for MTD if they receive DLI product with cells not meeting the dose-level criteria (**Section 5.1.3**), die of progressive disease without DLT, or have disease progression or other reason requiring removal from the study without DLT within the 6 week DLT observation period (**Section 5.4**).

Since the MTDs of CD25/Treg depleted DLI and Ipilimumab were previously defined in monotherapy studies. Dose level 0 will be the initial dose level for this combination therapy. To ensure at least 3 patients will be evaluable in each dose level, a cohort of 5 eligible patients will enter at each dose level. If there are fewer than 3 evaluable patients in a cohort, additional patients can be added at that dose level. At any dose level, if 2 or more patients experience DLT, MTD is considered exceeded. If this is dose level -1, then the study will be terminated. If this is dose level+1 or +2, the next lower level (dose level 0 or +1, respectively) will be declared to be the MTD. If this is dose level 0, dose de-escalation will take place (decision rule I). If no DLT is seen in the first 3, 4, or 5 evaluable patients at dose level 0 or +1, then dose escalation will take place. If no DLT is seen in the first 3, 4, or 5 evaluable patients at dose level -1 or +2, that dose level will be declared to be the MTD. If there are 3 evaluable patients in a cohort of 5 eligible patients and if 1 of the 3 evaluable patients experiences DLT then 2 additional evaluable patients will be treated at the same dose level. If 1 or more of the 2 additional patients experience(s) DLT then MTD is considered to have been exceeded, and the decision rule I will be applied. If there are 4 evaluable patients and if 1 of the 4 evaluable patients experiences DLT then 1 additional evaluable patient will be treated at the same dose level. If this additional patient experiences DLT then MTD is considered to have been exceeded, and the decision rule I will be applied. If 1 DLT is seen in a total of 5 evaluable patients treated at dose level 0, then dose escalation will take place. If 1 DLT is seen in a total of 5 evaluable patients treated at dose level -1 or +2, then this dose level will be declared to be the MTD. **Table 8** shows the probability of escalation under various true DLT rates for the cohort. With this design, there is 92-93% probability of dose escalation if the true rate of DLT is 10% and 74-76% probability if the true DLT rate is 20%. Of note, the probability of dose de- escalation is one minus the probability of dose escalation.

Table 8. Probability of dose escalation

True rate of DLT	Prob. of Escalation for (3+2) evaluable cases	Prob. of Escalation for (4+1) evaluable cases	Prob. of Escalation for 5 evaluable cases
10%	0.926	0.919	0.919
20%	0.758	0.737	0.737
30%	0.559	0.528	0.528
40%	0.372	0.337	0.337
50%	0.219	0.189	0.189

60%	0.11	0.087	0.087

Once the MTD is established, 10 more patients will be treated at the MTD in phase 1b to provide a better estimate of the toxicity and to describe preliminary efficacy at this doselevel.
To ensure the MTD determined in Phase 1a is safe for a larger Phase 2 study, we will monitor toxicity in this expansion cohort closely. That is, in a total of 10 evaluable patients in the expansion cohort, if ≤2 DLTs are observed, this MTD will be the recommended Phase II dose. Conversely, if 3 or more DLTs are observed, further accrual will be halted pending DSMC review. Depending on the result of this review, the DSMC may recommend permanent closure or testing of the next lower dose in the expansion cohort. With this design the probability of observing ≤2 DLTs in 10 is 0.07 if the true but unknown DLT rate is 10%, but 0.83 if the rate is 40%. Patients will receive maintenance Ipilimumab per their assigned dose level.

Amendment, December 23, 2020

The study team reviews AEs at each dose level closely on an ongoing basis. As of December 20, 2020, 10 patients have been treated on study: 3 at dose level 0, 6 at dose level 1, and 1 at dose level 2. At dose level 2, the first patient treated had DLT (severe aGVHD that was fatal), deemed attributable to study therapy. Due to a concern that we would observe more than 1 DLT in a cohort of 5 patients at this dose level if we continue the accrual, the protocol was amended to deem MTD exceeded at this dose level.

Further accrual of the expansion cohort was due for dose level 1, where 1 of 6 patients had experienced DLT (GVHD) within the DLT observation period. Recently, however, 1 additional patient has experienced delayed SAE with histology suggesting a possible role for remote Ipilimumab effect. Additionally, 1 other patient had a late SAE, albeit outside of the DLT window, that was concerning for treatment toxicity. We therefore further amend the protocol to enroll 2 more patients at dose level 0 to a total of 5 evaluable patients. If 1 or none of these 2 additional patients experiences DLT, dose level 0 will be declared to be the presumptive MTD and thereafter proceed to a phase 1b expansion cohort. If, however, we observe 2 DLTs at dose level 0, dose de-escalation will take place to dose level -1 and apply the decision rule above.

Amendment June 25, 2021

No DLTs were experienced during the DLT period in the final 2 patients enrolled at dose level 0. Therefore, the phase 1b expansion cohort will proceed at dose level 0.

13.5 Sample Size/Accrual Rate

The sample size will approximately range from 4-25 evaluable patients, depending on the number of dose levels tested. Based on the current practice, we anticipate that the accrual rate will be approximately 8-10 patients per year, thus requiring up to 38 months for the completion of the accrual. This projection does not count a mandatory waiting period prior to dose de-escalation. Follow-up after termination of accrual will be 1 year after the last patient ends therapy.

13.6 Stratification Factors

None

13.7 Analysis plan

Due to limited sample size, the analysis will be primarily descriptive. AEs/SAEs will be summarized by patient, type and grade as defined by the CTCAE v4.0. Rates will be described as proportions with exact binomial confidence intervals. CR/CRi rate at day 43 also will be described as a proportion with exact binomial confidence interval. Kaplan-Meier models will be used to estimate PFS and OS at day 100 and 1 year with exact pointwise confidence intervals. Acute and chronic GVHD will be described as cumulative incidence in the competing risks framework treating death without GVHD as a competing event. The association between clinical outcomes (response, PFS and OS and acute or chronic GVHD) and correlative endpoints (e.g., circulating Teff and Treg cells, number of AML/MDS/MPN blasts and other characteristics of the BM microenvironment pre-therapy and at post-treatment relapse) will be explored using univariable Cox, Fine and Gray regression analysis or exact Wilcoxon-Rank-Sum test, as appropriate. Analyses will be carried out in SAS 9.4 or R 3.3.2 or higher.

14. PUBLICATION PLAN

The initial study results will be made public within 12 months of the end of data collection. The study report is planned for publication in a peer-reviewed journal, and the initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A final report of study outcomes will be made public no later than 3 years after the end of data collection.

The study PI holds the primary responsibility for publication of study results. Permission of the study PI will be required before any unpublished study information can be used or passed on to a third party.

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APPENDICES

APPENDIX A PERFORMANCE STATUS CRITERIA

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

Appendix B: BODY SURFACE AREA AND CREATININE CLEARANCE

Body surface area (BSA) should be calculated using Dubois formula that yields the following results in meters squared (m^2):

$$BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$$

where the weight is in kilograms and the height is in centimeters.

Creatinine clearance (CrCl) can be calculated using the Cockroft-Gault equation as follows:

$$CrCl\ (ml/min) = \frac{(140 - age)\ (actual\ wt\ in\ kg)}{72 \times serum\ creatinine\ (mg/dl)}$$

For females, use 85% of calculated CrCl value.

Note: In markedly obese participants, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)

Appendix C: ACUTE GVHD GRADING

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (stool output/da
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexia	Adult: 500-999 mL/day or episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or > episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain w without ileus or grossly bl stool (regardless of stool volume).

Overall clinical grade (based on most severe target organ involvement):

- Grade 0: No stage 1-4 of any organ.
- Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
- Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
- Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
- Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

Adapted from Harris AC, et al. BBMT 2016; 22:4

Appendix D: NCI COMMON TOXICITY CRITERIA

Please refer to the NIH website:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_40

Forms available upon request

Appendix E: AML RESPONSE CRITERIA

(Cheson, *et al., 2003)*

E.1. Antitumor Effect: AML

E.1.1 Morphological Complete Remission (CR):
-Normalization of the peripheral blood absolute neutrophil count > $1.0x10^9$/L, platelets > than $100x10^9$/L no residual evidence of extramedullary disease and bone marrow aspirate with ≤ 5% blasts, no blasts with Auer rods.

E.1.2 Morphological Complete Remission with incomplete blood count recovery (CRi):
-Same as CR but without normalization of the peripheral blood absolute neutrophil and platelet Count

E.1.3 Relapse from CR or CRi:
-Reappearance of leukemic blasts in the peripheral blood; or > 5% blasts in the bone marrow not attributable to another cause (e.g. recovery of normal cells following treatment –induced aplasia or use of growth factors) OR
-Appearance or reappearance of extramedullary disease.
-If there are no circulating blasts and no extramedullary disease and the bone marrow blast percentage is >5% but <20%, then a repeat bone marrow performed at least 7 days after the first marrow examination and documenting bone marrow blast percentage >5% is necessary to establish relapse.

E.1.4 Partial Remission (PR):
-Normalization of the peripheral blood absolute neutrophil count $1.0x10^9$/L, platelets > than $100x10^9$/L, and at least a 50% decrease in the percentage of marrow aspirate blasts to 5-25% , or marrow blasts <5% with Auer rods.

E.1.5 Cytogenetic Responses
Complete cytogenetic response: An abnormal clone is detected in all metaphases prior to treatment and only normal metaphases are observed in all metaphases following treatment.
Partial cytogenetic response: An abnormal clone is detected in all metaphases prior to treatment
and the post-treatment sample has 50% or fewer abnormal metaphases compared to the pretreatment value.
Cytogenetic non-response: An abnormal clone is detected in all metaphases prior to treatment and neither complete nor partial cytogenetic response is observed in post-treatment specimens
Specimens with normal cytogenetic results before treatment will not be evaluated for cytogenetic
response. The method used to determine cytogenetic response will be standard metaphase cytogenetics.

E.1.6 Stable disease:
-Subjects who fail to achieve CR, Cri, or PR and who do not have criteria for PD will be defined as having the stable disease. If the subject dies prior to response assessment at the end of Cycle 1, then they will be classified as “indeterminate”.

E.1.7 Progressive disease (PD): may be defined as ONE of the following:
->50% increase in peripheral blood or bone marrow blasts from best assessment with minimum threshold of 20% blasts in the marrow or $1.0x10^9$/L blasts in peripheral blood.
-Development of biopsy proven extramedullary leukemia (if the subject has extramedullary

disease at baseline, then PD will be defined by blood and marrow criteria or if new sites of extramedullary disease appear).
-If subject who present with an initial marrow blast percentage sufficiently high to preclude the ability to base disease progression on a >50% increase in marrow blast percentage, disease progression should be based on peripheral blood criteria or development of extramedullary leukemia as above.

E.1.8 Persistent Disease

Hematologic Persistence – Bone marrow or peripheral blasts >5% after d+30 following HCT

Appendix F: MDS/MPN RESPONSE CRITERIA

(Cheson, *et al.*, 2006)

F.1 Antitumor Effect – Myelodysplastic Syndrome / Myeloproliferative Neoplasm

F.1.1. Complete Remission (CR)

Requires that all of the following be present:
Peripheral Blood Counts:
-Hemoglobin greater than 11 g/dL (untransfused, 4 weeks since last transfusion, subject not on erythropoietin).
-Neutrophils 1,000/mm^3 or more (not on a myeloid growth factor)
-Platelets 100,000/mm^3 or more (not on a thrombopoetic agent)
-Blasts, 0%
-No dysplasia
Bone Marrow Aspirate and Biopsy:
-Less than 5% myeloblasts
-Normal maturation of all cell lines
-No evidence for dysplasia
When erythroid precursors constitute less than 50% of bone marrow nucleated cells, the percentage of blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the percentage blasts should be based on the nonerythroid cells.

F.1.2 Partial Remission (PR):

Requires that all of the criteria for complete remission be satisfied except blasts decreased by 50% or more over pretreatment. Cellularity and morphology are not relevant. Persisting dysplasia is permitted.
Hematologic Improvement (HI)-major (must have at least one of the three listed below) must
persist for a minimum of 8 weeks to be considered a confirmed response.

· HI-major Erythroid: For subjects with pretreatment hemoglobin less than 11 g/dL, greater than 2 g/dL increase in hemoglobin; for RBC transfusion-dependent subjects transfusion independence.

· HI-major Platelet: For subjects with a pretreatment platelet count less than 100,000/mm^3, an absolute increase of 30,000/mm^3 or more; for platelet transfusion dependent subjects, stabilization of platelet counts and platelet transfusion independence.

· HI-major Neutrophil: For absolute neutrophil count (ANC) less than 1000/mm^3 before therapy, at least a 100% increase, or an absolute increase of more than 500/mm^3, whichever is greater.

· HI-major Trilineage: Trilineage response will require the demonstration of HI-major for each of the blood lineages (erythroid, platelet, neutrophil). Trilineage hematologic improvement will also apply to subjects who achieve adequate counts in all three lineages even if one or more lineages was adequate prior to treatment. Thus, all subjects who normalize all three lineages (within IWG definitions) for 8 weeks, but who do not meet the criteria for PR, will be considered HITrilineage.

F.1.3 Cytogenetic Responses

· Complete cytogenetic response: An abnormal clone is detected in all metaphases prior to treatment and only normal metaphases are observed in all metaphases following treatment. Partial cytogenetic response: An abnormal clone is detected in all metaphases prior to treatment and the post-treatment sample has 50% or fewer abnormal metaphases compared to the pretreatment value.

· Cytogenetic non-response: An abnormal clone is detected in all metaphases prior to treatment and neither complete nor partial cytogenetic response is observed in posttreatment specimens

· Specimens with normal cytogenetic results before treatment will not be evaluated for cytogenetic response. The method used to determine cytogenetic response will be standard metaphase cytogenetics.

F.1.4 Progressive Disease

At least one of the following four criteria must be met:

1. An increase of ≥ 50% in blasts relative to baseline, to at least the level shown below for different baseline levels:

2. Baseline blasts must increase to

- < 5% > 5%
- ≥ 5 and < 10% > 10%
- ≥ 10 and < 20% > 20%
- ≥ 50% reduction from maximum remission/response levels in granulocytes or platelets.
- Reduction in Hb concentration by at least 2g/dL or development of transfusion dependence.

F.1.5 Relapse

Relapse after CR or PR: one or more of the following:

-Return to pretreatment bone marrow blast percentage,

-Decrement of 50% or greater from maximum remission/response levels in granulocytes or platelets,

-Reduction in hemoglobin concentration by at least 2 g/dL or transfusion dependence.

-Progression after Hematologic Improvement: One or more of the following:

-Decrement of 50% or greater decrement from maximum response levels in granulocytes or platelets,

-A reduction in hemoglobin concentration by at least 2 g/dL or transfusion dependence.

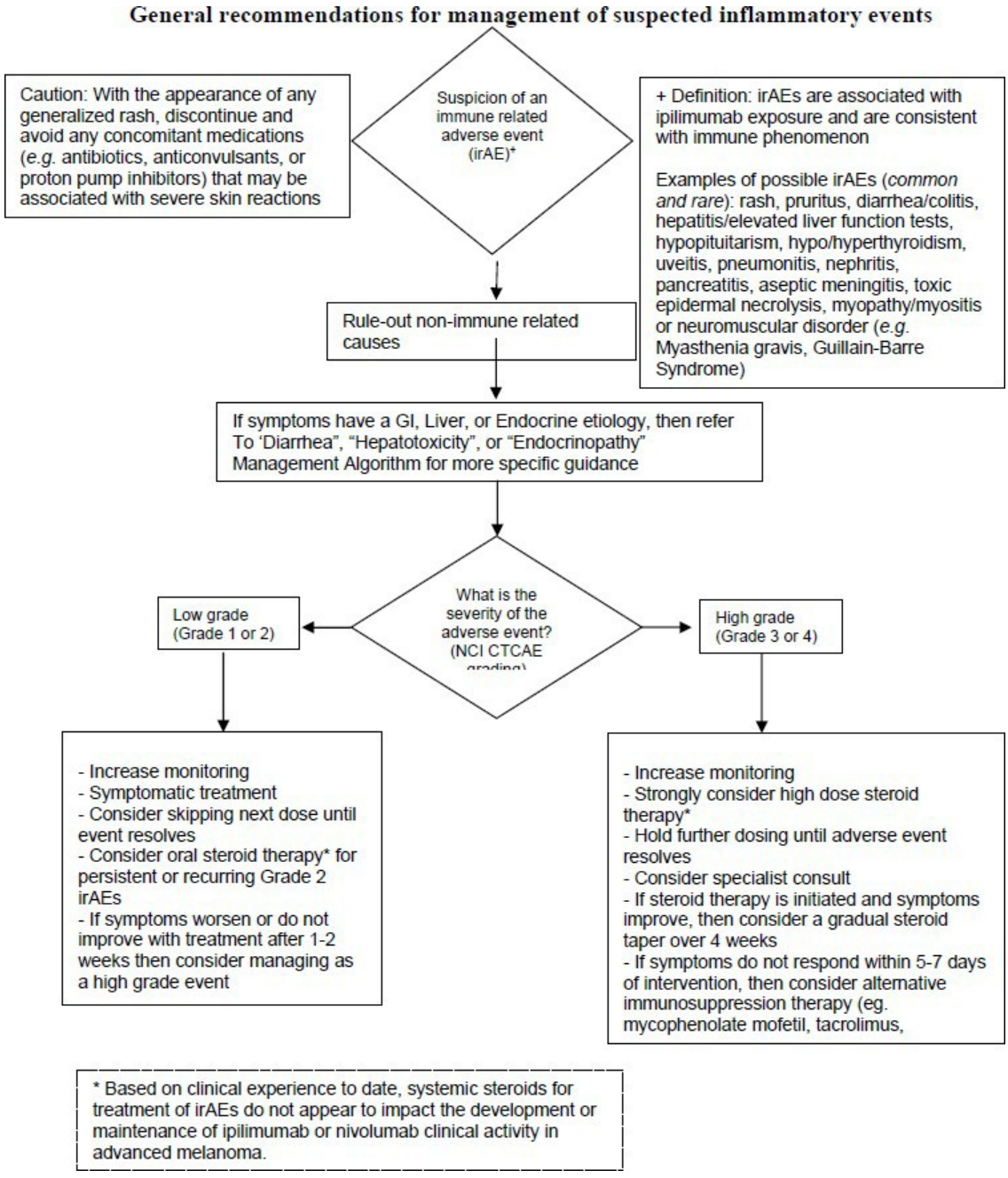
F.1.6 Persistent Disease

· Hematologic Persistence – Cytopenias not attributable to other post-transplant causes accompanied by characteristic morphological changes > d +90 following alloSCT.

· Cytogenetic Persistence – Persistence of clonal abnormality > d +90 following alloSCT

Appendix G: MANAGEMENT OF IMMUNE RELATED AEs DUE TO IPILIMUMAB

GI TOXICITY, HEPATOTOXICITY, ENDOCRINOPATHY, NEUROPATHY AND SKIN TOXICITY MANAGEMENT



GI Toxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.

Opiates/narcotics mask symptoms of perforation! Infliximab should not be used in case of perforation/sepsis!

Severity of diarrhea/ colitis	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue ipilimumab • Symptomatic treatment	• Close monitoring for worsening symptoms • Educate patient to report any worsening immediately
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; not interfering with ADL Colitis: abdominal pain; blood in stool	• Withhold/delay ipilimumab • Symptomatic treatment	**Symptoms improve/resolve** (grade 0/1): Resume ipilimumab **Symptoms persist for > 5-7 days, worsen, or recur:** • Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day) • Continue to hold/delay ipilimumab until grade 1 • When symptoms are grade 1 or less slowly taper steroids over at least 1 month and resume ipilimumab. **Symptoms worsen:** Treat as grade 3/4
Grade 3-4 Diarrhea (G3*): ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs;interfering with ADL Colitis (G3*): fever, ileus, peritoneal signs	• Permanently discontinue ipilimumab • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day) • Consider endoscopy	**Symptoms improve:** • Continue steroids (same dose) until grade 1 • Then taper over at least 1 month **Symptoms persist 3-5 days, or recur after improvement:** • 1 dose* of infliximab 5 mg/kg (if no contraindication)

*G4 = life-threatening, perforation

*Some patients have required a second dose of infliximab

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

Hepatotoxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.

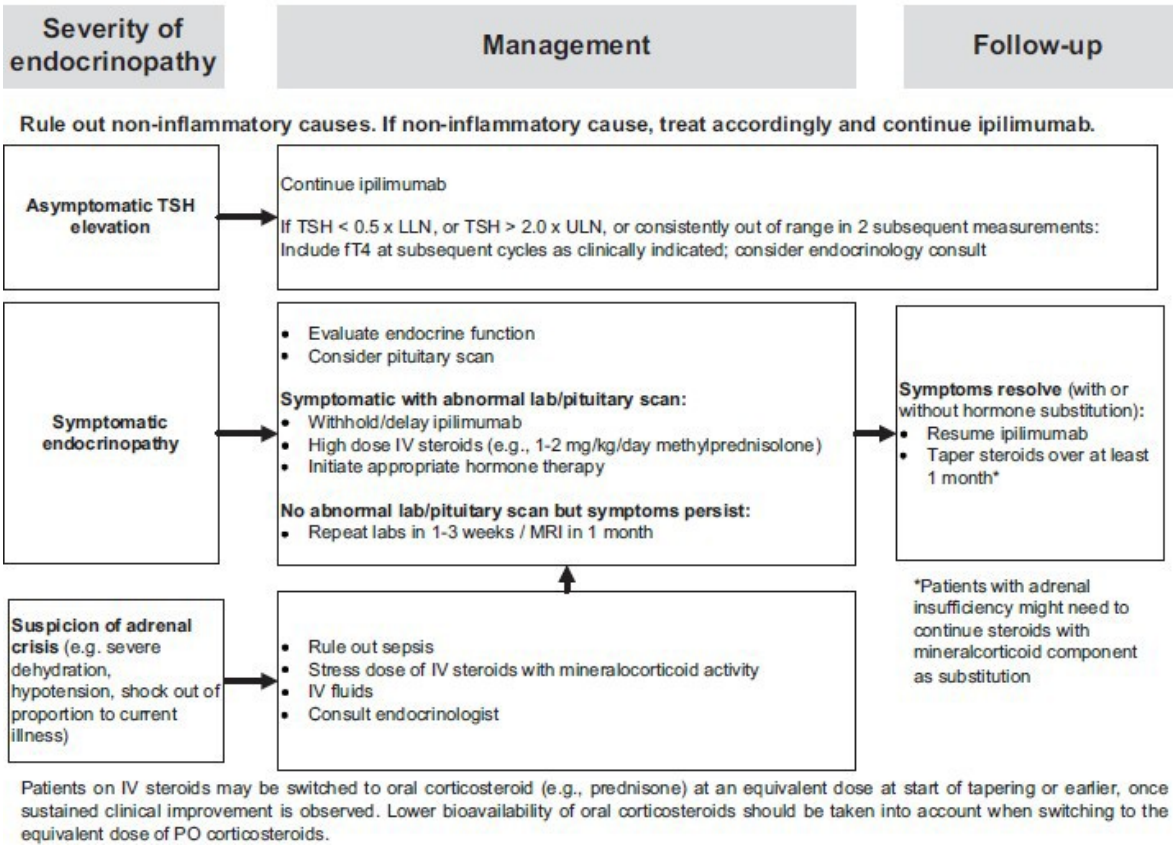
Caution: Infliximab is contraindicated in hepatic insufficiency!

Severity of hepatitis	Management	Follow-up
Grade 1 AST or ALT ≤ 2.5 x ULN or Total bilirubin ≤ 1.5 x ULN	• Continue ipilimumab	• Continue monitoring LFT prior to each dose
Grade 2 AST or ALT > 2.5 to ≤ 5.0 x ULN **or** Total bilirubin > 1.5 to ≤ 3.0 x ULN	• Increase frequency of monitoring (q3d) • Withhold/delay ipilimumab while investigating alternative etiology	**Return to baseline** • Resume q3w monitoring • Resume ipilimumab **Elevations persist for > 5-7 days or worsen, and no alternative etiologies are identified** • Consider moderate to high dose steroids PO (e.g., 0.5- 1 mg/kg/day prednisone or equivalent)
Grade 3-4 AST or ALT > 5.0 x ULN **or** Total bilirubin > 3.0 ULN	• Discontinue ipilimumab* • Increase frequency of monitoring (q1-3d) • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day**)	**AST/ALT/Tbili return to grade 2** • Taper steroids over at least 1 month **Labs do not decrease over 3-5 days, worsen or rebound:** • Add MMF*** 1 g BID • If no response within 3-5 days, consider other immunosuppressants per local guidelines

*Ipilimumab may be held/delayed rather than discontinued if AST/ALT ≤ 8 x ULN and Tbili ≤ 5 x ULN. Resume ipilimumab when AST/ALT/ Tbili return to grade 2 and meet protocol specific retreatment criteria.
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.
*** MMF, mycophenolate mofetil

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

Endocrinopathy Management Algorithm



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

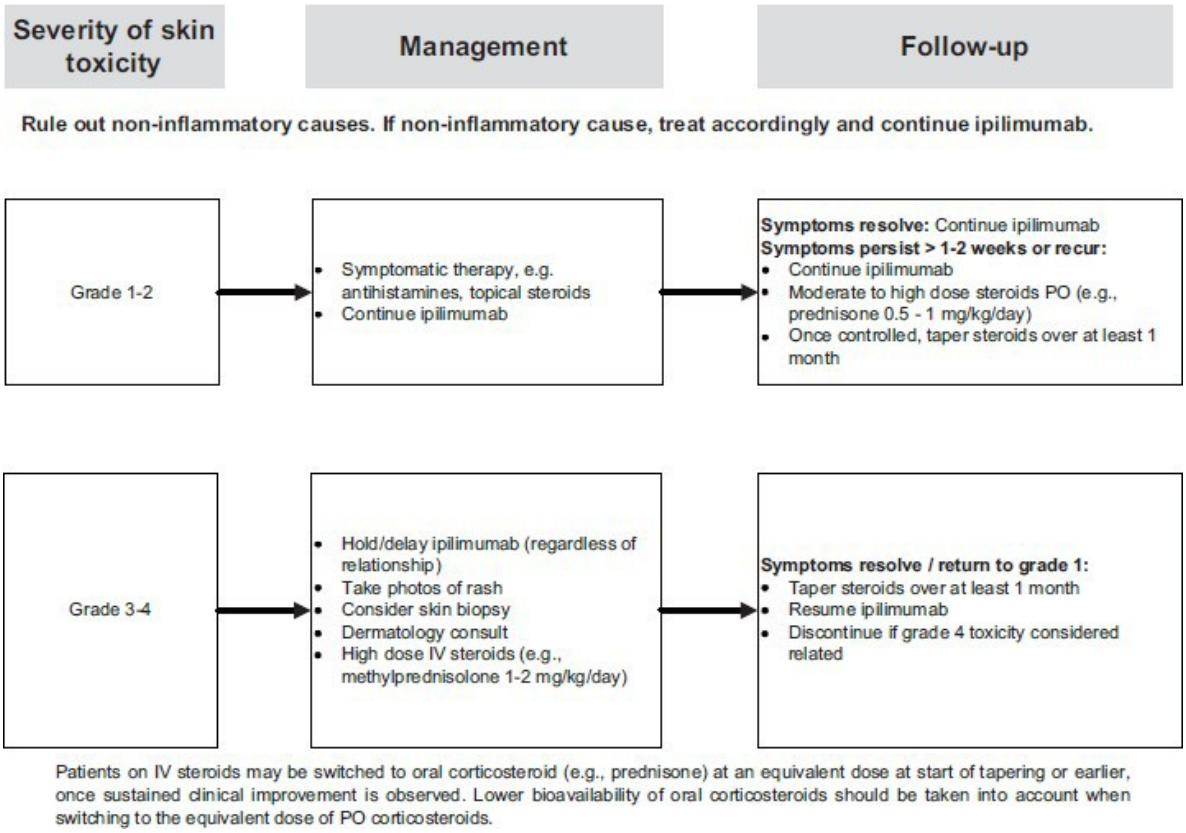
Neurological Toxicity Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab. Discontinue ipilimumab for any grade 3-4 motor neuropathy, regardless of relationship.

Severity of neurological toxicity	Management	Follow-up
Grade 1	• Continue ipilimumab	Continue monitoring the patient. If symptoms worsen, treat as below.
Grade 2	• Hold/delay ipilimumab • Treat symptoms per local guidelines	Resume ipilimumab when resolved / grade 1 If symptoms worsen, treat as below.
Grade 3-4 sensory	• Discontinue ipilmumab if considered related • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month
Grade 3-4 motor	• Discontinue ipilimumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month **Symptoms do not resolve or progress; atypical presention:** • Consider IVIg or other immunosuppressive therapies per local guidelines

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

Skin Toxicity Management Algorithm



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