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1. TITLE: Cellular Adoptive Immunotherapy Using Autologous Tumor-Infiltrating Lymphocytes Following Lymphodepletion with Cyclophosphamide and Fludarabine For Patients With Metastatic Melanoma

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TABLE OF CONTENTS

2. INTRODUCTION

This protocol proposes to examine the anti-tumor efficacy and *in vivo* persistence of adoptively transferred, autologous, tumor-infiltrating lymphocytes (TIL) following lymphodepletion with cyclophosphamide and fludarabine, in patients with metastatic melanoma. This study will also assess prognostic markers and safety of the infused autologous TIL following lymphodepletion.

3. BACKGROUND

3.1 Immunotherapy of Malignant Melanoma

3.1.1 Introduction

The incidence of melanoma is rising at a rate greater than any other cancer. Based on 2005-2009 data, it is estimated that 1 in 50 men and women will be diagnosed with melanoma during his or her lifetime[1]. Metastatic melanoma is particularly devastating, with a median survival of less than one year and low response rates to available chemotherapy and immunotherapy agents. Melanoma is notorious for affecting young men and women in their 30s and 40s, and thus, the loss of productive years is one of the most significant among cancers[2].

In a landmark year for melanoma therapy, two new drugs were FDA-approved in 2011 for advanced melanoma. Ipilimumab, an immune checkpoint blocker, offers long-term disease control for approximately 20% of patients, but complete responses are rare[3]. Vemurafenib, a BRAF inhibitor, produces rapid disease regression in roughly 50% of patients whose tumor carries a v600 mutation of BRAF, but these responses are almost always short-lived, with a median duration of only 6.7 months[4]. While both drugs represent huge advances in the melanoma field, neither agent will change the clinical outcome of the majority of melanoma patients in a meaningful way. Thus, the need for additional treatment options in melanoma remains urgent.

Melanoma has long been recognized as one of the most immunogenic cancers. Of all tumor types, melanoma has provided the most fertile ground for the development of immunotherapeutic strategies, such as interleukin-2, interferon-alpha, and ipilimumab. Over the past 10 to 15 years, there has been growing enthusiasm for adoptive cell therapy (ACT), a technical approach in which the patient's autologous T cells are expanded, manipulated *ex vivo*, and then re-infused into the patient to exert an anti-tumor response. Clinical trials of adoptive cellular therapy in our lab and others have demonstrated dramatic tumor regressions in an increasing number of melanoma patients with refractory metastatic disease[5-10].

3.2 Adoptive Cellular Therapy

3.2.1 Rationale as a treatment modality

Adoptive therapy involves the transfer of *ex vivo* expanded effector cells as a means of augmenting the anti-tumor immune response. In contrast to tumor vaccination strategies, adoptive T cell therapy can allow for far greater control over the magnitude of

the targeted response by appropriate generation in vitro of the T cells used for therapy[11,12]. Requisite numbers of high affinity effectors can be routinely achieved producing frequencies of tumor-specific T cells in the peripheral blood that are several-fold higher than that possible by current immunization regimens alone.

3.2.2 Adoptive Therapy of Melanoma Using Antigen-Specific $CD8^+$ T Cell Clones

The Yee Lab has recently reported the results of a Phase I study of adoptive therapy using $CD8^+$ T cell clones targeting the tumor-associated antigens, MART1/MelanA and gp100, for the treatment of patients with metastatic melanoma[13]. Eleven patients with bulky, refractory melanoma received cyclophosphamide 4000 mg/m^2 across 2 days, then 10^{10} antigen-specific, CD8+ cytotoxic T cell clones, followed by IL-2. Five of ten evaluable patients had stable disease at 8 weeks, and one patient had a complete remission that has persisted for greater than 3 years.

In another phase I study for patients with refractory metastatic melanoma, Yee and colleagues administered 4 infusions of autologous T cell clones (the first without IL-2) and subsequent infusions with low-dose IL-2 for 14 days[14]. Among 43 infusions at doses up to 3.3 x 10^9 cells/m^2 per dose administered to 10 patients, no serious toxicity was observed. They demonstrated that the adoptively transferred T cell clones persisted *in vivo* in response to low-dose IL-2, preferentially localized to tumor sites, and mediated an antigen-specific immune response characterized by the elimination of antigen-positive tumor cells and regression of individual metastases. Historically, patients with metastatic disease refractory to standard therapy experience an average median survival of 4 months. Among the initial cohort of 10 patients with progressive metastatic melanoma receiving T cell therapy in this study, 8 experienced minor, mixed or stable responses for periods of 2-21 months (average of 11 months).

3.2.3 Adoptive Therapy using Tumor-Infiltrating Lymphocytes

In 1988, Rosenberg and colleagues at the NIH demonstrated that tumor-infiltrating lymphocytes (TIL) derived from resected tumors and expanded in vitro were capable of specifically recognizing tumor antigens and mediating tumor regression in melanoma patients[15]. An analysis of 86 melanoma patients treated with TIL followed by high-dose IL-2 at the NIH from 1987 to 1992 demonstrated a 34% overall response rate, with similar activity in patients with prior IL-2 exposure or no prior IL-2[16]. Five patients (6%) had a complete response but only two were durable at 21 and 46 months. After the addition of a lymphodepleting regimen, response rates to TIL have reached 50-70% and durable complete responses have reached 20-40%.

An important advantage held by TIL over T cell clones is that TIL generation does not rely on exogenous peptide and therefore patient eligibility is not limited to the handful of HLA types to which the currently available peptides are restricted. In contrast, our trials with T cell clones are only open to patients with HLA type A2, which excludes approximately 60% of the population. The shorter generation time of 5-6 weeks for TIL versus 10-12 weeks for traditional T cell clones, also allows the inclusion of patients who have more rapidly growing disease and can't wait months for treatment. Additionally, because TIL can be generated more rapidly and with less manipulation that

traditional T cell clones, a TIL product may be comprised of younger T cells that are less terminally differentiated and with a greater proliferative capacity.

3.2.4 Addition of Lymphodepletion Prior to Adoptive Cell Therapy

In a compelling 2008 analysis, Dudley et al. presented the results of three sequential trials with increasing intensities of myeloablation prior to TIL infusion[17]. In the first trial, a nonmyeloablative regimen was administered using cyclophosphamide (60 mg/kg for 2 days) and fludarabine (25 mg/m^2 for 5 days) followed by TIL infusion and high-dose IL-2. The two subsequent TIL trials gave cyclophosphamide and fludarabine at the same doses as before, but with the addition of 2 Gy TBI for the second trial and 12 Gy TBI for the third trial. Although the trials were conducted sequentially, limiting the reliability of a comparison, they noted that the overall response rates and complete response rates were progressively higher with the greater intensity of lympho- and myeloablation. For the no-TBI, 2Gy-TBI, and 12Gy-TBI trials, the objective response rate was 49%, 52% and 72%, respectively, and the complete response rate was 12%, 20% and 40%, respectively[10]. All but one of the CRs have continued beyond 3 years.

While the response rates are impressive, the myeloablative regimens carried significant toxicity. The latter two trials required hematopoietic rescue with autologous stem cells after TIL infusion due to the intensity of the myeloablative regimen. There was one treatment-related death out of 93 patients and one patient with prolonged pulmonary hypertension; both were on the 2Gy-TBI trial. Five patients on the 12Gy-TBI arm developed long-standing microangiopathic nephropathy, though interestingly, all five patients also achieved CRs and recovered from their renal failure. Because the higher response rates of the 12Gy-TBI regimen is likely biased by differences in patient population and changes in TIL manufacture across time, a prospective randomized trial is ongoing in which patients will receive identical TIL cell and conditioning regimens, but half will also receive TBI with autologous stem cell support (study identifier NCT01319565, clinicaltrials.gov).

The creation of a lymphopenic environment prior to TIL infusion is believed to enhance TIL proliferation and activity by reducing the numbers of immunosuppressive regulatory T cells and myeloid derived suppressor cells that are otherwise promoted by many factors associated with tumor growth, such as TGF-β and apoptosis-inducing receptor-ligand interactions[18,19]. It is also widely believed that the elimination of other lymphocytes decreases the competition for homeostatic cytokines IL-7 and IL-15, providing both physical and biologic “space” for TIL and other potential effectors, such as NK cells, to proliferate and survive[20]. Total body irradiation contributes to lymphodepletion but also appears to increase the function of antigen-presenting cells by activating the innate immune system, in part due to bacterial translocation from gut mucosal damage, which provides activation signals to antigen-presenting cells through their toll-like receptors[21].

3.3 Rationale and Proposed Study of TIL Therapy for the Treatment of Patients with Metastatic Melanoma

TIL therapy in combination with cyclophosphamide and fludarabine conditioning and adjuvant high-dose IL-2 has been demonstrated by the NIH and other institutions to achieve impressive response rates of 50%, which is superior to all other available treatments for metastatic melanoma. Responses have been seen in patients who have failed high-dose IL-2, ipilimumab and vemurafenib. Current adoptive therapy strategies at our institution using T cell clones have been limited to the minority of melanoma patients who are HLA-A2 positive. In contrast, TIL therapy would be available to patients of all HLA types because it does not rely on HLA-restricted, exogenous peptide. In addition, the faster generation time makes TIL a more viable option for patients who are experiencing disease progression and can't wait months for T cell clones to grow.

These early successes of TIL therapy have also given rise to important unanswered questions regarding the variability in response among patients and the need to identify prognostic factors to determine which patients are likely to respond to TIL and thus avoid a costly manufacturing process for patients with little chance of benefit. The inherent requirement of a tumor biopsy for TIL generation provides a built-in guarantee of melanoma tissue for analysis of immunohistochemical features, molecular and genetic markers.

We propose a phase II study evaluating the anti-tumor activity, persistence, safety, as well as prognostic markers and immunologic correlates for durable benefit from TIL therapy following a lymphodepleting conditioning regimen of cyclophosphamide, and fludarabine, and adjuvant high-dose IL-2, for patients with metastatic melanoma.

4. OBJECTIVES

4.A. Primary Objectives

1. Examine the anti-tumor efficacy of cellular adoptive immunotherapy in metastatic melanoma patients using autologous tumor-infiltrating lymphocytes with a lymphodepleting conditioning regimen of cyclophosphamide and fludarabine, and followed by adjuvant high-dose IL-2.

4.B. Secondary objectives

1. Determine the *in vivo* persistence of transferred tumor-infiltrating lymphocytes.

2. Examine the safety of cellular adoptive immunotherapy in melanoma patients using autologous tumor-infiltrating lymphocytes, preceded by a lymphodepleting conditioning regimen of cyclophosphamide and fludarabine, and followed by adjuvant high-dose IL-2.

3. Evaluate for molecular tumor markers and immunohistochemical features that correlate with in vivo persistence and anti-tumor efficacy.

5. STUDY DESIGN

This will be a single-center, nonrandomized study that will enroll 20 patients with metastatic melanoma for the treatment phase (Step II) of this protocol. The protocol utilizes a pre-screening stage (Step I) to obtain the 20 treatment-eligible patients for whom TIL can be expanded to sufficient numbers. Enrollment on Step I will stop once there are 20 enrolled patients for Step II. The actual enrollment number for Step I is anticipated to be 80-120 patients based on an expected 50% success rate for TIL generation, mitigated by patient attrition between Step I and Step II due to external factors such as disease progression or response to other therapy.

Starting on day -7, patients will receive cyclophosphamide 60mg/kg/day intravenously for two days, then fludarabine 25mg/m^2/day intravenously for five days, followed by a single TIL infusion at a dose up to 1.5 x 10^{11} cells on day 0. Within 24 hours of the TIL infusion, high-dose IL-2 will be administered at 600,000 units/kg every 8 hours, up to 14 doses as tolerated. Patients will then be monitored with clinical visits and blood draws on a weekly basis until week 6, then at weeks 8, 12 and 24. Reimaging with CT or PET/CT will be performed at week 6, week 12 and week 24 after TIL infusion.

The chemotherapy of cyclophosphamide and fludarabine (days -7 to -1) will be administered at the University of Washington Medical Center inpatient oncology unit, or if the patient is deemed suitable by the PI, at the Seattle Cancer Care Alliance on the outpatient Immunotherapy Service. Treatment of tumor infiltrating lymphocytes (TIL) will be given at the UWMC inpatient oncology unit, or if the patient is deemed suitable by the PI, at the Seattle Cancer Care Alliance on the outpatient Immunotherapy Service IL-2 therapy (days +1 to +5) will given at the University of Washington Medical Center. in the intensive care unit (ICU) to allow close monitoring and evaluation of the patients.

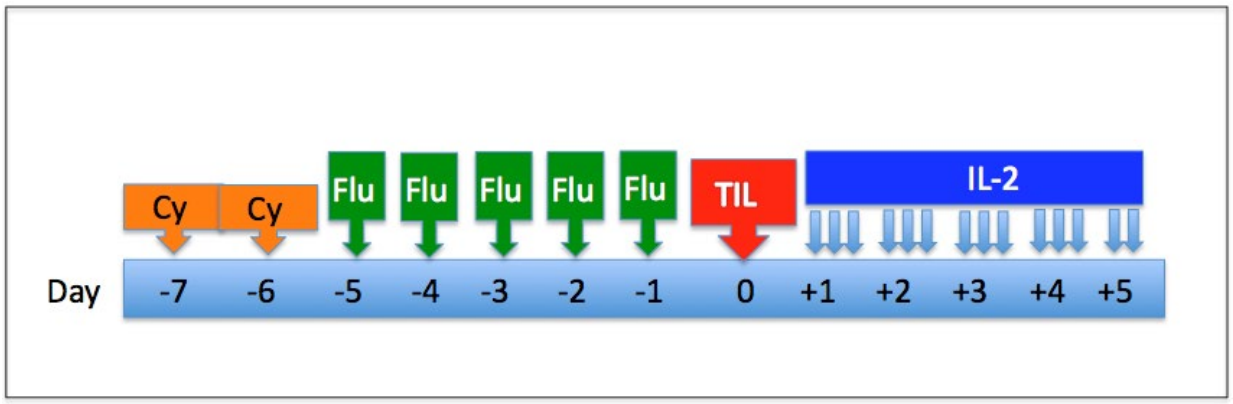

Figure 1. Treatment course

6. PATIENT SELECTION

Patients will be enrolled through the Seattle Cancer Care Alliance Melanoma Clinic (a clinical consortium of the Fred Hutchinson Cancer Research Center, Seattle Children's Hospital and University of Washington). This protocol is designed to treat patients with measurable metastatic melanoma. An average of 300 patients with stage IV disease are seen on a yearly basis in the melanoma clinic. With focused recruitment, we expect to evaluate roughly 50 patients per year for adoptive cell therapy, and expect 50% of these will meet eligibility criteria for Step 1. We anticipate we will succeed in expanding TIL for

60% of the patients enrolled on Step I of the protocol, and thus, the enrollment for the targeted of 20 treated patients will be completed within 30-36 months, and treatment and analysis of all patients completed by 36-42 months.

Patient evaluation for eligibility and enrollment will occur in a two-step process: Step I for pre-screen and TIL generation, and Step II for treatment. Results of tests and/or procedures conducted as per standard of care may be used for eligibility determination if conducted within an appropriate window prior to screening.

6.1 Step I: Pre-screen and initiation of TIL expansion.
Patients must fulfill all the following criteria to be eligible for Step I of the study.

6.1.1 Step I Inclusion Criteria

- **a.** Stage IV melanoma or stage III melanoma that is unlikely to be cured by surgery.
- **b.** Male or female subject, 18 years of age or older and able to tolerate high-dose cyclophosphamide, fludarabine and high-dose IL-2.
- **c.** ECOG performance status of 0-1 (Appendix A)
- **d.** Patients must have an MRI, CT, or PET of the brain within 2 months before consenting if known history of brain metastasis or if clinically indicated. If new lesions are present, PI or designee should make final determination regarding enrollment.
- **e.** Patients must have a site of metastatic disease that can be safely resected or biopsied for tissue sufficient for TIL harvest.

6.1.2 Step I Exclusion Criteria

- **a.** Men or women of reproductive ability who are unwilling to use effective contraception or abstinence for 4 months after treatment.
- **b.** Calculated creatinine clearance (eGFR) < 60ml/min. EGFR values can be determined by either MDRD or Cockcroft-Gault equation based on the investigator's discretion.
- **c.** Significant hepatic dysfunction (AST/ALT > 3x upper limit of normal; total bilirubin > 2.0 mg/dl, except in patients with Gilbert's Syndrome whose total bilirubin must not exceed 3.0 mg/dl) deemed by investigator to be irreversible.
- **d.** Clinically significant pulmonary dysfunction (FEV1<65% predicted, FVC <65% of predicted, DLCO (corrected for Hgb) <50% predicted). Pulmonary function tests (PFTs) within 4 months prior to consent for Step I will be required for patients with underlying risk factors such as smoking history >10 pack years, or a history of pre-existing symptomatic lung disease (not including melanoma metastases to the lung).
- **e.** Pre-existing known cardiovascular abnormalities as defined by any one of the following:
  - congestive heart failure,

- clinically significant hypotension,
- cardiac ischemia, or symptoms of coronary artery disease,
- presence of cardiac arrhythmias on EKG requiring drug therapy,
- ejection fraction < 45% (echocardiogram or MUGA), although any patient with an ejection fraction between 45-49% must receive clearance by a cardiologist to be eligible for Step II of the trial

f. Clinically significant autoimmune disorders or conditions of immunosuppression. Patients with AIDS or HIV-1 associated complex or known to be HIV antibody seropositive or known to be recently PCR+ for hepatitis B or C are not eligible for this study. The severely depressed or altered immune system found in these patients and the possibility of premature death would compromise study objectives.

g. Patients with active systemic infection requiring intravenous antibiotics.

h. Clinically significant psychiatric disease which, in the opinion of the PI or sub-I, would render immunotherapy and its potential sequelae unsafe or compliance with procedural requirements unlikely.

6.1.3 Procedures for Step I

All patients must sign an informed consent form for Step I. Patients will be screened for Hepatitis B surface antigen, hepatitis B core antibody, hepatitis B surface antibody, hepatitis C virus antibody, HIV 1/2 Antibody, and standard test for syphilis (STS) within 30 days of signing informed consent.

TIL expansion will be attempted on approximately 80-120 patients to obtain the 20 patients who continue on to receive treatment on Step II of this protocol. While TIL are being grown, patients may be treated with other therapies. However, patients must not have received other therapies within 4 weeks prior to lymphodepletion, with the exception of commercially available molecularly targeted therapies (e.g., vemurafenib), which are not allowed within 7 days prior to lymphodepletion.

6.2 Step II: Treatment with TIL.

All patients must complete screening procedures for Step I to be eligible for Step II. Patients must fulfill all of the following criteria to be eligible for Step II of the study. Patients must sign an informed consent form for Step II before receiving treatment with TIL therapy on this protocol.

6.2.1 Step II Inclusion Criteria

a. Patients must have measurable metastatic melanoma.

b. Male or female subject, 18 years of age or older and able to tolerate high-dose cyclophosphamide, fludarabine, and high-dose IL-2.

c. ECOG performance status of 0-1 (Appendix A).

d. Patients must have brain imaging by MRI, CT or PET within 30 days prior to lymphodepletion. Patients may have asymptomatic brain lesions that are ≤1cm each. Lesions that are >1cm that have been irradiated and in the opinion of the investigator no longer represents active disease will also be allowed.

e. A functional cardiac test (e.g., stress treadmill, stress thallium, MUGA, dobutamine echocardiogram) to rule out cardiac ischemia within 4 months prior to lymphodepletion is required for all patients.

f. PFTs are required of all patients within 4 months prior to lymphodepletion. FEV1 and FVC must be ≥65% predicted and DLCO must be ≥50% predicted.

g. Patients must have their tumor sent for BRAF mutational analysis.

h. Patients must have adequate TIL (at least 40 x 10^6 cells at the pre-expansion stage).

6.2.2 Step II Exclusion Criteria

a. Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception or abstinence. Women of childbearing potential must have a negative pregnancy test within 14 days prior to entry. Patients of both genders must practice birth control during treatment and for four months after treatment.

b. Calculated creatinine clearance (eGFR) < 60ml/min. EGFR values can be determined by either MDRD or Cockcroft-Gault equation based on the investigator's discretion.

c. Significant hepatic dysfunction (AST/ALT > 3x upper limit of normal; total bilirubin > 2.0 mg/dl, except in patients with Gilbert's Syndrome whose total bilirubin must not exceed 3.0 mg/dl).

d. Clinically significant pulmonary dysfunction (FEV1<65% predicted or FVC <65% of predicted, DLCO (corrected for Hgb) <50% predicted).

e. Pre-existing known cardiovascular abnormalities as defined by any one of the following:

- congestive heart failure,
- clinically significant hypotension,
- cardiac ischemia, or symptoms of coronary artery disease,
- presence of cardiac arrhythmias on EKG requiring drug therapy,
- ejection fraction < 45%, although any patient with an ejection fraction between 45-49% must receive clearance by a cardiologist to be eligible for Step II of this trial.

f. Absolute neutrophil count less than 1000/mm^3.

g. Platelet count less than 100,000/mm^3.

h. Hemoglobin less than 10.0g/dl.

i. Untreated central nervous system metastases that are either symptomatic or greater than 1 cm at time of therapy. Lesions that are >1cm that have been irradiated and in the opinion of the PI or sub-I no longer represent active disease may be allowed.

j. Patients with systemic infections requiring active therapy within 72 hours of lymphodepletion.

k. Systemic cancer therapy (standard or experimental), including cytotoxic chemotherapy, IL-2, or checkpoint blocking agents (e.g., CTLA-4 or PD1/PD-L1 inhibitors) received less than 4 weeks prior to lymphodepletion, with the exception of targeted therapies (see next).

l. Commercially available, molecularly targeted therapies (e.g., dabrafenib, trametinib, vemurafenib, imatinib) taken within 7 days prior to lymphodepletion.

m. Clinically significant autoimmune disorders or conditions of immunosuppression. Patients with AIDS or HIV-1 associated complex or known to HIV antibody seropositive or known to be recently PCR+ for hepatitis B or C virus are not eligible for this study. Virology testing will be done within 6 months of T cell infusion. The severely depressed or altered immune system found in these patients and the possibility of premature death would compromise study objectives.

n. Prior treatment with systemic steroids within 4 weeks prior to lymphodepletion (except for physiologic replacement doses, for adrenal insufficiency, premedication for contrast allergies for scans, and for drug fever related to targeted therapy).

o. Any other significant medical or psychological conditions that would make the patient unsuitable candidate for cell therapy at the discretion of the PI.

6.2.3 Procedures for Step II

- A complete history and physical examination noting in detail the exact size and location of any lesions appreciable by exam will be performed within 4 weeks prior to the initiation of chemotherapy.
- Blood work including a CBC, differential, complete metabolic panel with LDH, PT/PTT/INR, and a urinalysis (micro) will be performed within 14 days prior to initiation of chemotherapy.
- A pregnancy test (urine or serum) on all women of childbearing potential will be performed within 14 days prior to initiation of chemotherapy.
- Baseline radiographic studies must be obtained within 30 days prior to chemotherapy (CT or PET/CT of chest, abdomen, and pelvis; MRI, CT or PET/CT of brain). If a patient has no history of prior brain metastases and no symptoms concerning for brain disease, brain imaging can be performed within 60 days prior to chemotherapy.
- Pulmonary function tests must be completed within 4 months of lymphodepletion.
- An electrocardiogram must be completed within 2 weeks prior to lymphodepletion.
- Functional cardiac test is required, within 4 months prior to lymphodepletion.
- If prior virology studies for Step I were completed more than 6 months

prior to lymphodepletion, these tests must be repeated: Hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis C virus antibody, HIV 1/2 Antibody, standard test for syphilis (STS), CMV antibody, and EBV panel.

- HSV and VZV serologies within 2 months prior to lymphodepletion.
- HLA typing must be drawn at any time prior to lymphodepletion.
- Tumor must have been sent for BRAF mutational analysis at any time prior to enrollment on Step II.
- A research blood draw up to 80cc in ACD tubes for immunologic studies at any time prior to lymphodepletion, and at least three weeks after prior cytoreductive chemotherapy.

7. STUDY AGENTS

7.1 Tumor-infiltrating lymphocytes (TIL)

All infused TIL products will be autologous lymphocytes derived from excisional or incisional tumor biopsies of metastatic melanoma. Methods employed to generate and select TIL for infusion are outlined in Appendix B. In brief, tumor fragments are cultured in media containing IL-2 until sufficient lymphocytes grow out. The lymphocytes that demonstrate the fastest growth are maintained, and further selected based on CD8 expression and recognition of autologous tumor by ELISA. The selected TIL cultures are pooled for a 2-week rapid expansion. Prior to infusion they are tested for sterility. MD Anderson has found that TIL infusions up to 1.5×10^{11} cells are well tolerated.

7.2 Cyclophosphamide

The effectiveness of immunotherapy appears to be enhanced when combined with conventional cytotoxic agents, and among these, cyclophosphamide has been the most extensively studied [22]. Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands.

In clinical studies where no other chemotherapy drugs were administered, cyclophosphamide alone at doses of 30 – 120 mg/kg (1000 to 4000 mg/m^2) has a predictable hematologic profile with a period of neutropenia and leukopenia beginning 8-10 days after treatment lasting 7-10 days followed by a relatively rapid recovery. Thrombocytopenia may be more prolonged, but recovery to normal levels is usually complete. The dose limiting toxicity from cyclophosphamide is not hematopoietic, but cardiac at doses up to 8000 mg/m2- when given as an IV bolus[23]. Cardiac toxicities from cyclophosphamide include arrhythmias, potentially irreversible cardiomyopathy and pericarditis.

Sterile hemorrhagic cystitis occurs in approximately 20% of patients, ranging from microscopic hematuria to extensive cystitis with bladder fibrosis. Mesna (sodium 2-mercaptoethanesulphonate) is a synthetic sulfhydryl compound that can interact with the urotoxic metabolites of cyclophosphamide to decrease the incidence and severity of hemorrhagic cystitis.

7.3 Fludarabine

Fludarabine is a purine analog that inhibits DNA synthesis by impairment of ribonucleotide reductase and DNA polymerase alpha. Fludarabine induces lymphopenia in greater than 30% of patients treated with a standard 5-day course of fludarabine at 25 mg/m^2 [24]. Although fludarabine is an effective agent against hematologic malignancies, such as CLL, low-grade non-Hodgkin's lymphoma, and Waldenström's macroglobulinemia, it has no anti-tumor effect on solid tumors such as melanoma.

Lymphopenia is characterized by a CD4 > CD8 T cell depletion that results in lymphocyte counts falling to 30-40% of baseline or less that is sustained for periods of several weeks to months and is associated with T cell dysfunction-related infections [25,26]. The risk of lymphopenia, the severity and frequency of infectious complications observed in patients receiving multiple courses of fludarabine is much lower when patients receive a single five-day course of fludarabine[27]. Approximately 25% of patients receiving a 5-day course of treatment will develop an infection that is generally treatable. Patients receiving only a 3-day course of fludarabine exhibit a significantly lower incidence of infection per treatment course (14%) without a significant decrease in anti-lymphoproliferative effect[28]. Other common toxicities of fludarabine (< 10%) include edema, fever, chills, rash, pain, thrombocytopenia with a nadir of 16 days, malaise, fatigue, anorexia, nausea/vomiting, gastrointestinal bleeding, weakness, visual disturbances, sleep disorders, cough, dyspnea, and infection.

7.4 Interleukin-2

The major physiologic role of IL-2 is to promote the activation and proliferation of T and NK cells in an autocrine and paracrine manner [29]. It is secreted primarily by T cells in response to antigenic stimuli. Exposure of NK cells to IL-2 results in proliferation, enhanced cytolytic activity and secretion of other cytokines. B cells also express intermediate affinity IL-2 receptors and can secrete IL-2 in cooperation with other cytokines, resulting in B cell proliferation and differentiation[30].

Initial work by Rosenberg's group at the NIH found that adoptively transferred IL-2-activated peripheral blood mononuclear cells with the phenotype and functional characteristics of activated NK cells, supported with concomitant administration of IL-2 in high doses, resulted in significant tumor regression in patients selected for normal organ function and good performance status. Further investigation of these encouraging results suggested that therapeutic benefit could be seen in a subset of patients treated with high doses of IL-2 alone. This led to clinical trials that demonstrated high-dose IL-2 induces objective clinical responses in 15–20% of patients with advanced melanoma and durable complete responses in 5–7% of these patients [31,32]. The NIH group has been consistently administering a cycle of high-dose IL-2 after TIL infusion to stimulate the in vivo expansion of the TIL product. MD Anderson has been administering two cycles of high-dose IL-2 following their TIL infusions, spaced 21-days apart, and have reported in personal communication, that these are well tolerated.

The toxicity profile of IL-2 is largely associated with a capillary leak syndrome, which is characterized by hypotension, tachycardia and peripheral edema secondary to third space fluid accumulation. In addition to hypotension, IL-2 may also induce pulmonary edema, cardiac arrhythmias, myocarditis, reversible renal and hepatic dysfunction, pruritus, electrolyte abnormalities, thrombocytopenia, anemia and coagulopathy. IL-2

can also cause constitutional symptoms such as fever, chill and fatigue, gastrointestinal side effects such as nausea, vomiting, anorexia, transaminase elevation, cholestasis and diarrhea [33]. Rarely, IL-2 may induce confusion, disorientation or visual hallucinations. Although early studies with IL-2 reported a 2% mortality rate, generally related to gram-positive sepsis, current IL-2 centers that routinely use prophylactic antibiotics report no mortality [34-36]. In experienced centers such as University of Washington, IL-2-related toxicity can usually be easily managed and all side effects are reversible upon cessation of treatment.

8. INVESTIGATIONAL PLAN

8.1 Tumor Acquisition and TIL Generation

Once patients meet screening criteria for Step I, we will obtain a tumor specimen by a biopsy or surgery of a safely accessible melanoma lesion, ideally 2 cm^3. Whenever possible, this tumor will be acquired from a biopsy or surgery that is already being performed for medical reasons (e.g. to confirm metastatic disease or for palliation). Part of this tumor will be sent to pathology for diagnostic purposes, as well as additional immunohistochemical analysis to assess for prognostic features. If there is enough tissue, part of this tumor will be flash frozen and stored to enable future analysis. TIL will be generated, tested, selected and expanded across the next 5-6 weeks. If a patient is not ready for treatment, the TIL will be cryopreserved prior to the 2-week expansion phase. A portion of the TIL product pre- and post-expansion may also be removed for research purposes (e.g. immunophenotyping, gene expression analysis.)

8.2. Conditioning Regimen with Cyclophosphamide and Fludarabine

Patients will be admitted to the oncology inpatient service at the University of Washington Medical Center, or at the discretion of the PI, the outpatient Immunotherapy Service at Seattle Cancer Care Alliance on day -7. A PICC line will be placed, largely in anticipation of high-dose IL-2 but also for the administration of chemotherapy and TIL. If the PICC team is unavailable on admit, chemotherapy may be started using a peripheral IV and then switched to the PICC line once it is placed.

On day -7 and day -6, cyclophosphamide (Cy) at 120 mg/kg will be administered intravenously in split doses of 60 mg/kg/day x 2 days. Standard Practice Policy guidelines, including IV hydration and Mesna therapy for uroprotection, will be followed.

Fludarabine will be administered intravenously for 5 days from day -5 through day -1, at a dose of 25mg/m^2/day, and completed at least 24 hours prior to T cell infusion.

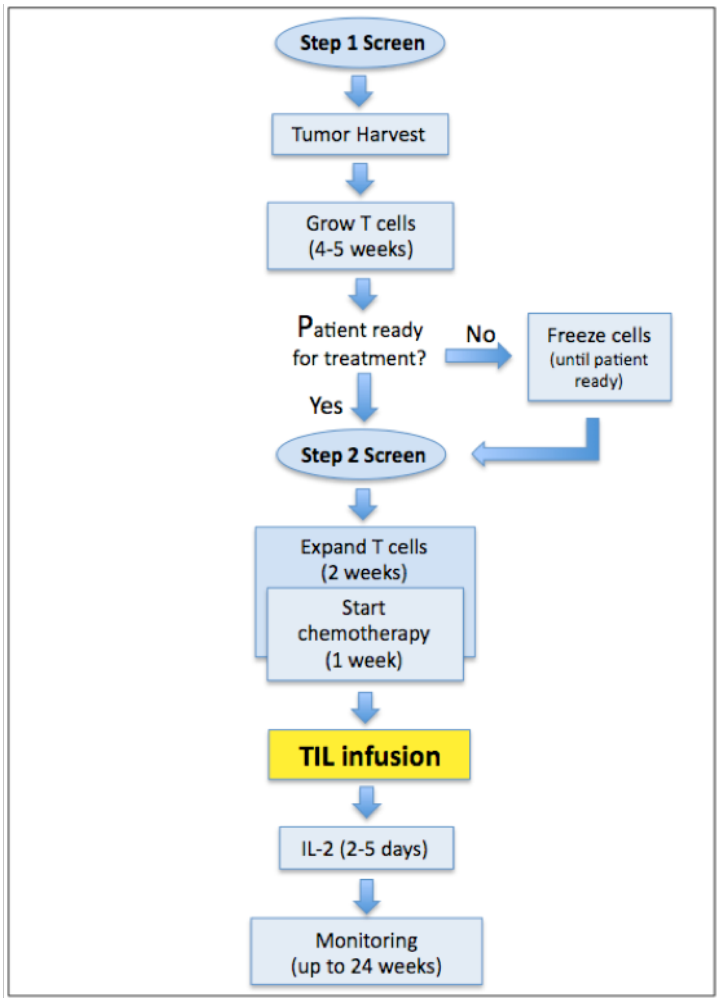

Figure 2. Investigational Plan

8.3 TIL infusion

On day 0, patients will receive the TIL infusion at the University of Washington Medical Center or at the discretion of the PI, the outpatient Immunotherapy Service at Seattle Cancer Care Alliance. Patients will receive up to 1.5 x 10^{11} cells, depending on the number that is generated in the laboratory. The remainder will be cryopreserved for research purposes, but will not be infused at a later date. The TIL will be suspended in a 500cc bag of normal saline and infused intravenously over 45 minutes (+/- 15 minutes) by a peripheral IV, a PICC line or central catheter. The infusion bag will be gently mixed, approximately every 15 minutes. Patients will have vital signs obtained pre-infusion, every 15 minutes during the infusion, at the end of the infusion and hourly for two hours following the infusion.

8.4 High-dose Interleukin 2

Within 24 hours of the TIL infusion, the patient will be transferred to the hematology-oncology intensive care unit at UWMC and started on high-dose interleukin 2 at 600,000 IU/kg as an IV bolus every eight hours up to a maximum of 14 doses, as tolerated, based on the High-Dose Interleukin-2 Administration and Toxicity Management Guidelines currently used at the University of Washington (Appendix C). Once the patient develops toxicities, the PI or designated surrogate will make a clinical decision when to stop interleukin-2. This decision will be based on the Toxicity Management Guidelines.

After completion of high-dose interleukin-2, standard supportive care will be provided including weaning off vasopressor support, discontinuing parenteral fluids, and monitoring. When the patient demonstrates hemodynamic stability and adequate pulmonary reserve, the patient will be discharged from the hospital, typically with 3-4 days of oral diuretics and potassium as indicated.

8.5 Prophylaxis

For all prophylactic medications below, dose adjustments and substitutions may be made if clinically warranted (e.g. drug allergy, renal impairment).

8.5.1 Bacterial Prophylaxis

Patients will receive levofloxacin 500 mg daily, starting on day +0 or once ANC falls below 500/mm^3, whichever is sooner. Levofloxacin will be continued until ANC recovers to greater than 500/mm^3.

8.5.2 Pneumocystis Jiroveci Pneumonia (PCP) Prophylaxis

Patients will receive the fixed combination of trimethoprim (TMP) and sulfamethoxazole (SMX) as double strength (DS) tablet [DS tabs = TMP 160 mg/tab and SMX 800 mg/tab] po bid on two consecutive days a week. TMP/SMX-DS will be taken by patients beginning on Day -7 and continued for 6 months after lymphodepletion. Patients with sulfa allergies may instead take dapsone 100mg po daily or atovaquone 1500mg po daily, starting one week prior to lymphodepletion until 6 months after lymphodepletion.

8.5.3 Herpes Simplex Virus and Varicella Zoster Virus Prophylaxis

Patients with a positive HSV and/or positive VZV serology will be administered valacyclovir 500 mg po bid, starting on day+0, for 6 months. If the patient cannot take oral medications, he will be given acyclovir 5 mg/kg IVPB every 8 hours, which is continued until absolute neutrophil count is greater than 1000/ml.

8.5.4 Fungal Prophylaxis

Patients will begin Fluconazole 200 mg po daily, starting on Day+0 or once ANC falls below 500/mm^3, whichever is sooner. Fluconazole will be continued for 2 months after lymphodepletion.

8.5.5 Empiric Antibiotics

Patients who develop fevers ≥38.5 C with an ANC less than 500/mm^3 will be treated on broad-spectrum antibiotics per standard UW practice for neutropenic fever as appropriate to the patient's signs and symptoms of infection. Aminoglycosides should be avoided unless clear evidence of sepsis.

8.5.6 Hematologic Support

In order to reduce neutropenia following chemotherapy and T cell infusion, G-CSF will be given at 5 µg/kg/day daily subcutaneously from Day +1 until neutrophil counts reach >500/mm^3x 3 days or >5000/mm^3 x 1 day. Using daily CBC's as a guide, the patient will also receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep hemoglobin >8.0 g/dl,

and platelets >20,000/ml. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection. Irradiated blood and blood products should be used.

8.6 Specific Guidelines for Patients on BRAF and MEK inhibition

Patients are not allowed to receive other anti-cancer treatments while on this protocol with the exception of BRAF and MEK inhibitors. Patients who are responding to targeted therapies such as vemurafenib and dabrafenib represent a very different patient category. It is well established that the responses to vemurafenib and dabrafenib are short-lived, with a 6-7 month median duration of response.[4] It often takes patients 1-2 months to reach best response, and this response remains stable until they ultimately experience disease progression. However, waiting for these patients to progress on BRAF/MEK inhibition or requiring they discontinue BRAF/MEK inhibitors for an extended period is not ideal, because patients in these two situations often experience rapid disease progression that would preclude subsequent immunotherapy that could offer more durable disease control.

Therefore, for those patients who are currently on BRAF and MEK inhibitors and are experiencing a response that has stabilized (two consecutive scans spaced at least one month apart that show no further regression), concurrent treatment on BRAF/MEK inhibitors will be allowed with the following guidelines:

- Patients must be off BRAF/MEK inhibitors for the 7 days prior to lymphodepletion.
- Patients may resume BRAF/MEK inhibitors no sooner than 7 days after the completion of IL-2, as long as the PI or her surrogate deems this safe based on the patient's clinical status at that time.
- If the patient achieves a complete response after T cell therapy that lasts at least 6 months, the BRAF/MEK inhibitors can be discontinued and a follow-up scan performed in 2-3 months.
- If the patient achieves a partial response or stable disease after T cell therapy, that is unchanged for at least 12 months and PET-negative, the BRAF/MEK inhibitors can be discontinued and a follow-up scan performed in 2-3 months.

9. SCHEDULE OF EVALUATIONS

9.1 General Toxicity Assessment

Patients will have vital signs taken, a physical exam, a comprehensive chemistry panel and a complete blood count and differential at each planned visit, daily while the patient is in the hospital/clinic, weekly until 4 weeks after the T cell infusion, and then on weeks 6, 8, 12 and 24 (Appendix D). Of note, the dates listed on the study calendar are approximate as many patients may reside out of the area and cannot always follow the time points as dictated by the protocol—however, patients must remain close to the study center until blood counts have recovered sufficiently to be at minimal risk of

infection or bleeding. All adverse events will be recorded and graded according to the NCI CTCAE version 4.0.

9.2 Immunologic Studies

For evaluation of peripheral blood cells following T cell infusion, 60 ml of blood into yellow top tubes (containing ACD solution) should be drawn within 60 days after signing of step I consent, prior to receiving chemotherapy on day -7, prior to infusion on day 0, and then day +1, +3, +7, +14, +21 +28, +35, +42, +49, +56 (+/- 3 days), and then week 12 and week 24, as well as any follow up visits when feasible. These dates are approximate as many of our patients reside out of the area and their oncologists and laboratories cannot always follow the time points as dictated by the protocol. Samples will be used to evaluate the duration of in vivo persistence, phenotype and function of infused TIL. Strategies for following in vivo persistence include deep sequencing of the T cell receptor CDR3 regions to follow frequencies of T cell populations, tetramer staining and ELISPOT analysis using melanoma peptide pools.

9.3 Efficacy Assessment

Radiographic imaging (CT, PET/CT or MRI) and clinical assessment of residual disease will be compared with pre-infusion assessment. This assessment will be performed at 6 weeks, 12 weeks and 24 weeks after T cell infusion, and for continued responders, every 3-6 months for the next two years, at the discretion of the primary provider.

Clinical response will be determined at 6 weeks, 12 weeks and 24 weeks based on RECIST version 1.1[37] definitions for CR, PR, SD and PD.

10. RE-TREATMENT

After TIL therapy, if a patient experiences disease control (complete response, partial response or stable disease) lasting 6 months, and then shows evidence of disease progression, and he experienced less than Grade III toxicity (except for the common expected toxicities listed below), the patient may be re-evaluated using the same screening procedures for re-treatment with TIL. The retreatment plan would be unchanged, with the same cyclophosphamide and fludarabine lymphodepletion regimen, TIL infusion and adjuvant high-dose IL-2. The patient would need to be re-consented and meet all eligibility criteria, as before. The patient would need to undergo a repeat tumor biopsy to harvest TIL, unless there are sufficient TIL cryopreserved from the prior TIL culture for expansion (at least 40 x 10^6 cells).

11. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

11.1 Evaluation of Toxicity

Toxicity grading will be evaluated according to guidelines in NCI Common Toxicity Criteria version 4.0[38]. The full text of the NCI CTCAE is available online at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html. Toxicities will be monitored on a daily basis beginning at day -7 and continuing until discharge from the hospital following IL-2 infusion and then on subsequent follow up visits through day +42. The principal

investigator will be responsible for monitoring the data and toxicities to identify trends, and for revising the protocol as needed to maintain safety.

A data safety and monitoring board () will be formed and meet after the treatment of the 5th patient, the 15th patient or in the event of 2 or more unexpected, serious (CTCAE grade 3 or greater), treatment-related toxicities *or at least annually whichever is sooner*. If the rate of unexpected, serious, treatment-related toxicities is observed in $\geq$ 33% of enrolled subjects in a cohort of 6 or more subjects, this will trigger the suspension of the study pending further review by the DSMB and FDA. Furthermore, any occurrence of unexpected grade 4 or greater toxicity will prompt the investigator to conduct a thorough investigation of available safety data to justify to the DSMB a decision to continue enrolling new subjects into the study.

Serious adverse events (Appendix E) will be reported to the IRB and the FDA, in accordance with regulations detailed in the IND package.

11.2 Regimen-Related Toxicity

If the patient develops an unexpected and serious toxicity attributable to the study regimen, the patient will not receive additional study treatment and a course of corticosteroids will be given if clinically indicated (see Section 11.3). A serious toxicity is defined as a non pre-existing Grade 3 or higher toxicity that develops after the start of treatment with the following exceptions:

Common and expected symptoms of chemotherapy and T cell infusion that are considered exceptions to criteria for discontinuation include:

A. For Cyclophosphamide at 60 mg/kg/day x 2 days and fludarabine 25mg/m^2/day x 5 days, expected toxicities are:

i. Non-life-threatening infections or febrile neutropenia (i.e. absence of hemodynamic compromise requiring vasopressor support)
ii. WBC < 1000 (Grade 4 toxicity) for less than 4 weeks
iii. ANC < 500 (Grade 4 toxicity) for less than 3 weeks
iv. Lymphocytes < 500 (Grade 3 toxicity) for less than 3 weeks
v. Platelets < 50,000 (Grade 3 or 4 toxicity) for less than 4 weeks
vi. Hyperbilirubinemia and transaminase elevation (Grade 3 toxicity) that resolves within 3 weeks
vii. Nausea, vomiting or diarrhea (Grade 3 toxicity) that resolves within 3 weeks
viii. Electrolyte and renal abnormality (Grade 3 toxicity) that resolves to Grade 1 or baseline within 3 weeks

B. Expected toxicities attributable to T cell infusions and considered exceptions to criteria for discontinuation include:

i. Cytokine Release Syndrome (CRS) Grade 3 or less, including but not limited to asthenia, flu-like symptoms, myalgia, lymphopenia and rigors
ii. Skin rash/Erythroderma (Grade 3 toxicity)

iii. Hypoxemia requiring continuous oxygen, but not mechanical ventilation or intubation < 72 h
iv. Fever that resolves within 72h after cessation of IL-2
v. (Lymphocytes < 500) (Grade 3 or 4 toxicity) that resolves within 4 weeks to baseline levels (pre-therapy)

C. Expected toxicities attributable to high-dose IL-2:

A variety of side effects and toxicities have been associated with high-dose IL-2 administration; a list of these along with guidelines for their management, which should be adjusted for the patient's clinical condition, is listed in Appendix C.

11.3 Management of Symptoms during TIL Infusion

Mild transient symptoms have been observed with LAK and TIL cell infusions, and with infusions of antigen-specific T cell clones. These symptoms occur during the first 24 hours following T cell infusion. The management of these symptoms is outlined below.

a. Myalgia, fever and chills will be managed with acetaminophen 650 mg p.o. q 4-6 hrs along with assessment of possible infection.
b. Headaches may be managed with acetaminophen and mild opiates following a neurologic examination.
c. Nausea, vomiting will be treated with a non-steroidal anti-emetic of choice.
d. Hypotension will initially be managed by intravenous fluid administration and further measures as dictated by standard medical practice.
e. Hypoxemia will initially be managed with supplemental oxygen and further measures as dictated by standard medical practice.

11.4 Potential Toxicities Warranting Ablation of Adoptively Transferred TIL

Ablation of transferred T cells by corticosteroid administration will be warranted by any grade 3 or greater toxicity (NCI Common Toxicity Criteria version 4.0[38]) occurring in any other organ system following administration of antigen-specific cytotoxic T cells not attributable to underlying metastatic melanoma and excluding exceptions associated with known toxicities of cyclophosphamide, fludarabine, T cells and IL-2 .

T-cell Ablation with Corticosteroids

a. All patients will be hospitalized for the first 48 hours for monitoring. The following guidelines are provided for steroid dose and taper but will be adjusted to patient's clinical situation.

Day 1	Intravenous Solu-Medrol at 2 mg/kg
Day 2	Intravenous Solu-Medrol at 2 mg/kg
Day 3-4	Prednisone at 30 mg po b.i.d.
Day 5-6	Prednisone at 15 mg po b.i.d.
Day 7-8	Prednisone at 10 mg po b.i.d.
Day 9-10	Prednisone at 10 mg po q.d
Day11-12	Prednisone at 5 mg po q.d.

b. Blood will be drawn for later analysis of the in vivo frequency of melanoma antigen-specific T cells will be assayed immediately prior to and 48 hours after the start of steroid therapy.

11.5 Concomitant Therapy

11.5.1 Infections occurring despite antibiotic prophylaxis should be treated according to the standard of care.

11.5.2 The following agents are not allowed while on study: systemic corticosteroids (except as outlined for management of toxicity of TIL or physiologic doses for adrenal insufficiency), chemotherapy that is not part of the treatment plan, other immunomodulatory agents, or any investigational agents, including unapproved BRAF inhibitors. Patients who are responding to vemurafenib (a BRAF inhibitor) at the time of enrollment on this trial may be allowed to resume vemurafenib ≥7 days after IL-2 is completed (refer to Section 8.6 for conditions and guidelines for patients on vemurafenib).

11.6 Premature Discontinuation

Subjects who do not complete the study procedures will be considered to have prematurely discontinued the study. The reasons for premature discontinuation (for example, voluntary withdrawal, toxicity, death) must be recorded on the case report form. A subject may re-enter the study after premature discontinuation only by approval of the Principal Investigator. If possible, final study evaluations should be completed at the time of discontinuation. Potential reasons for premature discontinuation include:

11.7.1 The development of a life-threatening infection.

11.7.2 Judgment of the principal investigator that the patient is too ill to continue.

11.7.3 Patient noncompliance with study therapy and/or clinic appointments.

11.7.4 Pregnancy.

11.7.5 Voluntary withdrawal; a patient may remove himself/herself from the study at any time without prejudice.

11.7.6 Significant and rapid progression of melanoma requiring alternative systemic therapy.

11.7.7 Grade III or IV toxicity judged to be possibly or probably related to study therapy according to criteria and exceptions as described above.

11.7.8 Termination of the study by the principal investigator, the DSMB, Institutional Review Office or the Food and Drug Administration. In this case, patients will be informed of the reasons and will continue to receive best available therapy of their disease.

12. GUIDELINES FOR ADVERSE EVENTS REPORTING

12.1 Reporting of Adverse Events (AEs)

All unexpected and serious adverse events which may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office (IRO) per their current reporting requirements.

AEs of grade 3 or greater (per IRB policy 2.6. section 1.a.) will be collected through 4 weeks after the T cell infusion. Beginning 4 weeks after the T cell infusion, only study-related toxicities will be collected.

All serious adverse events that are unexpected and related to study treatment will be reported to the FDA on a MedWatch 3500 reporting form that will be submitted to the FDA within 15 calendar days. The reports will include the date and time of onset, severity and duration of the event, the relationship to study treatment, the treatment given, and the eventual outcome.

12.2 Definitions

Definitions associated with reportable events can be found on the FHCRC IRO extranet website. (Relevant FHCRC policies include, but are not limited the documents listed [**Table 1**]. Please also refer to the FHCRC IRO website.)

Table 1. FHCRC IRB policies for reportable events

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects or Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

	Investigational Device Exemptions (IDE)	

13. STATISTICAL CONSIDERATIONS

The primary endpoint of this study will be clinical response, to be assessed at 6, 12 and 24 weeks, using RECIST 1.1 definitions for CR, PR, SD and PD. Formal guidelines will not be used to assess the potential efficacy of this treatment in terms of response rate. However, given data from FDA-approved therapies where response rates have ranged from 7% to 16%, we previously set a response rate of 20% for this trial as an informal benchmark that would support the clinical utility of TIL as an additional treatment for melanoma. The original version of this protocol had a targeted accrual of 40 patients with a goal of showing a statistically significantly better response rate than the benchmark of 20%. Accrual to this study, however, has been lower than hoped for and we therefore are modifying the study with a targeted accrual of 20 patients. In addition, with the introduction in recent years of the immune checkpoint inhibitors, we have modified our benchmark from 20% to a new benchmark of 35%, to be comparable with the response rates seen from the PD-1 immune checkpoint inhibitors from trials that included both treatment-naïve and previously treated melanoma patients, which reflects the patient groups eligible for this trial.[39-41] As a result of each of these changes, we will no longer require a statistically significant improvement over the benchmark to deem this treatment as worthy of further study. Instead, we shall consider the current treatment as worthy of further study if the observed response rate exceeds 35%. If the true response rate is 40%, then the probability of an observed response rate in excess of 35% among 20 patients (7 or more responses among 20) is 0.75; if the true response rate is 50%, the probability is 0.94. As with the original version, secondary endpoints include in vivo persistence of adoptively transferred T cells following TIL infusion and safety of the treatment protocol. Various markers will be examined for their association with response, among these % expression of central memory T cell markers, CD62L, CD27, and CD28. Logistic regression will be used to assess these correlations.

The original version of this protocol specified that if there are no responses observed after the first 20 patients are treated, the study will be suspended pending review by the DSMB. There have been 4 responses seen among the first 7 treated, so this modification will not have any stopping rules for futility.

14. ADMINISTRATIVE CONSIDERATIONS

14.1 Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

14.2 Termination of Study

The study will be stopped if any of the following events occur:

- All 20 patients have completed treatment.

- Stopping rules for toxicity have been met. Accrual will be put on hold pending discussion with the DSMB regarding whether a change in study design is warranted.

- The PI reserves the right to terminate the study at any time. The FDA may also terminate the study.

14.3 Consent

The Principal Investigator or her associate must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with federal regulations (21 CFR 312), all patients must sign the IRB-approved consent form in the presence of a witness. Prior to the start of the study, a copy of the IRB-approved consent form must be submitted to the Sponsor.

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APPENDIX A

ECOG / Zubrod Performance Status

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

APPENDIX B

B.1 GENERATION AND CHARACTERIZATION OF TUMOR-INFILTRATING LYMPHOCYTES FOR ADOPTIVE IMMUNOTHERAPY

Autologous tumor-infiltrating lymphocytes will be derived from a tumor biopsy from the patient with metastatic melanoma. The specimen will first go to the pathologist who will assist in distributing parts of the tumor to pathology for diagnostic purposes, to our lab for TIL generation, and cryopreservation for banking of remaining tissue.

The tumor sample obtained for the generation of TIL will be dissected into a goal of 24 fragments, 1-3mm^3 in size, which will then be placed in 24-well plates with culture media containing IL-2. The remaining tumor will be processed into a single cell suspension and then cryopreserved for later use as autologous tumor target cells for TIL testing.

The fragments are cultured in the wells, and inspected every few days for growth and evidence of contamination. Once the wells reach confluence, they are resuspended, counted and split into wells at a concentration of 1-1.5x10^6 cells/ml. TIL fragments must reach a minimum of 40 x 10^6 cells within 5 weeks of culture. The cells are tested for sterility, endotoxin and mycoplasma. The cells are analyzed for viability, cell number, phenotype, and may be assayed for autologous tumor recognition based on interferon-gamma release after co-culture with the thawed autologous tumor targets, if autologous tumor can be reserved at the time of initial tumor processing. Five or six wells are then selected based on the wells that demonstrate the best growth, phenotype (e.g., the highest CD3+ and CD8+ fractions), and if autologous tumors is available for testing, the most selective autologous tumor recognition by IFN-γ release assay. If the patient is ready for treatment, a rapid expansion is performed on the fresh, selected cells. If there are excess cells, a vial of 10 x 10^6 cells from each of the selected cultures is cryopreserved as a backup. If the patient is not ready for treatment, two vials of 10 x 10^6 cells are cryopreserved from each of the selected cultures, one for the future rapid expansion and one for the backup.

The requisite cell number to begin the expansion phase is 40 x 10^6 cells. The selected cells are expanded in Grex flasks in CTL media with anti-CD3 monoclonal antibody and gamma-irradiated feeder cells prepared from allogeneic peripheral blood mononuclear cells obtained from donors with a negative screen for infectious diseases. On day 7 of the rapid expansion, a cell count and visual inspection is performed. The patient begins the conditioning regimen on day -7, while the cells undergo the last 7 days of rapid expansion. Sterility testing is then repeated for bacteria, fungal and mycobacteria, as well as gram stain and endotoxin staining, prior to infusion into the patient.

APPENDIX C
ADMINISTRATION AND TOXICITY MANAGEMENT GUIDELINES FOR HIGH-DOSE INTERLEUKIN-2

(Adapted from University of Washington High-Dose IL-2 Administration and Toxicity Management Guidelines)

1) OVERVIEW

HDIL-2 is administered in 8-hour intervals as tolerated to a maximum of 14 doses. Doses that are skipped for management of toxicities are not compensated by lengthening the cycle, are not reduced, and are generally intended to be given at approximately 8-hour intervals. As a general rule, if a patient has severe enough toxicity to withhold 2 or more consecutive doses, the IL-2 will be discontinued.

2) SPECIFIC TOXICITIES AND CORRECTIVE INTERVENTION

A. Hemodynamics

- Hypotension

  - IV Fluids

Upon admission for HDIL-2 treatment, patients should be started on maintenance intravenous fluids in anticipation of ensuing capillary leak syndrome, vasodilation and decreased systemic vascular resistance. Colloid has no advantage over crystalloid, even when the albumin falls, and D5LR at 100cc/hr is recommended. Bolus IV fluids and increase in the continuous IV rate may be of value for initial management of hypotension to keep MAP > 60, attempting to limit the cumulative positive fluid balance by the end of 5 days to no more than 10% of the patient's dry weight by the end of each 5-day cycle of HDIL-2. This is accomplished by the judicious balance of fluids and vasopressor agents, depending on the patient's hemodynamic status, renal function, acid-base balance and pulmonary reserve.

  - Vasopressor agents

If hypotension persists despite judicious fluid resuscitation, vasopressor support with an α-sympathomimetic agent (generally phenylephrine) is indicated. Titration of phenylephrine should be between 0.5 and 2mcg/kg/min to keep the systolic blood pressure over 80-90 mm Hg (MAP > 60). Since the BP nadir tends to occur 4-6 hours after each dose of HDIL-2, a "reserve" should be available in the vasopressor dose, so that additional doses of HDIL-2 should be given only if the phenylephrine dose can be weaned to below about 1 mcg/kg/minute before the next IL-2 dose.

B. Renal

- Oliguria

  Oliguria is universal and is due to the combination of hypotension/hypoperfusion and decreased intravascular volume. It may be amplified in uninephric patients and patients on vasopressors. Oliguria per se is not an indication for fluids or diuresis; a bladder scan or urinary catheter may be useful to better assess the renal status if the patient is anuric, acidotic or has other reasons for urinary retention.

C. Pulmonary

- Hypoxia

  The need for supplemental oxygen will vary among patients depending on their underlying lung function and disease. Supplemental oxygen should be used to keep the patient's oxygen saturation > 90-92%, and intravenous volume should be limited in patients whose oxygenation is difficult to maintain and in those with significant crackles and/or chest radiographic findings of pulmonary edema or significant pleural effusion.

- Cardiogenic Pulmonary Edema

  Cardiogenic pulmonary edema is extremely rare in patients receiving HDIL-2 since patients are well screened and HDIL-2 rarely causes global cardiomyopathy. Any patient with sudden pulmonary decompensation should be evaluated for causes of cardiac origin.

D. Cardiac

- Ischemia

  Cardiac ischemia is also rare, but occasional focal myocarditis may occur, and arrhythmias or evidence of ischemia on EKG may mandate discontinuation of HDIL-2.

- Cardiac Arrhythmias

  Atrial cardiac arrhythmias during HDIL-2 treatment are uncommon, but occasionally supraventricular tachycardia and atrial fibrillation may be seen. Careful assessment should be performed for supervening causes such as ischemia, myocarditis, pericardial effusion or pulmonary events. Treatment should include antiarrhythmic medications, and does not preclude continuation of HDIL-2. Serious ventricular arrhythmias are rare and warrant discontinuation of HDIL-2 and resumption only if a minor, reversible/treatable cause was found.

- Edema

  Although patients often experience moderate amounts of peripheral edema at the end of each cycle, it is rare that they will experience severe symptoms from this extravascular fluid accumulation. Patients should be aggressively diuresed at the end of each cycle once they are hemodynamically stable.

E. Fever

Blood cultures will be drawn for all patients with fever (temperature > 38.5 C) and antibiotic prophylaxis will be given according to SPP guidelines associated with IL-2 therapy and neutropenia.

3) ADMINISTRATION GUIDELINES

Organ-by-organ guidelines for IL-2 dosing must be individualized and considered in the context of the whole patient rather than in isolation. Each dose should be given or withheld after an assessment of the patient by the RN and house officer, as supervised by the PI or designated surrogate.

System	Generally Safe to Give HDIL-2 (one or more present)	Consider Holding HDIL-2 Dose (Especially if >1-2 criteria present)	Unsafe to Given HDIL-2 (any criterion present)
Cardiac	Sinus tachycardia up to 130 bpm, occasional ventricular ectopy	Sustained sinus tachycardia after correction of contributing factor such as fever, chills, hypotension or significant ectopy	Ischemic ECG changes, atrial fibrillation, SVT, ongoing VT, elevated CPK/troponin
Dermatologic	Rash	Urticaria	Moist desquamation, bullae
Gastrointestinal	Mild diarrhea controlled with antimotility agents, isolated bilirubin elevation	Severe nausea, emesis, marked elevation/rise of transaminases	Ileus/abdominal distension, severe pain, intractable emesis
Hemodynamic	Phenylephrine support up to 1mcg/kg/min	Phenylephrine support up to 1.5mcg/kg/min – must be stable or titrating down before next dose	Phenylephrine support >1.5mcg/kg/min and or rising before next dose
Hemorrhagic/ Thrombotic	Thrombocytopenia	Clinically significant bleeding requiring platelet transfusions	Life threatening bleeding or clot
Infection	UTI, viral URI or localized HSV	Bacterial infection contributing to hemodynamic compromise	Infection requiring surgical intervention, or unresponsive to antibiotics
Metabolic	Asymptomatic and correctable: hypokalemia, hypocalcemia, hyperglycemia,	Symptomatic electrolyte or metabolic disturbance	Arrhythmia or other life threatening consequence or metabolic disturbance

	hypophosphatemia, hypomagnesemia,		
Neurologic	Mild somnolence, vivid dreams	Mild confusion, hallucinations, patient aware	Moderate – severe confusion, hallucinations, patient unaware or combative, seizures
Pulmonary	Mild dyspnea, hypoxia responsive to 4L or less of oxygen	Moderate dyspnea, hypoxia requiring more than 40% oxygen, significant crackles, significant effusion not requiring treatment	Severe dyspnea, hypoxia on oxygen, intubation, severe pulmonary edema, effusions requiring thoracentesis
Renal	Creatinine up to 3, Oliguria <100cc/in 8 hours, correctable acidosis	Creatinine > 3, anuria or < 100cc/8 hours, persistent acidosis	Persistent anuria, dialysis for metabolic or renal indications

4) MEDICATIONS USED DURING HDIL-2 THERAPY

1) Standard supportive care

In order to minimize the toxicities of HDIL-2 treatment the following medications should be prescribed:

Medications	**Indications**
Acetaminophen 650 mg PO q4h	Fever, myalgia
NSAID (Example: Naproxen 500 or 750 mg PO BID)	Fever, myalgia
Proton pump inhibitor (Pantoprozole 40 mg PO/IV daily, or equivalent)	Gastritis
5HT3 blocker (Ex: Ondansetron 8 mg PO/IV daily, or prior to each dose of IL-2 as needed)	Nausea, emesis
Broad spectrum antibiotic, usually Levofloxacin 500 mg PO/IV daily	Infection

2) Symptom management

For management of toxicity related symptoms, the following should be prescribed on an as needed basis:

Medications	**Indications**
5HT3 blocker, as above, if not given prophylactically	Nausea
Benzodiazepine, usually Lorazepam 0.5 mg IV or 1 mg PO q4h	Mild nausea, anxiety
Butyrophenone, usually Haloperidol 1-5 mg IV q1h	Agitation, hallucination
Loperamide or Atropine-Diphenoxylate 1-2 tablets PO q4h, maximum 6 tablets/24h	Diarrhea
Meperidine 25 mg IV or morphine sulfate 1-2 mg, repeat up to twice at 10 minute intervals	Chills

Tucks topically	Perianal discomfort
Diphenhydramine 12.5-25 mg IV or 25-50 mg PO q4h	Pruritus
Hydroxyzine 10-25 mg PO q4h	Pruritus if paradoxical reaction to diphenhydramine
Aveeno bath, lotion	Severe pruritus
Lubriderm-based lotion	Pruritus, skin irritation
"Magic" mouthwash (Diphenhydramine/Aluminum hydroxide/Lidocaine)	Mouth irritation
Pseudoephedrine 30 mg PO q4h	Nasal congestion
Zolpidem or temazepam	Sleep
Phenylephrine 25 mg/250 mL concentration, Titrate to BPs 80-90, range 0.1-2 mcg/kg/min	Hypotension
Magnesium sulfate	Hypomagnesemia
Potassium or sodium phosphate	Hypophosphatemia
Potassium chloride	Hypokalemia

5) ADDITIONAL MANAGEMENT GUIDELINES

Symptom/sign	**Intervention**
Anemia	Transfuse to keep Hct 27-30
Arrhythmia	Correct other factors, R/O ischemia, effusions; arrhythmia specific treatment; Consider resuming IL-2 on or off anti-arrhythmic treatment
Acidosis	Keep bicarb level >20
Edema, threatening compartment syndromes	Elevation, attention to neuropathic symptoms
Fever pattern atypical (late spike)	Culture, look for sources of bacterial infection
Hypoalbuminemia	No specific treatment
Hypocalcemia	Correct for low albumin, replace to normal values
Infection documented	Continuation of IL-2 depends on severity, interventions needed
Other electrolyte disturbances	Look for contributors, replace as needed

6) MONITORING

1) Monitoring guidelines: ICU routine

2) Labs for IL-2 monitoring: According to UWMC standard of care for high-dose IL-2

VIII. PATIENT MANAGEMENT FOLLOWING COMPLETION OF HDIL-2

Since the hemodynamic effects of HDIL-2 begin to wane within hours of the last infusion, it is usually sufficient to wean the patient off pressors as rapidly as tolerated, followed by or accompanied by (depending on the patient's particular hemodynamic needs and volume status) prompt discontinuation of parenteral fluids and then active diuresis. Patients should have sufficient pulmonary reserve at the time of discharge to tolerate the possibility of mobilizing a few liters back into the vascular space (which can result in pulmonary edema) AND should be given at least 3-4 days of oral diuretics, with K as indicated, to return them to near dry weight (which is likely to be about 2-3 kg below their prior weight). Patients can stop the diuretics on their own when their weight normalizes and/or edema resolves. All patients experience desquamation and dry, pruritic skin, which is managed symptomatically with lubricants and anti-pruritics (avoiding steroid).

APPENDIX D
MONITORING SCHEDULE

Date*		Event	History and PE	Comprehensive Panel	CBC	Research Lab	Tumor biopsy	Radiographic evaluation	Additional screening tests
Step 1 Screen		Evaluation for TIL generation	x	x	x	x	x		Brain imaging <2mo; PFTs for high risk pts, Virology panel**
Step 2 Screen		Evaluation for TIL infusion	x	x	x	x		x	Brain imaging <30 days; cardiac test; PFTs; EKG
	Day -7 to Day -14	Admit to UWMC or SCCA	x	x	x	x			
	Day -7	Lymphodepletion: Cy	x***						
	Day -6	Lymphodepletion: Cy		x	x				
	Day -5	Lymphodepletion: Flu		x	x				
	Day -4	Lymphodepletion: Flu		x	x				
	Day -3	Lymphodepletion: Flu		x	x				
	Day -2	Lymphodepletion: Flu		x	x				
	Day -1	Lymphodepletion: Flu		x	x				
	Day 0	TIL infusion	x	x	x	x			
	Day +1	Transfer to ICU; IL-2 600,000 U/m^2 q8 up to 14 doses, as tolerated	x	x	x	x			
	Day +2		x	x	x				
	Day +3		x	x	x	x			
	Day +4		x	x	x				
	Day +5		x	x	x				
Week 1	Day +7		x	x	x	x			
Week 2	Day +14		x	x	x	x			
Week 3	Day +21			x	x	x			
Week 4	Day +28		x	x	x	x			
Week 5	Day +35			x	x	x			
Week 6	Day +42		x	x	x	x		x	
Week 7	Day +49			x	x	x			
Week 8	Day +56		x	x	x	x			
Week 12	Day +84		x	x	x	x		x	
Week 24	Day+168		x	x	x	x		x	

* The dates listed on the study calendar above are approximate as many of our patients reside out of the area and their oncologists and laboratories cannot always follow the time points as dictated by the protocol.
**Patient's will be screened for Hepatitis B surface antigen, Hepatitis B core antibody, Hepatitis C virus antibody, HIV 1 /2 antibody, standard test for syphilis (STS), CMV antibody and EBV panel within 30 days of signing Step 1 consent.
*** H & Ps during lymphodepletion will be performed according to institutional guidelines/practice.

APPENDIX E
DATA AND SAFETY MONITORING PLAN

Primary Monitoring

The principal investigator (PI) will be responsible for monitoring the data and toxicities to identify trends, and for revising the protocol as needed to maintain safety. The PI will be responsible for ensuring that the protocol is conducted as approved by the Scientific Review Committee and Institutional Review Board, that the monitoring plan is being followed, and that all adverse events are reported according to protocol guidelines.

Data and Safety Monitoring Board

A dedicated data and safety monitoring board (DSMB) will be formed to review the conduct of the trial to date and assess the safety and toxicity of the study intervention. At each meeting, all grade III or greater toxicities as defined by version 4.0 of NCI Common Toxicity Criteria will be reviewed. The DSMB will determine if the study should be prematurely discontinued due to excessive toxicity or if a change in study design is warranted.

DSMB Meeting Schedule

The DSMB will meet after the treatment of the 5^{th} patient, the 15^{th} patient or if there are ≥2 unexpected, serious, treatment-related toxicities (see Section 11.2 for definitions) *or at least annually whichever is sooner*.

As described in Section 11, if the rate of unexpected, serious, treatment-related toxicities is observed in ≥ 33% of enrolled subjects in a cohort of 6 or more subjects, this will trigger the suspension of the study pending further review by the DSMB and FDA. Furthermore, any occurrence of unexpected grade 4 or greater toxicity will prompt the investigator to conduct a thorough investigation of available safety data to justify to the DSMB a decision to continue enrolling new subjects into the study.

DSMB Reporting

The CTSO provides staff to assist with minutes for the DSMB meeting. A report from the DSMB is submitted to the FHCRC IRB and Regulatory Affairs Manager. The FHCRC also has a Protocol Data Monitoring Committee (PDMC) that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.