CC Protocol Number: 14-C-0022K
Version Date: 02/15/2019
NCT Number: NCT01993719

PROTOCOL TITLE

A Phase II Study for Metastatic Melanoma using High Dose Chemotherapy Preparative Regimen followed by Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes Plus IL-2 with the Administration of Pembrolizumab in the Retreatment Arm

NCI Principal Investigator: Stephanie L. Goff, M.D. [E]
Surgery Branch, CCR, NCI
Building 10, CRC, Room 3-5840
9000 Rockville Pike, Bethesda, MD 20892
Phone: 240-760-6214;
Email: Stephanie.Goff@nih.gov

Investigational Agents:

Drug Name:	Young TIL
IND Number:	IND 14265
Sponsor:	Center for Cancer Research
Manufacturer	Surgery Branch Cell Processing Facility

Commercial Agents: Cyclophosphamide, Fludarabine, Aldesleukin, and Pembrolizumab

PRÉCIS

Background:

- Adoptive cell therapy (ACT) using autologous tumor infiltrating lymphocytes can mediate the regression of bulky metastatic melanoma when administered along with high-dose aldesleukin (IL-2) following a non-myeloablative lymphodepleting chemotherapy preparative regimen consisting of cyclophosphamide and fludarabine.
- In a series of consecutive trials using this chemotherapy preparative regimen alone or with 2 Gy or 12 Gy total body irradiation (TBI) objective response rates using RECIST criteria were 49%, 52%, and 72%, respectively. Of the 20 complete regressions seen in this trial, 19 are on-going at 70 to 114 months.
- The chemotherapy alone preparative regimen required in-patient treatment and was associated with significant neutropenia and thrombocytopenia requiring multiple transfusions and treatment for febrile neutropenia.

Objectives:

- With amendment D, to determine if there is a difference in the rate of response between patients who have received prior anti-PD1 and those who have not; both groups will receive non-myeloablative lymphoid depleting preparative regimen followed by autologous young TIL and administration of high dose aldesleukin.
- To determine the toxicity of the treatment.

Eligibility:

- Age greater than or equal to 18 and less than or equal to 70 years
- Evaluable metastatic melanoma
- Metastatic melanoma lesion suitable for surgical resection for the preparation of TIL
- No contraindications to high-dose aldesleukin administration
- No concurrent major medical illnesses or any form of immunodeficiency.

Design:

- Patients with metastatic melanoma will have lesions resected and after TIL growth is established, patients will receive ACT with TIL plus aldesleukin following high dose chemotherapy preparative regimen.
- Up to 64 patients may be enrolled over 4-5 years.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Study Objectives:

1.1.1 Primary Objectives

- With amendment D, to determine if there is a difference in the rate of response between patients who have received prior anti-PD1 and those who have not; both groups will receive non-myeloablative lymphoid depleting preparative regimen followed by autologous young TIL and administration of high dose aldesleukin.
- To determine the toxicity of the treatment.

1.1.2 Secondary Objectives

- Evaluate progression free and overall survival
- Evaluate the safety and efficacy of pembrolizumab in combination with TIL therapy

1.2 Background and Rationale:

1.2.1 Surgery Branch, NCI Studies of Tumor Infiltrating Lymphocytes for the Treatment of Patients with Metastatic Melanoma

Melanoma is the sixth leading cancer in both men and women[1]. Metastatic melanoma has a poor prognosis with five-year survival of less than 5%. FDA-approved treatments for metastatic melanoma include aldesleukin, ipilimumab and dacarbazine chemotherapy. Aldesleukin has an objective clinical response rate of about 16% and a complete response rate of 6%[2]. In a recent trial that led to FDA approval, ipilimumab had an objective response rate of 7% in 540 patients with 0.55% complete responses, though a 3.6 month improvement in survival was seen. Dacarbazine-based chemotherapy has a clinical response rate of up to 15% but few complete responders or long-term survivors[3]. More effective treatment for metastatic melanoma is needed. Previous studies in animal models demonstrated that the cellular arm of the immune system plays an important role in tumor surveillance and may be recruited to destroy tumor[4]. Therefore, most therapeutic strategies focusing on immunotherapy against metastatic melanoma have focused on the ability of effector T-cells to mediate tumor destruction.

The Surgery Branch of the National Cancer Institute has pioneered Adoptive Cell Therapy (ACT) for the treatment of patients with metastatic melanoma. We have reported the results of ACT therapy in 93 patients with metastatic melanoma who received autologous TIL following a lymphodepleting regimen plus aldesleukin administration[5]. Forty-three patients received a non-myeloablative chemotherapy consisting of 60 mg/kg cyclophosphamide qd x 2 and 25mg/m^2 fludarabine qd x 5 prior to cell transfer and aldesleukin administration. Twenty-five patients each also received the same chemotherapy agents in conjunction with either 200 or 1200 cGy total body irradiation (TBI) prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%. The clinical results in these three trials are shown in **Table 1** the toxicities shown in **Table 2** for the initial study without TBI (99-C-0158).

In a recent prospective randomized trial with 91% of patients accrued, the objective response rate using the same cyclophosphamide and fludarabine regimen (60 mg/kg qd x 2d and 25 mg/m^2 qd x 5d) was 48% compared to 62% for patients with 12 Gy whole body radiation added. There was no significant difference in progression free and overall survival between these two arms. We

have thus selected the chemotherapy preparative regimen alone as the basis of comparison in future trials (**Table 3** and **Figure 1**).

Despite this high rate of objective responses in previously treated patients, ACT is available predominantly in the Surgery Branch, NCI and although several other institutions in the United States have begun to explore ACT therapy for melanoma, very limited numbers of patients have been treated elsewhere. The non- myeloablative chemotherapy regimen that we have been using results in significant lymphopenia, as is desired, but also causes substantial neutropenia as well as thrombocytopenia. Thus, most patients have neutrophil counts less than 500/ mm^3 for approximately eight days most also require platelet and red blood cell transfusions to keep these cell types at adequate levels. In a prior study 43% of patients required more than 6 RBC transfusions and 23% of patients required more than 10 platelet transfusions (**Table 2**). This severe neutropenia and thrombocytopenia often required prolong hospitalization and a substantial expense is involved in managing neutropenic fevers and administering the required blood and platelet transfusions.

In an effort to make this treatment more widely available we have decided to evaluate a chemotherapy preparative regimen that is commonly used in patients with lymphoma and leukemia. This regimen is associated with less neutropenia and thrombocytopenia and patients are able to tolerate multiple cycles with little morbidity[6]. In this trial, we will only be giving one cycle of this regimen and thus expect the hematologic side effects of this regimen to be minimal. To adequately evaluate a less morbid chemotherapy preparative regimen, we are thus proposing a prospective randomized trial comparing our standard cyclophosphamide/fludarabine regimen (60 mg/ kilogram QD x 2 days and 25 mg/ meter squared QD x 5 days, respectively) with a decreased chemotherapy preparative regimen of cyclophosphamide (300 mg/meter squared QD X 3 days) and fludarabine (30 mg/meter squared QD x 3 days). These regimens will be evaluated to determine their anti-tumor impact as well as the hematologic toxicities associated with treatment.

Given the proliferation of new agents available for the treatment of melanoma, the majority of patients referred to us have been treated with anti-PD1 or anti-PDL1 antibodies and this treatment has ultimately failed. Patients who experience disease progression following both conventional TIL therapy and anti-PD1/PDL1 treatment provide us with the opportunity to study the effect of anti-PD1/PDL1 therapy in combination with TIL therapy. Laboratory evidence shows that TIL highly express PD1 and can express PD1 as they expand in vivo. In the retreatment arm of this study we would like to see if the administration of an anti PD1 agent (pembrolizumab) given a few hours prior to the administration of the cell product and continuing for 4 months will cause a response in patients who have progressed following anti PD1/PDL1 therapy and TIL therapy on this protocol.

The NCI SB participated in the early trials evaluating the use of ipilimumab in patients with melanoma, renal cell cancer and pancreatic cancer[7]. Ipilimumab is a recombinant human IgG1 monoclonal antibody that blocks cytotoxic T-lymphocyte associated antigen 4 (CTLA-4). Pembrolizumab is a fully humanized IgG4 kappa immunoglobulin monoclonal antibody that blocks the interaction between PD-1 and its ligands. Both agents have very similar toxicity profiles with the major toxicities being immune related adverse events (IRAEs). Protocol 03-C-0109 "*An open-label study of MDX-010 combined with interleukin-2 for the treatment of patients with metastatic melanoma"* included treatment with a combination of IL-2 and ipilimumab. This study enrolled 36 patients with metastatic melanoma using high dose IL-2 and escalating doses of ipilimumab. Two other studies included ipilimumab and vaccination with gp100 peptides (02-

C-0106 and 04-C-0083). The number of IRAEs did not increase with the dose escalation of ipilimumab and the incidence of IRAEs was lower in this trial than in the trials that included ipilimumab plus the gp 100 peptides (17% vs 29% and 32%). Of note, the overall response rate in this trial was 25% with a 17% Complete Response rate – all of which (CRs) are ongoing.

We consider this to be the ideal opportunity to study combination therapy as we will have the ability to treat patients with essentially the same TIL product in both the primary and retreatment arms and the patients will not have received any additional treatments in the interim. If this treatment does elicit a response, we can be confident that the response is very likely due to the combination treatment.

In September 2014 anti-PD1 (pembrolizumab) was approved showing a high response rate in patients with metastatic melanoma. Because our adoptively transferred T cells can re-express PD1 following administration, it raised the very important question of whether adding anti-PD1 to the standard dose preparative regimen might be beneficial. We are thus preparing a new protocol to supplant the present protocol (14-C-0022) with a protocol in which patients will receive adoptive cell therapy with TIL following our standard preparative chemotherapy (no radiation) and be randomized to either receive or not receive anti-PD1 (pembrolizumab).

Given this trend favoring the high dose arm (as analyzed by our statistician) and the fact that we are now preparing a new protocol and plan to stop this protocol within the next few months, we will stop randomizing patients on the low dose chemotherapy arm but keep the high dose chemotherapy arm open with separate accrual objectives for patients who received prior anti-PD1 or not, so that we can continue to accrue information about the relative responsiveness of patients who have had or have not had prior PD1. These patients will help us answer that question until the new protocol can be instituted.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

a. Measurable metastatic melanoma with at least one lesion that is resectable for TIL generation and at least one other lesion that can be measured by RECIST criteria

b. Confirmation of diagnosis of metastatic melanoma by the Laboratory of Pathology of NCI.

c. Patients with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for 1 month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.

d. Greater than or equal to 18 years of age and less than or equal to 70 years of age.

e. Ability of subject to understand and the willingness to sign the Informed Consent Document

f. Willing to sign a durable power of attorney.

g. Clinical performance status of ECOG 0, 1 or 2.

h. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after treatment.

i. Serology:

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

j. Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

k. Hematology

- Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim
- WBC $\geq 3000/mm^3$
- Platelet count $\geq 100{,}000/mm^3$
- Hemoglobin > 8.0 g/dl

l. Chemistry:

- Serum ALT/AST $\leq$ to 2.5 times the upper limit of normal
- Serum Creatinine $\leq$ to 1.6 mg/dl
- Total bilirubin $\leq$ to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

m. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo). Patients must have progressive disease after prior treatment.

Note: Patients may have undergone minor surgical procedures within the past 3 weeks, as long as all toxicities have recovered to grade 1 or less.

n. Subjects must be co-enrolled in 03-C-0277

2.1.2 Exclusion Criteria

a. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

b. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

c. Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

d. Active systemic infections (e.g.: requiring anti-infective treatment), coagulation disorders or any other active major medical illnesses.

e. Concurrent systemic steroid therapy.

f. History of severe immediate hypersensitivity reaction to any of the agents used in this study.

g. History of coronary revascularization or ischemic symptoms.

h. Any patient known to have an LVEF less than or equal to 45%

i. Documented LVEF of less than or equal to 45%, note: testing is required in patients with:

- Age $\geq$ 65 years' old
- Clinically significant atrial and or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block, or history of ischemic heart disease or chest pain

j. Patients who are receiving other investigational agents

k. Documented FEV1 less than or equal to 60% predicted tested in patients with:

- A prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years).
- Symptoms of respiratory dysfunction

2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under our companion protocol, 99-C-0128.

2.2.1 Within 3 months prior to enrollment:

- HIV antibody titer and HBsAg determination, and anti HCV
- Confirmation of diagnosis of metastatic melanoma by the Laboratory of Pathology of NCI. (Note: Testing is permitted to be conducted at any time prior to this point.)

2.2.2 Within 8 weeks prior to enrollment:

- Pulmonary Function Testing for patients with a prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years) or symptoms of respiratory dysfunction.
- Cardiac evaluations in patients who are greater than or equal to age 65, or have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart block, will undergo cardiac evaluation (stress thallium, echocardiogram, MUGA etc.,). Patients with a LEVF of less than or equal to 45% will not be eligible. Patients under the age of 65 who have cardiac risk factors may also undergo cardiac evaluations as noted above (e.g., diabetes, hypertension, obesity).

2.2.3 Within 4 weeks prior to enrollment:

a. Complete history and physical examination, including weight, and vital signs, and noting organ system involvement and any allergies/sensitivities to antibiotics. (Note: patient history may be obtained within 8 weeks)

b. Baseline imaging to determine the status of disease. This may include CT, MRI, PET, or Photography.

2.2.4 Within 14 days prior to enrollment:

a. Baseline blood tests

- Chemistries: Creatinine, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin
- CBC with differential and platelet count

b. Urinalysis and culture, if indicated

2.2.5 Within 7 days prior to enrollment:

a. β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

b. ECOG performance status of 0, 1 or 2

2.3 REGISTRATION PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will initially be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to tumor resection for TIL generation, by the clinical fellow or research nurse within 24 hours of the patient signing the consent and sending the completed Eligibility Checklist via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the COA the patient will be consented for this protocol.

2.3.2 Registration Procedure

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.3.3 Treatment Assignment Procedures (for registration purposes only)

Cohorts

Number	Name	Description
1	Cohort 1 (Closed with amendment D)	Patients with metastatic melanoma
2	Cohort 2	Patients with metastatic melanoma who have not received prior treatment with anti PD-1/PD-L1
3	Cohort 3	Patients with metastatic melanoma who have received prior treatment with anti PD-1/PD-L1

Arms

Number	Name	Description
1	Arm 1 (Closed with amendment D)	Standard preparative regimen + Young TIL Cells
2	Arm 2 (Closed with amendment D)	Lower dose preparative regimen + Young TIL Cells
3	Arm 1N	Standard preparative regimen + Young TIL Cells
4	Arm 1P	Standard preparative regimen + Young TIL Cells + possible retreatment with standard preparative regimen + Young TIL Cells +pembrolizumab

Stratifications, Randomization and Arm Assignment

Prior to Amendment D: Subjects in Cohort 1 were randomized to receive the standard non-myeloablative chemotherapy regimen ARM 1 or the lower dose chemotherapy regimen in ARM 2. Randomization was conducted at 1:1 fashion using variable block sizes.

Patients were stratified for M1a vs. M1b + M1c disease

Name	Distinct Options
Disease	• M1a • M1b+M1c

Starting from Amendment D: Subjects in Cohort 2 are directly assigned to Arm 1N and subjects in Cohort 3 are directly assigned to Arm 1P.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Performed on 03-C-0277

Patients with evaluable metastatic melanoma will undergo resection of tumor under protocol 03-C-0277. TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. TIL cultures will be monitored regularly and when approximately $5x10^8$ cells are available, the cells will undergo a rapid expansion.

3.1.2 Treatment Phase

Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the COA (approximately 7 days after the REP procedure has been initiated), the patient will receive the assigned lymphocyte depleting preparative regimen, followed by infusion of between $1x10^9$ to $2x10^{11}$ lymphocytes and high dose aldesleukin. Patients will be evaluated for response approximately 4-6 weeks following the administration of the cell product. Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation. Patients may undergo a second treatment as described in Section **3.5**.

3.1.3 Protocol Stopping Rules

The study may be halted if any of the following conditions are met during initial treatment or retreatment:

1. If 1 or more treatment related death occurs in either arm, we will promptly discuss this with the NIH Intramural IRB and the FDA but we may not immediately stop accrual.
2. Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen or aldesleukin (or circumstances unrelated to the study).
3. If one of the first three patients (or 2 of the first 6 patients, or 3 of the first 9 patients, or 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.

3.2 DRUG ADMINISTRATION:

Treatment schedule will be according to the following schedule (See study calendars in section **3.3**). (Starting on day -6, study medication start times for drugs given once daily may be within 2 hours of the scheduled time. Administration of diuretics, electrolyte monitoring and replacement, and hydration should all be performed as clinically indicated. Chemotherapy infusions maybe slowed or delayed as medically indicated.)

3.2.1 Preparative Regimen

The following will comprise a course of therapy for Day -7 through day -3:

Day -7and -6

Approximately 6 hours Prior to Cyclophosphamide

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 1.5 - 2.6 ml/kg/hr (starting approximately 6 hours' pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if the urine output <1 ml/kg/hr or if body weight >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV maybe administered. The hydration rate will be capped at 250mL/hr.

Approximately 1 hour pre-Cyclophosphamide

Ondansetron (0.15 mg/kg/dose [*rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight]* IV q 8 hours X 3 days) will be given for nausea.

Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day over 1 hr X 2 days. If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**.

A decreased dose of cyclophosphamide at 30mg/kg/day (x2 days) will be considered for patients who have a history of prolonged hematologic recovery from prior chemotherapy treatments.

Immediately following the end of Cyclophosphamide

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section **11**) over 23 hours after each cyclophosphamide dose. If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**.

Day -7 to Day-3

Fludarabine 25 mg/m^2/day IVPB daily over 15-30 minutes for 5 days.

If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**. (*The fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6).*

3.2.2 Pembrolizumab (RETREATMENT ONLY)

Patients in the RETREATMENT ARM will receive 4 doses of Pembrolizumab at the NIH Clinical Center

- Day -2 (One day after the last dose of fludarabine) -Pembrolizumab 2mg/kg IV approximately given over approximately 30 minutes
- Day 21 (+/- 2 days) following cell infusion – Pembrolizumab 2mg/kg IV given over approximately 30 minutes
- Day 42 (+/- 2 days) following cell infusion - Pembrolizumab 2mg/kg IV given over approximately 30 minutes
- Day 63 (+/- 2 days) following cell infusion - Pembrolizumab 2mg/kg IV given over approximately 30 minutes

  Please note: Dosing may be held or stopped at the discretion of the treating investigator

3.2.3 Cell Infusion and Aldesleukin Administration

The patient's autologous TIL is delivered to the patient care unit by an authorized staff member. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD

or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15-minute period approximately every 8 hours beginning within 24 hours of cell infusion and continuing for up to 4 days (maximum 12 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered with supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix 3**. Toxicities will be managed as outlined in **Appendix 4**. In addition, dosing may be held or stopped at the discretion of the treating investigator.

Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

Day 0 (two to four days after the last dose of fludarabine):

- Autologous young TIL infusion will be administered intravenously over 20 to 30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.
- Aldesleukin as described in section **3.2.3** above.

Day 1-4 (Day 0 is the day of cell infusion):

- Aldesleukin as described in section **3.2.3** above.
- **For all patients:** When neutrophil count is less than .5 $x10^9$/L: Filgrastim will be started at 5 mcg/kg/day daily subcutaneously until neutrophil count >1$x10^9$/L X 3 days or > 5$x10^9$/L. The maximum filgrastim dose will be 300 mcg per day.

3.3 TREATMENT SCHEDULE

A Initial Treatment

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Therapy												
Cyclophosphamide 60 mg/kg	X	X										
Fludarabine 25 mg/m^2	X	X	X	X	X							
Young TIL Cells[1]								X				
Aldesleukin[2]								X	X	X	X	X
Filgrastim[3]									X	X	X	X

5 mcg/kg/day												
TMP/SMX[4] 160mg/800mg (example)								X		X		X
Fluconazole[5] 400 mg po								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

B Retreatment

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Therapy												
Cyclophosphamide 60 mg/kg	X	X										
Fludarabine 25 mg/m^2	X	X	X	X	X							
Pembrolizumab 2mg/kg						X[7]						
Young TIL Cells[1]								X				
Aldesleukin[2]								X	X	X	X	X
Filgrastim[3] 5 mcg/kg/day									X	X	X	X
TMP/SMX[4] 160mg/800mg (example)								X		X		X
Fluconazole[5] 400 mg po								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]Two to four days after the last dose of Fludarabine

[2]Initiate within approximately 24 hours after cell infusion

[3] When neutrophil count is < .5x10^9 start Filgrastim and continue until neutrophils count > 1X10^9/L X 3 days or > 5x10^9/L.

[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2, starting day 0 or within one week of anticipated lymphopenia

[5]Continue until ANC > 1000/mm^3

[6]In patients positive for HSV or VZV continue until CD4 > 200 X 2

[7] additional doses to be given on days 21, 42, and 63 +/- 2 days

3.4 ON STUDY EVALUATION

Note: Refer to section **5** for research evaluations.

3.4.1 Within 14 days prior to starting the preparative regimen

- Apheresis as indicated
- Baseline blood test
    - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
    - Complete Blood Count with differential
    - PT/PTT
    - TBNK
    - Thyroid Panel
    - Urinalysis
- Anti CMV antibody titer, HSV serology, VZV antibody IgG, IgM, and EBV panel. (Note, may be performed within 3 months of chemotherapy)
- Chest x-ray
- EKG

3.4.2 During the preparative regimen: DAILY

- Complete Blood Count with differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Urinalysis as needed
- Daily weight as indicated
- PT/PTT (every 3 days)
- Thyroid panel (every 7 days)

3.4.3 After Pembrolizumab (Retreatment only) and Cell Infusion

- Following pembrolizumab (retreatment arm only) - Vital signs will be monitored every 15 minutes' x 2 then routinely
- Following cell administration- Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated

3.4.4 Prior to each Pembrolizumab infusion (days 21, 42, 63) (Retreatment only)

- A complete review of systems and physical exam

- Review of patient toxicities
- CBC with differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Other tests will be performed as clinically indicated.

Note: Patients may receive doses 2-4 at the NIH Clinical Center day hospital.

3.4.5 Management of Immune Related Adverse Events (IRAEs) Refer to Appendix 5

- IRAEs are most commonly seen following the second and third dose however they may occur at any time during the course of treatment.
- Patients will undergo a complete physical examination prior to each infusion and will be monitored closely for signs of IRAEs. Events will be managed as described in **Appendix 5**.

3.4.6 During Hospitalization

Every 1-2 days

- A review of systems and physical exam as clinically indicated
- CBC with differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- PT/PTT (every 3 days)
- Thyroid panel (every 7 days)
- Other tests will be performed as clinically indicated.
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). Please refer to section **5** for additional post cell infusion evaluations.
- Vital signs will be monitored as clinically indicated.

3.5 RETREATMENT

Patients who do not respond or who experience a partial or complete response and subsequently progress and have received prior therapy with either pembrolizumab or nivolumab may receive a second treatment (Arm 1P). **Note**: patients who were enrolled prior to Amendment C, may be retreated with standard dose chemotherapy regardless of prior treatment status. These patients will be reconsented prior to treatment.

Patients must continue to meet the original eligibility criteria stated in Section **2** to be considered for retreatment. All toxicities related to cyclophosphamide, fludarabine, or aldesleukin should be stable and resolved to less than grade 1 prior to retreatment [with the exception of alopecia.]

Retreatment benefits and risks will be carefully explained to the patient. A maximum of 1 retreatment course may occur.

Patients will receive standard dose Chemotherapy as described in Section **3.2.1** followed by pembrolizumab as described in Section **3.2.2** and high-dose aldesleukin in Section **3.2.3**.

Patients who developed grade 3 or grade 4 toxicity due to cell infusion and/or who experienced Immune Related Adverse Events requiring treatment with steroids following treatment with anti PD-1 agents will not be retreated.

All response data will be captured in C3D however only the assessments following the initial treatment will be used in the overall evaluation of response. (The response to the retreatment for all patients who are not retreated will not be used in the evaluation of response.)

3.6 POST TREATMENT (FOLLOW UP) EVALUATION

- All patients will return to the NIH Clinical Center for their 1st evaluation for response 6 weeks (+/- 2 weeks) following administration of the cell product.
- Patients who have received multiple transfusions during the treatment phase or have been discharged with grade 3 or greater significant adverse events should be evaluated by their referring physician within 2 weeks of discharge and repeat labs drawn as appropriate to be faxed to the Research Nurse. Patients will receive appropriate treatment as determined by their treating physician.

3.6.1 Time period of evaluations:

- Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:
  - Week 12 (+/- 2 weeks)
  - Every 3 months (+/- 1 month) x3
  - Every 6 months (+/- 1 month) x 5 years
  - As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated

3.6.2 At each scheduled evaluation for response patients will undergo:

- Physical examination, including weight and vital signs
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Complete blood count with differential
- PT/PTT
- Urinalysis as needed
- Thyroid panel as clinically indicated
- TBNK, until CD4 > 200 x 2

- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis per baseline assessment. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- A 5-liter apheresis may be performed at the first follow up visit, if the patient is unable to undergo pheresis, approximately 96 ml of blood may be obtained. Subsequently, approximately 60 ml of blood will be obtained at follow up visits for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed. This will be performed under 03-C-0277.
- Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contact. A request will be made to send laboratory, imaging and physician exam reports performed by their treating physician; and any outstanding toxicities will be reviewed with the patient.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

Patients will be taken off treatment (and followed for survival) for the following:

- Completion of protocol therapy (including pembrolizumab in the retreatment arm only)
- Participant requests to be withdrawn from active therapy
- IRAEs related to pembrolizumab as noted in **Appendix 5**.
- PI discretion (e.g., persistent grade 1 or 2 events, patient preference, rapid disease progression at any time)
- Positive pregnancy test

3.7.2 Off Study Criteria

Patients will be taken off study for the following:

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Lost to follow-up
- Death

Note: patients who are taken off study for progressive disease or study closure may be followed on Protocol 09-C-0161 - "Follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies."

Note: once a subject is taken off study, no further data can be collected.

3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be

completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator.

4.1.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days, beginning day 0 or within 1 week of anticipated lymphopenia.

Dapsone (in G6PD sufficient patients), Atovaquone or Pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

4.1.2 Herpes or Varicella Zoster Virus Prophylaxis

Patients with positive HSV or VZV serology will be given Valacyclovir orally at a dose of 500 mg daily starting on the day of cell infusion, or acyclovir, 250 mg/m^2 IV q 12 hrs if the patient is not able to take medication by. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for Pneumocystis Varicella Zoster and Herpes will continue for 6 months' post chemotherapy. If the CD4 count is less than 200 at 6 months' post chemotherapy, prophylaxis will continue for at least 6 months and until the CD4 count is greater than 200 for 2 consecutive measures.

4.1.3 Fungal Prophylaxis (Fluconazole)

Patients will start Fluconazole 400 mg p.o. starting on the day of cell infusion and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.1.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics in accordance with current institutional guidelines for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.1.5 Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. As a general guideline, patients may be transfused for:

- Hemoglobin < 8 gm/dl
- Platelets < 10,000/mm^3

Note: Patients may be transfused at a higher platelet count as clinically indicated, e.g.:

- Increased risk for bleeding such as undergoing an invasive procedure or presence of metastatic lesion likely to bleed
- fever greater than 38.5°C
- sepsis

All blood products will be irradiated. 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.

4.2 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and ranitidine (150 mg g12h). If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long term research efforts within the Surgery Branch and with collaborators as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

5.1 SAMPLES SENT TO DR. FIGG'S LAB

- Venous blood samples will be collected in either a 4ml or an 8ml SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pickup.
- For sample pickup, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.
- The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.2 SAMPLES SENT TO SURGERY BRANCH CELL PROCESSING LABORATORY

- Venous blood samples will be collected in 8ml CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pickup.
- Samples will be pick-up by the research nurse or designee and transported to the SB Cell Processing Laboratory within 24 hours of blood draw.
- The samples will be processed, barcoded, and stored in SB Cell Processing Laboratory.

5.3 PRIOR TO CHEMOTHERAPY ADMINISTRATION

- 5 CPT tubes (8 ml each) – SB's lab
- 1 SST tube (8ml) – Figg's lab
- 1 SST tube (4ml) daily; starting day of chemotherapy – Figg's lab

5.4 PRIOR TO CELL INFUSION

- Baseline blood sample for cytokine analysis (one 8 ml SST) – Figg's lab

5.5 POST CELL INFUSION EVALUATIONS:

- Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday and Friday x5, then weekly (while the patient is hospitalized):
  - 5 CPT tubes (8 ml each) SB's lab
  - 1 SST tube (8 ml) – Figg's lab

5.6 IMMUNOLOGICAL TESTING:

- A variety of tests including evaluation of specific lysis and cytokine release, intracellular FACS of cytokine production, ELISA-spot assays, and lymphocyte subset analysis may be used to evaluate the immunological correlates of treatment. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences.
- Samples of all infused cell products will be cryopreserved, and extensive retrospective analysis of infused cell phenotype and function will be performed to attempt to find in vitro characteristics of the infused cells which correlate with in vivo antitumor activity. Analyses of TIL samples will include evaluation of the activity, specificity, and telomere length of the infused TIL.
- Peripheral blood lymphocytes (PBL) will be purified by centrifugation on a Ficoll cushion, then evaluated for function and phenotype. Lymphocytes may be tested by cytolysis assays, cytokine release, limiting dilution analysis and by other experimental studies. Immunological monitoring will consist of quantifying T cells reactive with HLA-matched tumor cells using established techniques such as intracellular FACS, cytokine release assays, and Elispot assays. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the T cells cryopreserved at the time of infusion. TCR gene usage may be quantitated in samples using conventional sequencing techniques of the T cell receptor variable regimen of the beta chain.

5.7 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR SB CELL PROCESSING LABORATORY

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Blood Processing Core (BPC) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Note: Blood and tissue collected during the course of this study will be stored, tracked and disposed of as specified in our companion protocol 03-C-0277, (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

5.8 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR DR. FIGG'S LAB

5.8.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the Surgery Branch central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.8.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Note: Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts.

All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for 30 days after removal from study treatment or until off-study, whichever comes first.
An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention

- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Exclusions to Routine Adverse Event Recording:

With amendment D, patients will be receiving multiple agents, which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin and supportive medications) in combination with the investigational agent; therefore, all grade 1 events not related to the cell product will not be reported/recorded.

6.1.2 Additional Reporting Requirements:

The following parameters will be captured in C3D for this study:

- RBC and platelet transfusions

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

Coded, linked human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long term research efforts within the Surgery Branch and coded, linked data may also be shared with collaborators as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response at 6 and 12 weeks (+/- 2 weeks), then every 3 months (+/- 1 month) x3, then every 6 months' (+/- 1 month) x 5 years. In addition to a baseline scan, confirmatory scans should also be obtained approximately 4 (not less than 4) weeks following initial documentation of objective response.

Clinical response will be determined using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.0).

6.3.1 Definitions

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

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

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: >20 mm;
- By CT scan:
  - Scan slice thickness 5 mm or under as >10 mm with CT scan
  - Scan slice thickness >5 mm: double the slice thickness

With calipers on clinical exam: >10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters) .

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters

will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and >10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication.

However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 Response Criteria

6.3.4.1 Evaluation of target lesions[1]

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.
- Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

6.3.4.2 Evaluation of non-target lesions[2]

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.
- Non-Complete Response: Persistence of one or more non-target lesions
- Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

6.3.5 Evaluation of best overall response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR

1 All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

2 All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

6.3.6 Confirmatory Measurement/Duration of Response

6.3.6.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

6.3.6.2 Duration of Overall Response

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

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

6.3.6.3 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event:

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research .

7.1.2 Suspected Adverse Reaction:

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable

possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected Adverse Reaction:

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event:

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

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

7.1.6 Disability:

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-Threatening Adverse Drug Experience:

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition):

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non Compliance (NIH Definition):

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem:

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
    a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
    b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NIH INTRAMURAL IRB AND NCI CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths:

The Protocol PI will report in the NIH Problem Form to the NIH Intramural IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. The protocol PI will report to the NIH Intramural IRB:

- All Grade 2 unexpected events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NIH Intramural IRB Reporting of IND Safety Reports:

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

7.3 IND SPONSOR REPORTING CRITERIA

From the time of the first study treatment through the first 30 days after the subject receives the last investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For events that occur more than 30 days after the last administration of investigational agent/intervention, only report serious adverse events that have an attribution of at least possibly related to the agent/intervention.
Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events including deaths due to progressive disease must be reported within one business day .

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Wavier of expedited reporting to CCR

The investigators are requesting a waiver from reporting specific events in an expedited manner to the CCR. Patients will be receiving commercially available agents, such as fludarabine, cyclophosphamide, and aldesleukin. The majority of toxicities observed on Surgery Branch Adoptive Cell Therapy protocols are expected toxicities of the non-myeloablative chemotherapy regimen or IL-2 and occur in approximately 95% of the patients enrolled, therefore, we are requesting a waiver from reporting the following events in an expedited manner to the CCR.

- Grade 3 or greater myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Grade 3 or greater nausea, vomiting, mucositis - oral, anorexia, diarrhea, fever, chills, fatigue, and rash maculo-papular.
- Grade 3 hypoxia, dyspnea, hematuria, hypotension, sinus tachycardia, urine output decreased, confusion, infections, and febrile neutropenia.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of patients treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov

7.3.2 Reporting Pregnancy

7.3.2.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as a SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.2.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of aldesleukin or pembrolizumab.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

7.4 DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team:

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be reported to the IRB using iRIS .

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the

investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Sponsor Monitoring Plan:

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects' protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

8 STATISTICAL CONSIDERATIONS

The primary objective of this protocol will be to determine whether the regimen will result in objective clinical responses, separately according to whether patients received prior anti-PD1 or not.

The secondary objective is to determine the survival of patients receiving this treatment regimen.

Of 23 patients that have been randomized and treated in this protocol prior to amendment D, preliminary analysis performed of the ordered categorical responses (PD < SD < PR) using the Cochran-Armitage trend test demonstrated a two-tailed p-value in favor of the high dose chemotherapy arm of 0.11. In patients that did not have prior anti-PD1, the p-value in favor of the high dose arm was 0.047, although in patients that had prior anti-PD1 there was no difference between the high and low dose arm (p=1.00). The difference between the response rates in the two arms could not be accounted for by the small difference in patients who had prior PD1 in the two arms. In addition, progression free survival curves using this small number of patients favored the high dose chemotherapy arm with p=0.0084 (median progression free survival 4.7 months vs. 1.9 months). Therefore, on the basis of these interim results, we have eliminated the low dose chemotherapy arm and will treat all subsequently enrolled patients with high dose chemotherapy.

Prior to amendment D, 8 patients who did not have prior anti-PD1 received high dose chemo on this protocol, and 3 of these 8 patients responded (38%); 0 of 3 patients who had received prior anti-PD1 and received high dose chemo on this protocol responded. These patients who were enrolled in the randomized phase of the trial and received high dose chemo will be included in newly created separate phase II evaluations of standard (high) dose chemo according to the following design:

For the patients who did not have prior anti-PD1, a single stage design with 21 total evaluable patients will be used. Including the initial 8 patients (with 3 responders), this design will have 80% power to reject a 25% response rate in favor of a 50% response rate, with a one-sided 0.10 alpha level exact binomial test. In practice, the number of patients who respond out of the total number evaluated will be determined and presented along with 80% and 95% two-sided confidence intervals. As an example, if there are 8 responses in 21 evaluable patients in this cohort, an exact two-sided 80% confidence interval extends from 25.4% to 52.4%, thus demonstrating results potentially superior to 25% and consistent with 50%.

For patients who have received prior anti-PD1 therapy, a two-stage Simon MinMax design will be used. This cohort will include the initial 3 patients (with 0 responses) as part of the first stage. This portion of the trial will seek to rule out a 10% response rate (p0=0.10) and target a 30% response rate (p1=0.30). With alpha=0.10 (probability of accepting a poor treatment =0.10) and beta=0.10 (probability of rejecting a good treatment=0.10), this part of the trial will initially enroll 16 evaluable patients (including the 3 from the randomized portion) and if there is only 0 or 1 response out of 16 patients, no further patients will be enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 or more responses in the initial 16 patients, then accrual may continue until there are 25 total evaluable patients in this cohort. If there are 2 to 4 responses in 25 patients, this would be an unacceptably low rate, while if there are 5 or more responses in 25 patients, this would be worthy of further evaluation. Under the null hypothesis (10% response rate), the probability of early termination is 51%.

The response results will primarily be reported separately according to their prior use of anti-PD1. Survival and progression free survival will also be estimated for the patients, overall and by prior anti-PD1 exposure.

Toxicity will be evaluated by tabulating the types of toxicity observed and describing the results obtained. This will be done for all patients evaluated as part of the phase II designs as well as the initial patients randomized to receive low dose treatment.

Patients who are retreated on arm1P and receive pembrolizumab will be considered to be part of a pilot subset of the main protocol, intended for evaluation for responses separately from patients treated without pembrolizumab, with separate 95% confidence intervals formed around the response fractions of both groups. If the addition of this agent demonstrates the ability to produce at least a small fraction of patients with a clinical response, the combination may be tested in a more definitive subsequent study.

The initial accrual under the randomized portion of the trial was 24 total patients. Eleven of these 24 who were treated with high dose chemo will be included among the phase II cohorts' maximum accrual of 21+25=46 patients. Thus, up to 13 (randomized to low dose or not treated) + 11 (randomized and treated with high dose, and to be included in phase 2) + 35 additional patients in phase 2 may be required (to arrive at 46 total in phase 2). In order to enroll up to these 59 patients, the accrual ceiling will be set at 64. Since 24 patients have already been enrolled, approximately 35 to 40 total additional patients may be enrolled. If the remaining patients are able to be accrued at the rate of approximately one per month, accrual should be completed within approximately 3 to 4 years after approval of amendment D, or 4 to 5 years after the trial initially was open for accrual.

9 COLLABORATIVE AGREEMENTS

We have a CRADA (#02734) with Iovance Biotherapeutics, Inc. (formerly Lion Biotechnologies, Inc.) and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR PATIENT SELECTION

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.2 PARTICIPATION OF CHILDREN

The use of the nonmyeloablative and myeloabalative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **10.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though this is unknown. The NCI Surgery Branch has extensive experience with ACT following treatment with high dose

aldesleukin, however this experimental treatment is only available at a very few centers throughout the country. The risks associated with ACT are substantial, including, a delay in treatment due to the need to harvest and grow the cells, a surgical procedure (possible major) to obtain tumor for the cell product, the possibility that a cell product cannot be generated, infection and sepsis due to non-myeloablative chemotherapy, intubation, and renal toxicities due to aldesleukin, and death. The risks in this treatment are detailed in section **11**.

10.5 RISKS/BENEFITS ANALYSIS

Because all the patients in this protocol have metastatic or recurrent melanoma and limited life expectancies, the potential benefit is thought to outweigh the potential risks.

The risks and benefits of participation for adults who become unable to consent, are no different than those described for patients who are less vulnerable.

10.6 CONSENT PROCESS AND DOCUMENTATION

If the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

11 PHARMACEUTICAL INFORMATION

11.1 INVESTIGATIONAL REGIMEN

11.1.1 Young TIL

The procedure for growing and expanding the autologous young TIL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration and used in other Surgery Branch, NCI TIL clinical studies. This product will be provided for investigational use only under a sponsor-investigator IND. The Certificate of Analysis is in protocol **Appendix 1** and the Standard Operating Procedures for the growth of TIL are found in the IND. Cells will be administered at a dose of between 1x109 to 2x1011 lymphocytes. Note: Penicillin, Streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

11.1.2 Aldesleukin (Interleukin-2, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Aldesleukin (Interleukin-2) is manufactured by the Novartis Pharmaceuticals Corporation, Florham Park, NJ and will be purchased by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin is provided as single-use vials containing 22 million IU (≙1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl

contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

Storage: Intact vials are stored in the refrigerator (2° - 8°C) protected from light. Each vial bears an expiration date.

Dilution/Stability: Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of RIL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2° – 30°C.

Administration: The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in **Appendix 3** and 4. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix 3**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 4**.

11.1.3 Fludarabine

Description: (Please refer to package insert for complete product Information) Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).

Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with

or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.1.4 Cyclophosphamide

(Refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

Administration: It will be diluted in 250 ml D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute

myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

11.1.5 Pembrolizumab

(Refer to FDA-approved package insert for complete product information)

Description: Pembrolizumab is a humanized monoclonal IgG4 antibody directed against the human cell surface receptor, programmed death-1, (PD-1). Following administration, pembrolizumab binds to PD-1 and blocks the interaction between PD-1 and its ligands.

How Supplied: Keytruda (pembrolizumab) will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Pembrolizumab is supplied in a single-use 50 mg vial as pembrolizumab lyophilized powder and as a single use 25mg/mL ready-to-use solution containing 4mL (100mg/vial).

Reconstitution/Dose Preparation: The lyophilized powder is reconstituted with 2.3 ml Sterile Water for injection, USP, and the resultant concentration is 25 mg/ml. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Let contents sit for up to 5 minutes to allow all bubbles to clear. Do not shake.

The ready-to-use solution or reconstituted lyophilized powder must be further diluted prior to administration. It may be diluted in 0.9% Sodium Chloride injection, USP or 5% Dextrose Injection, USP. The final concentration of the dilution solution should be between 1 mg/ml to 10 mg/ml.

Stability/Storage: The reconstituted lyophilized power is stable for up to 6 hours at room temperature or up to 24 hours at when stored under refrigeration (2-8°C or 36-46°F).

The diluted solution is stable for up to 6 hours at room temperature or up to 24 hours at when stored under refrigeration (2-8°C or 36-46°F).

Administration: Pembrolizumab will be given at a dose of 2 mg/kg (based on actual body weight) as an intravenous infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.

Toxicities: The most common adverse reactions are fatigue (47%), nausea (30%), pruritus (30%), cough (30%), rash (29%), decreased appetite (26%), constipation (21%), diarrhea (20%), arthralgia (20%), and peripheral edema (17%). Because of pemrolizumab's effect on the immune system, serious, sometimes fatal, immune-related adverse events (IRAEs) have been observed, which include: immune-mediated pneumonitis, immune-mediated colitis, immune-mediated hepatitis, immune-mediated nephritis/renal dysfunction, immune-mediated endocrine disorders (hypothyroidism, hyperthyroidism, hypophysitis, and pancreatitis).

11.2 SUPPORT MEDICATIONS

11.2.1 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 2**. Toxicities include nausea, vomiting and diarrhea.

11.2.2 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/ml and 480 ug/1.6 ml vials. Filgrastim should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. Filgrastim will be given as a daily subcutaneous injection. The side effects of filgrastim are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

11.2.3 Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (on NON-consecutive days) beginning day 0 or within one week of anticipated lymphopenia and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur. Should allergies develop, the following medications may be used in place of TMP/SMX DS:

11.2.3.1 Dapsone:

Dapsone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia. The dose is 100mg by mouth daily, starting on day 0 (± 7 days) and continuing at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. It is supplied as 25mg and 100mg tablets. Dapsone contains a sulfa group, although the cross reactivity in patients with sulfa allergies is quite low. Dapsone may be considered in patients with mild to moderate sulfa allergies. Dapsone should be avoided in patients with severe (i.e., a history of anaphylaxis or other equally serious reaction) reactions to sulfa drugs. Additionally, dapsone has been reported to cause hemolytic anemia is patients with G6PD deficiency. It is recommended that patients be tested for G6PD deficiency prior to the initiation of dapsone therapy. Dapsone is generally well tolerated, but may cause a number of hematologic adverse reactions, including increased reticulocyte counts, hemolysis, decreased hemoglobin, methemoglobinemia, agranulocytosis, anemia, and leukopenia. Other rare but serious adverse reactions include bullous exfoliative dermatitis,

Stevens-Johnson syndrome, toxic epidermal necrolysis, pancreatitis, interstitial pneumonitis, and pulmonary eosinophilia. For more detailed information about adverse reactions, consult the package insert.

11.2.3.2 Atovaquone:

Atovaquone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or pentamidine. Atovaquone may be given as a single daily dose of 1500mg orally or the dose may be split into 750mg given orally twice daily. Atovaquone will be started on day 0 (± 7 days), and will continue for at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. Atovaquone is supplied as an oral suspension containing 150mg/mL. Common adverse reactions to atovaquone include: headache, rash, diarrhea, nausea, vomiting, abdominal pain, cough, and fever. Rare but serious adverse reactions include acute renal failure, hepatitis and hepatic failure, angioedema, pancreatitis, and Stevens-Johnson syndrome. For more detailed information about adverse reactions, consult the package insert.

11.2.3.3 Aerosolized Pentamidine:

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer within one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months' post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

11.2.4 Herpes and Varicella Zoster Virus Prophylaxis

11.2.4.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

11.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of

acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. gancyclovir. In renal disease, the dose is adjusted as per product labeling.

11.2.5 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 MG/ML solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

11.2.6 Ondansetron hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

11.2.7 Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of all side effects.

12 REFERENCE LIST

1. Jemal, A., et al., *Cancer statistics, 2007.* CA Cancer J. Clin, 2007. **57**(1): p. 43-66.
2. Atkins, M.B., et al., *High-dose recombinant interleukin-2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993.* J. Clin. Oncol, 1999. **17**: p. 2105-2116.
3. Gogas, H.J., j.M. Kirkwood, and V.K. Sondak, *Chemotherapy for metastatic melanoma: time for a change?* Cancer, 2007. **109**(3): p. 455-464.
4. Wunderlich, J.R. and R.J. Hodes, *Principles of tumor immunity:Biology of cellur immune responses.* Biologic Therapy of Cancer, DeVita, Hellman and Rosenberg, eds, 1991: p. 3-21.
5. Dudley, M.E., et al., *Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens.* J. Clin. Oncol, 2008. **26**: p. 5233-5239.
6. O'Brien, S.M., et al., *Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia.* J. Clin. Oncol, 2001. **19**(5): p. 1414-1420.
7. Prieto, P.A., et al., *CTLA-4 blockade with ipilimumab: Long-term follow-up of 177 patients with metastatic melanoma.* Clin Cancer Res, 2012. **(In Press)**.

13 FIGURES, TABLES & APPENDICES

13.1 TABLE 1
Objective Response Rates for 3 ACT Studies

Cell Transfer Therapy (5/1/13)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (114+, 112+, 111+, 97+, 86+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (101+, 98+, 93+, 90+, 70+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (81+, 78+, 77+, 72+, 72+, 71+, 71+, 70+, 70+, 19)	18(72%)

(20 complete responses: 19 ongoing at 70 to 114 months)

13.2 TABLE 2

Time in Hospital and Non-hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

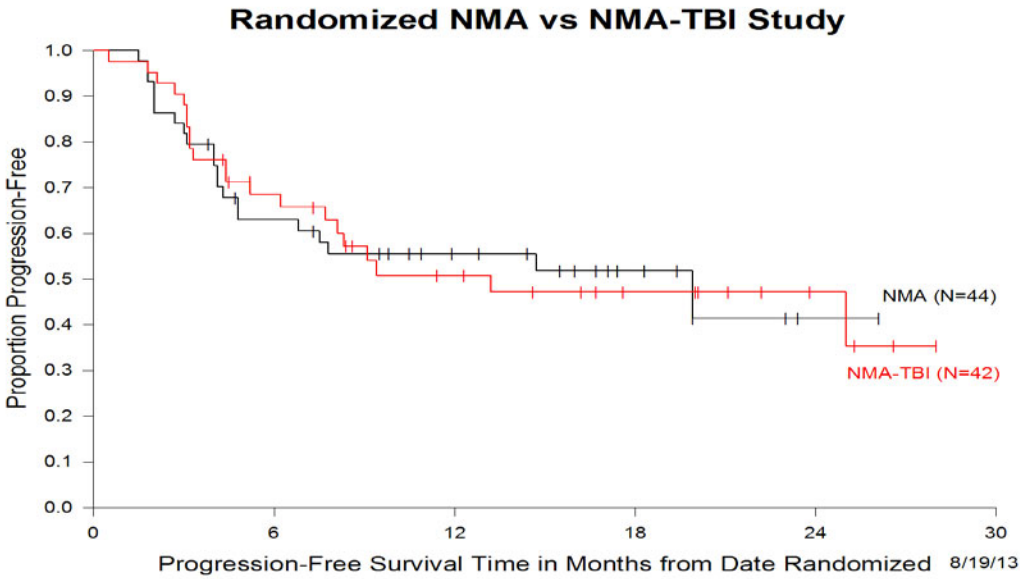
13.3 TABLE 3

Randomized NMA vs NMA-TBI Study (8/19/13)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
NMA	44	16 (36%) 26+, 19, 19+, 18+, 17+, 17+, 17+, 16+, 14+, 12+, 11+, 7, 7+, 6, 4, 3+	5 (11%) 23+, 19+, 16+, 15+, 10+	21 (48%)
NMA-TBI	42	23 (55%) 26+, 25, 25+, 21+, 20+, 19+, 17+, 16+, 16+, 13, 12+, 11+, 9, 9, 8, 8+, 7+, 6, 5, 5+, 4, 4+, 4+	3 (7%) 27+, 23+, 22+	26(62%)

(8 complete responses: 8 ongoing at 10 to 27 months)
(1ST patient randomized 3/24/11)

13.4 FIGURE 1


Randomized NMA vs NMA-TBI Study
Proportion Progression-Free
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
NMA (N=44)
NMA-TBI (N=42)
Progression-Free Survival Time in Months from Date Randomized
8/19/13

13.5 APPENDIX 1
Certificate of Analysis:

Young TIL

Patient:

Date of preparation of final product:

Unique TIL identifier (tumor and culture number):

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests performed by*	*Initials/Date*
Cell viability[1]	trypan blue exclusion	>70%			
Total viable cell number[1]	visual microscopic count	between 10^9 and 2×10^{11}			
Identity	FACs	> 80 % CD3+ on REP cells			
TIL potency[2]	OKT3-stimulated IFN release	>200 pg/ml per 10^5 cells and > 2 times background			
Microbiological studies	aerobic culture[5]	no growth			
	anaerobic culture[5]	no growth			
	gram stain[1,3]	no micro-organisms seen			
	aerobic culture[3,4]	no growth			
	fungal culture[3,4]	no growth			
	anaerobic culture[3,4]	no growth			
	mycoplasma test[2]	negative			
Endotoxin[1]	limulus assay	≤5 E.U./kg			
Presence of tumor cells[2]	Cytopathology	No tumor cells per 200 cells examined			

[1] Performed on the final product prior to infusion. Results are available at the time of infusion.

[2] Performed 2 - 10 days prior to infusion (test performed prior to final manipulation). Results are available at the time of infusion.

[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

[4] Sample for test collected on the final product prior to infusion. Results will not be available before cells are infused into the patient.

[5] Sample for test collected on the in process cells prior to the REP. Results will be available before cells are infused into the patient.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____

Qualified laboratory or Clinical Supervisor

13.6 APPENDIX 2

Modification of Dose Calculations* in Patients whose BMI is > 35

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

BMI = weight (kg) / [height (m)]^2

2. Calculation of ideal body weight

Male = 50 kg + 2.3 (number of inches over 60 inches)

Example: ideal body weight of 5'10'' male

50 + 2.3 (10) = 73 kg

Female = 45.5 kg + 2.3 (number of inches over 60 inches)

Example: ideal body weight of 5'3'' female

45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight"

Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.

13.7 APPENDIX 3
ADVERSE EVENTS OCCURING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (n=525)[1]

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

[1]Source: Proleukin® Prescribing Information – June 2007

13.8 APPENDIX 4

Expected Aldesleukin Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg q4h p.r., prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest interspersed with activity	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all	No

			supportive measures	
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose

Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours

** Unless the toxicity is not reversed to grade 2 or less by next re-treatment.

13.9 APPENDIX 5

Guidelines for the Management of Immune Related Adverse Events (IRAEs)

Guidelines for the Management of Immune Related Adverse Events (IRAEs) **The following are guidelines only - patient treatment should be at the discretion of the PI or designee**					
Toxicity	**Grade**	**Hold Pembrolizumab**	**Treatment**	**Restart**	**Treatment Discontinuation**
Diarrhea/Colitis	2	Yes	Supportive care x 3 days - if no improvement- high dose oral steroids - taper when stable over 4 weeks	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Diarrhea/Colitis	3-4	Yes	IV steroids followed by high does oral steroids taper when stable over 6-8 weeks	Permanently discontinue	Permanently discontinue
AST, ALT, or increased Bilirubin	2	Hold pembrolizumab when AST or ALT >3.0 to 5.0 times ULN and/or total bilirubin >1.5 to 3.0 times ULN.	0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month,	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
AST, ALT, or increased Bilirubin	3-4	same as above	high dose IV glucocorticoids x 24-48 hours then continue as above	Permanently discontinue [1]	Permanently discontinue[1]
Type 1 diabetes mellitus (if new onset)		Yes	as appropriate -Consider local testing for islet cell antibodies and antibodies to GAD, IA-2, ZnT8, and insulin may be obtained	Resume pembrolizumab when patients are clinically and metabolically stable.	
Hyperglycemia associated with evidence of beta cell failure	3-4	Yes	same as above	Resume pembrolizumab when patients are clinically and metabolically stable.	

Hypophysitis	2-4	No- asymptomatic Yes- symptomatic	Treat with prednisone 40 mg p.o. or equivalent per day. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted. If held, restart when toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Hyperthyroidism	2	no	Hormone replacement as indicated	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Hyperthyroidism	3-4	Yes	initial treatment with steroids followed by hormone replacement as appropriate	Permanently discontinue	Permanently discontinue
Hypothyroidism		no	hormone replacement as indicated	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted.
Infusion Reaction	3-4	Yes		Permanently discontinue	Permanently discontinue
Pneumonitis	2	Yes	Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Pneumonitis	3-4	Yes	Immediately treat with intravenous steroids (methylprednisolone 125 mg IV). When symptoms improve to Grade 1 or less, continue as above	Permanently discontinue	Permanently discontinue

Renal Failure or Nephritis	2	Yes	Prednisone 1-2 mg/kg/day- once resolved to grade 1 taper over at least 4 weeks	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Renal Failure or Nephritis	3-4	Yes	IV steroids then as above	Permanently discontinue	Permanently discontinue
Rash/pruritus	2	No	Symptomatic treatment		Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
Rash/pruritus	3-4	Yes	Oral steroids as indicated with appropriate slow taper		Permanently discontinue for grade 4
All Other Drug-Related Toxicity[2]	2	Yes	Oral steroids as indicated with appropriate slow taper	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
All Other Drug-Related Toxicity	3-4	Yes	IV steroids as indicated then as above when resolves to grade 1	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
Note: clinical symptom management and evaluation to rule out other causes of the event should be ongoing
[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.
[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.