Abbreviated Title: 1200 TBI TIL Randomized Study
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PROTOCOL TITLE

A Prospective Randomized Study of Cell Transfer Therapy for Metastatic Melanoma Using Short-Term Cultured Tumor-Infiltrating Lymphocytes Plus IL-2 Following Either a Non-Myeloablative Lymphocyte Depleting Chemotherapy Regimen Alone or in Conjunction with 12Gy Total Body Irradiation (TBI)

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Investigational Agents:

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

Commercial Agents: Cyclophosphamide, Fludarabine, and Aldesleukin

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. Complete regression rates in these three consecutive trials were 12%, 20%, and 40%, respectively—strongly suggesting that the addition of TBI could improve the complete regression rate. Of the 20 complete regressions seen in this trial, 19 are on-going at 37 to 82 months.
- Because of the complexity of developing selected TIL for use in adoptive transfer, we have recently developed a simplified method for producing TIL that is more applicable to use in outside institutions. Utilizing "young TIL" cells (sometimes with CD8 purification) in 105 patients, the objective response rate was 34% with a 6.6 % incidence of complete regressions. All patients in this trial received the cyclophosphamide fludarabine regimen alone.
- Because of the strong suggestion that the addition of TBI to the chemotherapy regimen could increase durable, complete regression rates in patients with metastatic melanoma, we are now attempting to definitively determine whether the addition of TBI to the chemotherapy preparative regimen can improve complete response rates, and overall survival in patients receiving "young TIL".

Objectives:

- To determine, in a prospective randomized trial, the complete response rate and survival of patients with metastatic melanoma receiving ACT using young TIL plus aldesleukin treatment following either a chemotherapy preparative regimen alone, or the same chemotherapy preparative regimen plus TBI.

Eligibility:

Patients who are 18 years or older must have:

- Evaluable metastatic melanoma;
- Metastatic melanoma lesion suitable for surgical resection for the preparation of TIL;
- No contraindications to high-dose aldesleukin administration or total body irradiation;
- 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 with will be prospectively randomized to receive ACT with young TIL plus aldesleukin following either a non-myeloablative chemotherapy preparative regimen or this same regimen plus TBI.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To compare in a prospective randomized trial, the complete response rate and survival in patients with metastatic melanoma receiving adoptive cell transfer (ACT) using young TIL plus aldesleukin treatment following a preparative regimen consisting of lymphodepleting chemotherapy with or without total body irradiation.

1.1.2 Secondary Objectives

- To determine response rates and progression free survival in patients in the two treatment arms.
- To determine the toxicity of these two treatment regimens.

1.2 BACKGROUND AND RATIONALE

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 and dacarbazine chemotherapy. Aldesleukin has an objective clinical response rate of about 16% and a complete response rate of 6%[2]. It is the only treatment known to cure some patients with metastatic melanoma. Dacarbazine-based chemotherapy has a clinical response rate of up to 20% but almost no complete responders or long-term survivors[3]. With the incidence of melanoma increasing in the United States, 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 ACT therapy 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 2 or 12 Gy whole body irradiation prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%. The characteristics of these patients are shown in **Table 1**, and the toxicities shown in **Table 2** for the TBI studies (04-C-0288, 06-C-0136) and **Table 3** for the initial study without TBI (99-C-0158). There was one treatment related death in these 93 patients which occurred in a patient in the 2 Gy trial who had an undetected diverticular abscess prior to beginning therapy. In the 12 Gy study, five patients had hematuria and elevated creatinine levels. Four of these patients underwent a renal biopsy which indicated thrombotic microangiopathy. The consulting nephrologists indicated that they believed this toxicity would progress to renal failure and all these patients would eventually need dialysis. However, this has not occurred and all 5 patients are doing well.

The objective and complete response rates in these three consecutive trials are shown in **Table 4**. The objective response rates (RECIST) were 49%, 52%, and 72% and the complete response rates were 12%, 20%, and 40% in the NMA study, 2 Gy study and 12 Gy study, respectively. Of the 20 complete responders in the three trials, 19 are ongoing from 37 to 82 months. These represent by far the highest complete response rates seen in patients with metastatic melanoma (**Table 5**). There appeared to be an increase in survival with increasing lymphodepletion although the consecutive nature of these non-randomized trials and possible selection bias of patients entering the trials cause doubt concerning the validity of this conclusion (**Figure 1** and **Figure 2**). Of the 52 responding patients in this trial, 42 were previously shown to be refractory to aldesleukin therapy and 21 were refractory to prior aldesleukin plus chemotherapy.

Despite this high rate of objective responses in previously treated patients, ACT is available only in the Surgery Branch, NCI and although two other institutions have begun to explore ACT therapy very limited numbers of patients have been treated elsewhere. The generation of TIL is time consuming, expensive and labor intensive. In addition, the costs for cell preparation as well as patient treatment are not covered by third party payers and these factors have discouraged the application of the ACT approach. In the original clinical trials, all patients received highly selected TIL, grown from multiple individual cultures with expansion of cultures shown to have specific reactivity against the autologous tumor or HLA matched tissue culture lines. This requirement substantially increased the difficulty in generating the appropriate reagents necessary to treat a large number of patients.

Because of the difficulties in generating highly selected reactive TIL we have put considerable effort into the development of techniques for the generation of effective TIL using procedures that do not require the extensive laboratory manipulations and testing required to develop highly selective TIL. In addition, intensive post-hoc analysis of some TIL cultures revealed tumor recognition that was not discovered in time to treat patients. This revealed that some TIL were considered “non-reactive” due to inadequacies in current assay methods.

Furthermore, murine and human evidence suggest that TIL cultured for shorter times (young TIL) may have better in vivo activity against tumors than extensively cultured TIL. In the initial report of the treatment of melanoma patients with adoptive transfer of TIL, we noted that patients treated with TIL from younger cultures had a higher frequency of clinical response to treatment than patients treated with TIL from older cultures ($p = 0.0001$)[6]. More recent studies examining the role of telomere length support this hypothesis. Differentiation and expansion of T cells is associated with telomere shortening[7,8] and with altered and loss of cell functions, including

proliferation[9,10]. Zhou et. al. reported that TIL which were associated with objective clinical responses in treated patients had longer telomere lengths than TIL that were not associated with clinical responses ($p < 0.01$)[11]. TIL which persisted in vivo had longer telomere lengths than TIL which did not persist ($p < 0.001$).

To determine the relationship between telomere length and time in culture, we measured telomere lengths of 495 TIL cultures generated from 48 consecutive patients by quantitative fluorescent in-situ hybridization (QFISH)[12]. The TIL were evaluated after eight to 56 days in culture. **Figure 3** shows the close relationship between the age of TIL in culture (days) and their mean telomere length (kb). Though the telomere lengths varied widely, there was a strong inverse correlation between time in culture and telomere length of TIL ($p < 0.001$). This observation is consistent with earlier observations that shorter time in culture is associated with improved response and improved response is associated with longer telomere lengths.

The hypothesis that younger TIL have improved in vivo survival and anti-tumor activity is corroborated by studies demonstrating that younger T cells have phenotypes with more favorable characteristics for in vivo persistence and antitumor activity. CD27 and CD28 are associated with proliferation and survival of T cells and are often used as markers of less-differentiated T cells[13]. Huang, et al reported that T-cell clonotypes with long-term in vivo persistence expressed higher levels of CD27 and CD28 than clonotypes with short-term persistence[14]. In a murine model of adoptive immunotherapy, Gattinoni et al demonstrated that the differentiation of effector T cells in culture had a severe negative effect on their capacity to function in vivo, and T cells expressing higher levels of CD27 had better in vivo antitumor activity than CD27-low cells[15].

We thus developed an approach to rapidly generate TIL for treatment that involved growth of the entire complement of infiltrating lymphocytes for about two weeks and then expanding them to numbers sufficient for cell treatment. We called these cells “young” TIL. We studied the expression of CD27 and CD28 in young and standard TIL to examine the differences in phenotypic expression of these TIL[12]. Matched pairs of young TIL (mean age = 14 days) and standard TIL (mean age = 31 days) were generated from 14 tumor specimens. Expression of CD27 and CD28 as well as CD3, CD4, and CD8 were measured in both sets of TIL by fluorescent-activated cell sorting (FACS). **Figure 4** shows representative FACS plots comparing the phenotypic expression of one young TIL culture with its matched standard TIL culture. **Figure 5** demonstrates the differences in phenotypic expression of CD27 and CD28 by young and standard TIL. Young TIL have significantly higher expression of CD27 ($p < 0.0001$) and CD28 ($p < 0.003$) than standard TIL. In addition, FACS analysis of gated CD8+ cells from these TIL populations demonstrated a strong relationship between culture age and the expression of CD27 and CD28. These observations suggested that younger effector T cells have phenotypic expression of CD27 and CD28 which may indicate better in vivo proliferation, survival, and antitumor activity compared to older effector T cells.

A potential advantage of the use of this simpler method of producing TIL is that they will be more heterogeneous in their reactivity than highly selected TIL which have grown for longer periods of time. Another major advantage of the young TIL procedure is that the great majority of patients with resectable lesions can be treated. Thus, young TIL can be successfully grown from approximately 80% of all patients with resectable lesions. This high rate of cell production makes it possible to treat the great majority of patients with resectable lesions that come to the Surgery Branch for treatment.

We have performed a series of clinical trials to evaluate ACT using these young TIL preparations. In the first of these consecutive trials, 24 patients were treated with young TIL and in a second cohort, 39 patients were treated with young TIL that had been enriched for CD8+ cells. Although there was a suggestion that CD8 enriched young TIL gave higher response rates, we then performed a prospective randomized trial in which patients were randomized to receive either bulk young TIL or CD8 enriched young TIL. This trial has almost completed accrual and there is no suggestion of a difference in either overall response rates, complete response rates, or toxicities between the young TIL and CD8 enriched young TIL arms. The CD8 enrichment procedure can be quite expensive, including the use of the GMP quality Miltenyi separation apparatus. Thus, for future trials we will be using bulk young TIL. Since there were no differences between these two cell preparations, combined data of 105 patients that received young TIL or CD8 enriched young TIL are presented. In this combined group of 105 patients, all of whom received the cyclophosphamide fludarabine alone non-myeloablative preparative regimen, the objective response rate was 34.3% with 6.6% complete regressions. There is no significant difference in overall or complete response rates in patients receiving young TIL or the selected TIL used in previous trials (p2=0.14 and p2=0.33, respectively) in patients receiving the same chemotherapy non-myeloablative preparative regimen alone. The toxicity profile is also not significantly different than that using highly selected TIL. A post-hoc analysis of the activity of young TIL in-vitro revealed that many of these preparations had specific anti-tumor activity quite similar to that of the selected TIL used in prior trials. A pilot trial was conducted in 23 patients using young TIL and aldesleukin following cyclophosphamide/fludarabine and a 6 GY intermediate dose of TBI. Objective responses were seen in 48% of patients with 13% of patients experiencing a complete regression. This complete regression was double the 6.6% complete regression rate in patients receiving young TIL and the chemotherapy preparative regimen alone. Toxicities were similar to that in the trials without TBI.

Interestingly, 2 additional groups have now reported their results utilizing the cyclophosphamide/fludarabine non-myeloablative preparative regimen followed by young TIL and IL-2 administration. Besser et al., treated 31 patients with an objective response rate of 48.4% and a complete regression rate of 12.9 %. Joseph, et al., reported at the last meeting of the International Society for the Biologic Therapy of Cancer on 25 patients treated with young TIL with an objective response rate of 52% with 4% complete regressions. Thus, the conglomerate data available concerning the use of young TIL and the chemotherapy alone preparative regimen in 161 patients revealed an objective response rate of 39.8 % with a 7.5 % incidence of complete regressions.

In summary, this data reveals that the results of the use of young TIL is quite similar to that of the previous trials with selected TIL utilizing the cyclophosphamide fludarabine preparative regimen. In our previously published trials using selected TIL, the complete regression rate using the chemotherapy preparative regimen alone was 11.6% compared to 40% when 12Gy TBI was (p2=0.01). Thus, in the current trial we will attempt to definitively answer the question as to whether the 12 Gy TBI does indeed improve results by prospectively randomizing patients to receive TIL plus IL-2 following either the chemotherapy preparative regimen alone or the chemotherapy/12 Gy TBI regimen.

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. The lesion must be of at least 1 cm in diameter that can be surgically removed with minimal morbidity (defined as any operation for which expected hospitalization ≤ 7 days).

b. Patients with 3 or less brain metastases are eligible. **Note:** If lesions are symptomatic or greater than or equal to 1 cm each, these lesions must have been treated and stable for 3 months for the patient to be eligible.

c. Greater than or equal to 18 years of age and less than or equal to 66 years of age.

d. Willing to practice birth control during treatment and for four months after receiving all protocol related therapy.

e. Life expectancy of greater than three months

f. Willing to sign a durable power of attorney.

g. Able to understand and sign the Informed Consent Document

h. Clinical performance status of ECOG 0 or 1.

i. Hematology:

- Absolute neutrophil count greater than 1000/mm^3
- Hemoglobin greater than 8.0 g/dl
- Platelet count greater than 100,000/mm^3

j. 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 be less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, or hepatitis C antibody or antigen.

k. Chemistry:

- Serum ALT/AST less than three times the upper limit of normal.
- Calculated creatinine clearance (eGFR) > 50 mL/min.
- Total bilirubin less than or equal to 2 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3 mg/dl.

l. More than four weeks must have elapsed since any prior systemic therapy at the time of randomization, and patients' toxicities must have recovered to a grade 1 or less (except for alopecia or vitiligo). Patients must have stable or progressing 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 or as specified in the eligibility criteria in Section **2.1.1**.

m. Six weeks must have elapsed since any prior anti-CTLA4 antibody therapy to allow antibody levels to decline.

Note: Patients who have previously received ipilimumab or tremelimumab, anti- PD1 or anti-PD-L1 antibodies, and have documented GI toxicity must have a normal colonoscopy with normal colonic biopsies.

2.1.2 Exclusion Criteria

a. Prior cell transfer therapy which included a non-myeloablative or myeloablative chemotherapy regimen.

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

c. Systemic steroid therapy requirement.

d. Active systemic infections, coagulation disorders or other active major medical illnesses of the cardiovascular, respiratory or immune system, as evidenced by a positive stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.

e. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease and AIDS).

f. 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.)

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

h. History of coronary revascularization or ischemic symptoms.

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

j. In patients > 60 years old, documented LVEF of less than or equal to 45%.

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

l. Prior radiation therapy that, in the judgment of the radiation oncologist, precludes the administration of total body irradiation.

2.2 RESEARCH ELIGIBILITY EVALUATION

2.2.1 Within 2 Months Prior to Randomization

a. HIV antibody titer and Hb_sAG determination, anti HCV (may be performed within 3 months of chemotherapy start date)

b. Anti CMV antibody titer, HSV serology, and EBV panel. (may be performed within 3 months of chemotherapy start; patients who are known to be positive for any of the above do not need to be retested.

c. Verification that HLA typing is completed (testing is permitted to be conducted at any time prior to this point).

d. Stress cardiac (MUGA or echocardiogram) in all patients > 60 years old regardless of history, and in patients meeting requirements noted in Section **2.1.2**i.

e. Pulmonary evaluation (PFTs) for patients meeting requirements in Section **2.1.2**k.

f. Colonoscopy for patients meeting requirements noted in Section **2.1.1**n.

2.2.2 Within 3 Weeks Prior to Randomization AND Treatment

Note: The following evaluations should be repeated for safety if treatment will not begin within 3 week of randomization.

a. Complete physical examination including height, weight and vital signs and eye exam, noting in detail the exact size and location of any lesions that exist. (**Note:** Patient history may be obtained within 8 weeks)

b. Chest x-ray

c. EKG

d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.

e. Chem 20 [Sodium (Na), Potassium (K), Chloride (Cl), Total CO2(bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid]and thyroid blood chemistry panel.

f. CBC, differential, PT/PTT, platelet count

g. Urinalysis and culture, if indicated

h. Calculated creatinine clearance

2.2.3 Within 7 Days Prior to Treatment

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

b) ECOG

2.3 Participant Registration and Status Update Procedures

When approximately $5x10^8$ cells are available, patients will enter the study, be registered, and then stratified by M1a versus M1b and M1c. Following stratification, patient will be randomized to receive the non-myeloablative chemotherapy regimen of cyclophosphamide and fludarabine, young TIL and high dose aldesleukin (arm 1) or the same regimen plus 12 Gy total body irradiation (TBI). Randomization will be conducted a 1:1 fashion using variable block sizes.

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

Patients with evaluable metastatic melanoma will undergo resection of tumor under protocol 03-C-0277. Patients with evaluable metastatic melanoma who have TIL successfully growing from a metastatic lesion will be prospectively randomized to arm 1 or arm 2 as described in Section **2.3**. TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. Volunteers will also undergo apheresis to obtain mononuclear cells to be used as feeder cells in cell culture according to protocol 03-C-0277. The procedures used are the same as those in all prior ACT protocols and are in routine use in the Department of Transfusion Medicine in the Clinical Center. Separate consents will be obtained from all apheresis volunteers. TIL cultures will be monitored regularly and when approximately $5x10^8$ cells are available the cells will undergo a rapid expansion. Prior to starting the rapid expansion, patients will be randomized. Patients randomized to TBI (Arm 2) will undergo collection of CD34+ hematopoietic stem cells as indicated in 03-C-0277 prior to beginning the preparative regimen. Prior to the start of the preparative regimen (approximately one week prior to infusion) TIL will be assessed for potency by interferon gamma release as specified in the Certificate of Analysis shown in **Appendix 1**.

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 lymphocyte depleting preparative regimen, followed by infusion of between $1x10^9$ to $2x10^{11}$ lymphocytes and the administration of the FDA approved standard regimen of aldesleukin at 720,000 IU/kg every eight hours to tolerance starting within 24 hours of cell infusion. Patients will be evaluated for response approximately 4-6 weeks following the completion of aldesleukin. 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 date of the first post-treatment evaluation.

Patients who are randomized to the TBI arm for whom the MINIMUM dose of CD34+ cells cannot be obtained will cross over to the chemotherapy preparative regimen without TBI arm (arm 1).

3.2 PROTOCOL STOPPING RULES

The study may be halted if any of the following conditions are met:

1. If 1 or more treatment related death occurs in either arm, we will promptly discuss this the protocol with the IRB and the FDA but we may not immediately stop accrual.
2. The study will be halted pending discussions with the FDA and IRB if the following conditions are met:
    - Two or more patients remain neutropenic (grade 3 or 4 ANC) 4 weeks following treatment.
    - Two or more patients remain thrombocytopenic (grade 3 or 4 platelets) 8 weeks following treatment.
    - If 2 or more patients have a greater than or equal to grade 3 creatinine level at 3 months' post treatment.

3. The NCI DSMB futility evaluation determines that the trial is unlikely to find an effect at the 0.025 two-tailed significance level with continued accrual, based on the fraction surviving at one year.
4. The NCI DSMB efficacy evaluation determines that the p-value at the interim point (based on approximately 56 total patients enrolled and followed for 6 months) is <0.0054, then the trial will stop accrual for better than expected efficacy.

3.3 AUTOLOGOUS STEM CELL COLLECTION

Prior to beginning the preparative regimen, patients randomized to the TBI (Arm 2) will have stem cells collected and stored for re-infusion after the myeloablation and cell therapy as per protocol 03-C-0277. Patients will receive filgrastim at 8 mcg/kg dose BID (total = 16 mcg/kg/day) by subcutaneous injection for 5 days. The morning dose of filgrastim will be given at approximately 7:00 am each day, except for the last morning dose which will be at 5 am. Eleven hours prior to the apheresis procedure, a single dose of plerixafor at 240 µg/kg may be administered by SQ injection to improve stem cell mobilization. Stem cells will be collected by apheresis.

Autologous apheresis will start on the fifth day of filgrastim administration, and filgrastim and plerixafor administration and apheresis will be repeated one more day if necessary to achieve a sufficient dose of CD34+ cells after ex vivo processing (Miltenyi ClinicMACs). For each daily apheresis a blood volume of 15- 25 liters of blood will be processed, using ACD-A anticoagulation, peripheral or central venous access, and calcium replacement as needed, per standard operating procedure of the NIH Clinical Center DTM. Sufficient CD34+ cell doses after Miltenyi ClinicMACs positive selection are defined as a TARGET of $> 4 \times 10^6$/kg recipient weight, and a MINIMUM of $\geq 2 \times 10^6$/kg recipient weight. Patients who do not reach the MINIMUM dose of selected CD34+ cells after two apheresis collections may be considered for a second cycle of filgrastim mobilization and apheresis collections.

If the MINIMUM dose of CD34+ cells has not been reached after 2 cycles of mobilization and collection, the patient in the TBI arm will receive the chemotherapy preparative regimen without TBI.

CD34+ cells will be processed and cryopreserved according to DTM policy and procedure until needed for cell infusion.

3.4 DRUG ADMINISTRATION

Treatment schedule will be according to the following schedule (see Section **3.5**). Only patients in arm 2 will receive 12Gy TBI. (Times are offered as examples and may be changed as long as a similar time relationship between administrations of the drugs is maintained. Study medication start times for drugs given once daily may be within 2 hours of the scheduled time. All other medications may be given ± one hour of the scheduled time; the length of administration may be ± 15 minutes. Administration of diuretics, electrolyte monitoring and replacement, and hydration should all be performed as clinically indicated – the times noted below are offered only as examples.)

3.4.1 Preparative Regimen

The following will comprise a course of therapy for Day -7 through Day 2:

<u>Day -7 and -6</u>

1 am: Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 2.6 mL/kg/hr. (starting 11 hours' pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if the urine output <1.5 mL/kg/hr or if body weight >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV maybe administered. Serum potassium should be monitored and treated as indicated following administration of furosemide.

11 am: 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.

12 pm (NOON): 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**.

1 pm: Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see Section **14**) 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). To allow as much time between the last dose of fludarabine and the administration of the cell treatment, the timing of the fludarabine administration can be moved to an hour earlier each day.)*

For patients randomized to TBI (Arm 2) the following will be added to the preparative regimen:

Day –3 to Day -1

Prior to TBI, patients will receive a single dose of IV ondansetron (ondansetron 0.15 mg/kg IV [*rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight]* x 1 dose pre-TBI).

Patients will receive 2Gy of TBI twice a day for 3 days (total dose 12 Gy) using a linear accelerator in Radiation Oncology. Lungs will be shielded to a mean dose of 6 Gy.

Day-2 (Arm 2 only)

For infection prophylaxis, levofloxacin will be administered at a dose of 500mg PO once daily starting on day -2 and will continue daily as clinically indicated in the best clinical judgment of the attending physician.

3.4.2 Cell Infusion and Aldesleukin Administration

Day 0 (one to three days after the last dose of agent in the preparative regimen):

- Autologous young TIL infusion will be administered intravenously over 20 to 30 minutes (minimum 1 X 10^9 and up to a maximum of 2 X 10^{11} lymphocytes).
- Within 24 hours of cell infusion administration of aldesleukin will be initiated

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

- Aldesleukin 720,000 IU/kg IV (based on total body weight) over 15 minute approximately every eight hours (± 1 hour) for up to 5 days (maximum 15 doses).
- **For patients in cohort 2 only (TBI cohort):**
  - On day 1, the cryopreserved autologous CD34+ selected stem cell product will be thawed and administered intravenously immediately. The minimum dose will be > 2 x 10^6 CD34+ cells per kg.
- **For all patients:**
  - Beginning on day 1 or 2: Filgrastim will be started at 5 mcg/kg/day daily subcutaneously until neutrophil count >1x10^9/l X 3 days or > 5x10^9/L. The maximum filgrastim dose will be 300 mcg per day.

3.5 STUDY CALENDAR

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							
TBI - 2Gy BID (cohort 2 only)					X	X	X					
Young TIL Cells[1]								X				
Aldesleukin[2]								X	X	X	X	X
CD34+ Cells (Cohort 2 only)									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	X	X
Valacyclovir po or Acyclovir IV[6]						X	X	X	X	X	X	X

[1]One to three days after the last dose of agent in the preparative regimen
[2]Initiate within approximately 24 hours after cell infusion
[3]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
[5]Continue until ANC > 1000/mm^3
[6]In patients positive for HSV continue until CD4 > 200 X 2

3.6 TOTAL BODY IRRADIATION (TBI)

3.6.1 Energy

All patients should be treated with a linear accelerator using energies higher than 4MV.

3.6.2 Timing

It is anticipated that TBI will be delivered on day -3, -2, -1.

3.6.3 Technique

TBI will be delivered with lateral fields using extended SSD/SAD values of 200-500cm (depending on machine/vault size) and partial transmission lung blocks. Patients will then receive AP/PA Mediastinal Boost fields using full blocks in the lung area.

3.6.4 Dose/Fractionation

Patients will be treated with TBI to a total dose of 1200 cGy delivered in 200 cGy fractions bid, at least 6 hours apart (six total treatments in three days). The mediastinal boost fields will be delivered at a dose of 100cGy per fraction for a total dose of 600cGy, also at least six hours apart.

3.6.5 Dose Modifications

Occasionally, the total dose/technique of TBI may require modifications due to patient factors (unexpected or severe (grade 4-5) adverse events, serious medical illnesses not conducive to stable patient transfer, patient refusal, etc) or treatment factors (linear accelerator machine offline, etc.) Modifications to the radiation treatment will be at the discretion of the treating radiation oncologist and will be discussed with the Principal Investigator or Study Chairperson.

3.6.6 Toxicities

Toxicities associated with TBI include mucositis, infections, cataracts, pulmonary insufficiency, liver toxicity and secondary late malignancies. Treatment-related mortality in series receiving comparable doses are approximately 2 to 5%.

3.7 INFECTION PROPHYLAXIS

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

3.7.1 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 on the first Monday, Wednesday, or Friday on or after the first dose of chemotherapy.

Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week prior to admission and continued monthly until CD4 count is above 200 and for at least 6 months' post chemotherapy.

3.7.2 Herpes Virus Prophylaxis

Patients with positive HSV serology will be given Valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, 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 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 until the CD4 count is greater than 200 X 2.

3.7.3 Fungal Prophylaxis (Fluconazole)

Patients will start Fluconazole 400 mg p.o. the day after chemotherapy concludes 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.

3.7.4 Infection Prophylaxis for Patients in Cohort 2 (TBI Cohort)

Patients who receive the 1200 TBI regimen are myeloablated and consequently more susceptible to infection. Therefore, patients in cohort 2 only will receive levofloxacin as follows: 500mg PO once daily starting on day -2. Dosing will continue daily as clinically indicated in the best clinical judgment of the attending physician.

3.7.5 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone 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. Aminoglycosides should be avoided unless clear evidence of sepsis. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

3.8 Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >20,000/mm^3. All blood products with the exception of the stem cell product 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.

3.9 Aldesleukin: Intravenous Administration

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 5-minute period every eight hours eight hours (± 1 hour) beginning on the day of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be skipped 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**. If these toxicities can be easily reversed within 24 hours by supportive measures then additional doses may be given. If greater than 2 doses of aldesleukin are skipped, aldesleukin administration will be stopped. 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.

3.10 On Study Evaluation

3.10.1 Prior to Starting the Preparative Regimen

- Apheresis as indicated

- Within 21 days prior to starting the preparative regimen, patients will have a complete blood count, electrolytes, BUN, creatinine, liver function tests and serum chemistries performed. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.10.2 During the Preparative Regimen (Daily)

- Complete Blood Count
- Chem 20: [Sodium (Na), Potassium (K), Chloride (Cl), Total CO_2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid]
- Urinalysis
- CMV antigen assay will be assessed if clinically indicated (e.g. unexplained fevers, pulmonary changes).
- Review of systems and physical exam at least 3 times/week
- Vital signs as per routine

3.10.3 After Cell Infusion

- Vital signs will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4 -6 hours) unless otherwise clinically indicated

3.10.4 During and After Aldesleukin Administration until Hospital Discharge

Every 1-2 days

- Complete Blood Count
- Chem 20: [Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid].
- Review of systems and physical exam at least 3 times/week
- Vital signs as per routine

3.10.5 Severe Immune Reactions

Cytokine levels may be assayed on patients who experience rapidly escalating grade 3 or 4 events assessed as being likely related to the cell product. If the cytokine levels are greatly elevated, and the patient is decompensating, aggressive supportive measures will be instituted as appropriate until cytokine levels are on a significant downward trend.

3.11 POST TREATMENT EVALUATION

3.11.1 Routine Follow up

The initial post treatment evaluation will be conducted approximately 6 weeks (± 2 weeks) following administration of the cell product.

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.

3.11.1.1 At each scheduled evaluation patients will undergo:

- Physical examination
- Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Complete blood count
- Thyroid panel as clinically indicated
- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis as clinically indicated. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.
- 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.
- Patients who develop renal failure will undergo a nephrology consultation and a renal biopsy, if indicated.

3.11.1.2 Patients who develop progressive disease or are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contact. The following information may be requested from the patient:

- Summary of treatment received, including adverse events, since the previous contact
- Estimation of performance status
- Request for imaging studies, physical exam and laboratory reports to be sent to the PI

3.12 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

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

3.12.1 Criteria for Removal from Protocol Therapy

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

- Completion of treatment
- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).

- If a patient experiences grade 3 or 4 toxicity due to cell infusion as referred to under Section **6.4** occurring within 24 hours post cell infusion that does not reverse to grade 2 or less within 8 hours with 2 doses of 650 mg p.o. of acetaminophen or two dose of 50 mg po of diphenhydramine the patient will receive no further cells but may continue to receive IL-2.
- Grade 2 or greater allergic reaction including bronchospasm or generalized urticaria
- Grade 3 or greater toxicity due to cytokines (as referred to under **Appendix 4**) that does not decrease to grade 2 or less within 96 hours of management according to study site SOP.
- Patients with progressive disease who elect not to undergo further active treatment.
- If the PI determines that continued treatment is not in the best interest of the patient.

3.12.2 Off-Study Criteria

Patients will be taken off-study for the following:

- The patient voluntarily withdraws
- There is significant noncompliance
- General or specific changes in the patient's condition render continued participation the patient unacceptable for further follow up in the judgment of the investigator.
- Death

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

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

4 CONCOMITANT MEDICATIONS/MEASURES

Patients will have blood transfusions in order to maintain a minimum hemoglogin of greater than 8.0 g/dL. Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chills develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg q12h). Patients who require transfusions will receive irradiated blood products. Additional antiemetic therapy will be administered for breakthrough nausea and vomiting. Patients will receive supportive care as indicated for aldesleukin toxicities as listed in **Appendix 4**.

5 CORRELATIVE STUDIES FOR RESEARCH

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).

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

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. Tubes/media

may be adjusted at the time of collection based upon materials available or to ensure the best samples are collected for planned analyses.

5.1 Samples Sent to Figg Lab Blood Processing Core (BPC)

- Venous blood samples will be collected in either a 4 mL or an 8 mL SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube.
- For sample pickup, page [redacted].
- For immediate help, [redacted]
- For questions regarding sample processing, contact the BPC at [redacted]
- The samples will be processed, barcoded, and stored in the Figg lab until requested by the investigator.

5.2 Samples Sent to Surgery Branch Cell Production Facility (SB-CPF)

- Venous blood samples will be collected in 8-mL CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube.
- Samples will be transported to the Surgery Branch Cell Production Facility (SB CPF).
- The samples will be processed, barcoded, and stored in SB CPF.

5.3 Prior to Start of Chemotherapy Administration

- 5 CPT tubes (8 mL each): SB CPF
- 1 SST tube (8 mL): Figg Lab
- 1 SST tube (4 mL): Daily starting day of chemotherapy. Send to Figg Lab.

5.4 Prior to Cell Infusion

- Baseline blood sample for cytokine analysis (one 8 mL SST): Figg 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 SB CPF lab on Monday, Wednesday and Friday x5, then weekly (while the patient is hospitalized):

- 5 CPT tubes (8 mL each): SB CPF
- 1 SST tube (8 mL): Figg Lab

5.6 Follow-up

- 5 CPT tubes (8 mL each): SB CPF

5.7 Immunological Testing

- 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.

- 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.

5.8 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR SURGERY BRANCH CELL PRODUCTION FACILITY (SB-CPF)

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB CPF. 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.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be as per the requirements in Section **7.2**.

5.9 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR FIGG LAB (BPC)

5.9.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the Labmatrix 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 Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix 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.).

5.9.2 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 Labmatrix . 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.

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 Labmatrix. 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.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be as per the requirements in Section **7.2**.

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).

5.10 ADDITIONAL RESEARCH EVALUATIONS

To determine predictors of renal toxicity and possible biodosimeters of radiation dose, the following samples will be collected, and then delivered to Dr. Deborah Citrin, M.D., Radiation Oncology Branch, NCI.

- For patients in **Arm 2** (receiving TBI)
  - Prior to the first dose of radiation (Day -3):
    - 3 tubes of blood consisting of 1 red top tube (4 mL), 1 purple top tube (6 mL), and 1 CPT tube (10 mL)
    - Urine specimen collected in blue cup (20-30 mL)
  - Prior to the second dose of radiation (Day -3, 6 hrs. after the 1st radiation dose)

        - 3 tubes of blood consisting of 1 red top tube (4 mL), 1 purple top tube (6 mL), and 1 CPT tube (10 mL)
    - Prior to the last dose of radiation on day -1
        - Urine specimen collected in blue cup (20-30 mL)
    - If renal toxicity occurs:
        - 1 tube of blood consisting of 1 purple top tube (6 mL),
        - Urine specimen collected in blue cup (20-30 mL)
    - Six months (± 1 month) following the last dose of treatment
        - 1 tube of blood consisting of 1 purple red tube (4 mL),
        - Urine specimen collected in blue cup (20-30 mL)
- For patients in **Arm 1** (not receiving TBI)
    - On Day -3
        - 3 tubes of blood consisting of 1 red top tube (4 mL), 1 purple top tube (6 mL), and 1 CPT tube (10 mL)
        - Urine specimen collected in blue cup (20-30 mL)
    - If renal toxicity occurs:
        - 1 tube of blood consisting of 1 purple top tube (6 mL),
        - Urine specimen collected in blue cup (20-30 mL)
    - Six months (± 1 month) following the last dose of treatment
        - 1 tube of blood consisting of 1 purple top tube (6 mL),
        - Urine specimen collected in blue cup (20-30 mL)

With approval of amendment C, to analyze metabolites of vitamin A in the serum, the following samples may be collected from patients treated on both arms of this study and sent to the Dept. of Nutritional Science and Toxicology, University of California at Berkley. One tube of blood (1 mL) may be collected in a 2.5 mL red top tube at the following times:

- On day -8
- Between day +10 and day +14
- At follow-up visits

In addition, to assess dendritic cell subsets, 4 tubes of blood (10 mL) may be collected in CPT tubes at the following times:

- On day -8
- Between day +10 and day +14
- At follow-up visits

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR 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. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. All events occurring during the treatment phase of the study will be followed until resolution to grade 2 or baseline. During the follow up period, only grade 3 and 4 and unexpected grade 2 events that are related the treatment will be captured in C3D. Patients will be followed for adverse events from registration through 30 days after the last treatment.

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 and FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.1.1 Exclusions to Routine Adverse Event Recording

Patients will be receiving multiple agents which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin, and supportive medications) in combination with the investigational agents. Therefore, all grade 1 and all expected grade 2 adverse events do not require reporting/recording.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

De-identified 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 de-identified 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 RECIST version 1.0.

6.3.1 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.

6.3.2 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

6.3.3 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. The time to progression will be measured from the date of randomization.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR

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."

CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

6.3.4 Confirmatory Measurement/Duration of Response

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.

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.

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.

6.3.5 Image Acquisition Plan

Images will be obtained digitally and reviewed by a radiologist in a blinded fashion for the determination of tumor response.

6.4 TOXICITY CRITERIA

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of the treatment regimens will be performed. This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the website http://ctep.cancer.gov. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0.

Over 100 patients have been treated in the Surgery Branch, NCI with TIL. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients. In 93 patients treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGy TBI) due to an unexpected but preexisting diverticular abscess.

The standard approach to the administration of high-dose aldesleukin in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long-term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

To ensure safety using this treatment, the NCI SB will review safety data on all protocols semi-annually at the time of continuing review. Data will be presented for both the recent 6 month period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

- All toxicities attributed to the cells,
- All incidences of intubation including the duration of and reason for intubation,
- All grade 2 unexpected adverse events, and all grade 3 or greater events regardless of attribution.

Toxicities seen on protocols using this non-myeloablative regimen and aldesleukin, that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine.

The major discomforts of the research are those of nausea and vomiting, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this regimen include:

- Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.
- Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.
- Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression, photosensitivity.
- TBI: Mucositis, infections, cataracts, pulmonary insufficiency, liver toxicity, thrombotic microangiopathy, and secondary late malignancies.
- High-dose aldesleukin administration: A listing of these side effects in 652 patients who received 1,039 treatment courses are listed in **Appendix 3**.

SAEs will be reported as per section **8.3**.

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found **here**.

7.2 OHSRP Office of Compliance and Training/IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-Compliance Human Subjects Research found **here**. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found **here**.

7.3 NIH Required Data and Safety Monitoring Plan

7.3.1 Principal Investigator/Research Team

The clinical research team will meet on a regular 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 immediately 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.

7.3.1 Data Safety Monitoring Board (DSMB)

This protocol requires monitoring by the NCI CCR Data Safety Monitoring Board (DSMB) as described in Section **10**. Interim outcome results will not be revealed to the investigators of the trial; results will be presented to the investigators prior to final accrual to the trial only if the DSMB recommends early termination of the trial. (**Note:** The study statistician is responsible for providing the description of how the monitoring will take place, including endpoints to be monitored and the frequency or timing of monitoring.)

8 SPONSOR PROTOCOL/ SAFETY MONITORING PLAN

8.1 Definitions

8.1.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease, temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 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 (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- A persistent or significant disability/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.

8.1.3 Life-Threatening

Any adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 3.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs

occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.4.1.1 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 no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.4.1.2 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.

8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.6 SPONSOR PROTOCOL DEVIATION REPORTING

Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

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.

10 STATISTICAL CONSIDERATIONS

The primary objectives of the trial are to compare in a prospective randomized study both the complete response rate and the overall survival of patients with metastatic melanoma receiving ACT using young TIL plus aldesleukin treatment following either a chemotherapy preparative regimen alone, or the same chemotherapy preparative regimen plus TBI.

The secondary objectives are to determine clinical response rate and progression free survival in patients in the two treatment arms, and to determine the toxicity of these two treatment regimens.

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. Complete regression rates in these three consecutive trials were 12%, 20%, and 40%, respectively— suggesting that the addition of TBI could be associated with a greater complete regression rate. Of the 20 complete regressions seen in this trial, 19 are on-going at 37 to 82 months.

A total of 112 evaluable patients (56 per arm) will be randomized to ACT following a chemo preparative regimen + 12 Gy TBI vs. ACT following a chemo preparative regimen alone. The patients will be stratified for M1a vs. M1b+M1c disease.

By enrolling 112 patients, the trial will have the following statistical characteristics.

As there are two primary endpoints, the study will have sufficient patients for each of the two main outcomes to be evaluated using a 0.025 alpha level two-tailed test in order to conservatively adjust for the effect of two endpoints while maintaining an overall 0.05 alpha level test. In practice, a Hochberg adjustment to the p-values may be performed, which is less overly stringent than the Bonferroni correction using 0.025 for each test.

With 56 patients per arm, there will be 80% power to detect a difference between an 8% complete response rate and a 33% complete response rate using a two-tailed Fisher's exact test at the 0.025 significance level. These rates were selected since each represents a modest decline from that identified using a more standard TIL regimen.

With respect to overall survival, it will be assumed that the arm without TBI will be associated with an 8-month median survival, since the overall survival using standard TIL without TBI was approximately 12 months based on 43 patients. This arm would have an associated hazard rate of 0.0866, or approximately a 9% death rate per month. The arm including TBI will be estimated to have a 16 month median survival time, based on the median survival of approximately 24 months using standard TIL. This arm would have an associated hazard rate of 0.0433, or approximately 4% death rate per month. Assuming a 36-month accrual period and an additional 24 months of follow-up to evaluate survival after the last patient has been enrolled, 56 patients per arm will have 88% power to detect this hazard ratio of 2.00 using a 0.025 two-tailed log rank test. The primary analysis with respect to this endpoint will thus be determined with Kaplan-Meier curves and a log-rank test.

Analysis will be done on an intent-to-treat basis; patients will be included in the primary analysis according to the arm on which they have been randomly assigned, whether they are able to receive the intended therapy or not.

Progression free survival will be estimated with a Kaplan-Meier curve and results between the curves compared with a log-rank test, as a secondary endpoint. The rates of clinical response (PR+CR) will also be estimated and compared as a secondary endpoint, with a two-tailed Fisher's exact test. Finally, toxicities will also be compared using a Cochran-Armitage test for those types of toxicities which are experienced by greater than 5 patients on each arm.

Although accrual is expected to be fairly rapid, and approximately 40 or more patients per year are expected to enroll on the trial, the study will be monitored by the NCI/CCR Data Safety and Monitoring Board on an annual basis to evaluate the toxicity on the two arms and be sure that the toxicity on the TBI arm is not appreciably worse than what is observed on the other arm. In addition, at the first DSMB meeting held following the point at which approximately one half of the required total subjects have been enrolled (56 total evaluable patients) and potentially followed for 6 months, a single evaluation for futility and for better than expected efficacy will be undertaken. Note that this may mean that approximately 75-80 patients may have been enrolled by the time that 56 have been potentially followed for 6 months.

The futility evaluation will be performed as follows: based on the overall survival percentage of the first 56 patients randomized and potentially followed for 6 months, a conditional power analysis will be performed to determine if the trial is unlikely to find an effect at the 0.025 two-tailed significance level with continued accrual. Using 40% and 60% as the projected probability values for 12 months overall survival for the patients who will be accrued on the two arms under the alternative, and using the actual observed proportions alive at 12 months from among those patients who are randomized and potentially followed for 6 months, the conditional power of the trial will be determined assuming accrual of the remaining patients to achieve a total of 112 patients. If the conditional probability of finding a difference at the 0.025 two-sided level at the conclusion of the trial is less than 10%, then it will be reasonable to recommend that no further patients will be enrolled.

The evaluation for better than expected efficacy will be performed as follows: an O'Brien-Fleming interim evaluation boundary will be used. If the p-value at the interim point (based on approximately 56 total patients enrolled and followed for 6 months) is <0.0054, then the trial will stop accrual for better than expected efficacy. By using this interim evaluation, the final significance level should be <0.0246 instead of 0.025 to declare the result to be significant at the 0.05 level.

It is expected that 40 to 50 patients per year can be accrued onto this trial. Allowing for a very small number of inevaluable patients, the accrual ceiling will be set at 118 patients. Thus, it is anticipated that the trial can complete its accrual in approximately 3 years.

11 COLLABORATIVE AGREEMENTS

With Amendment C, the NCI Surgery Branch will enter into an agreement (MTA #33284-11) with colleague Joseph L. Napoli, Ph.D., Professor and Chairman of Nutritional Science and Toxicology, University of California at Berkeley, to analyze metabolites of vitamin A, including all trans retinoic acid, retintyl ester, and retinol in serum samples from patients treated on both arms of this study. We are interested in analyzing metabolites of vitamin A since using a mouse model that closely mimics our current adoptive T cell protocols in patients, we found that host conditioning (irradiation) caused an acute vitamin A-deficient state and a loss of a vitamin A-

responsive dendritic cell subset. Moreover, we found that in this setting, replacement of the vitamin A-derivative (called ATRA) back to the animals profoundly enhanced the efficacy of the treatment and restored this dendritic cell subset. Our objective is to attempt to document whether a similar loss of vitamin A occurs in patients receiving either NMA conditioning or TBI. De-identified specimens will be analyzed and the results will be sent to the NCI Surgery Branch. The NCI Surgery Branch will release coded serum samples collected in association with this protocol to Dr. Joseph L. Napoli for analysis of these samples as specified in NCI Material Transfer Agreement # 33284-11. Specimens will be labeled with the study identifier only and will be shipped to the address below on dry ice.

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

12 HUMAN SUBJECTS PROTECTIONS

12.1 Rationale for Patient Selection

The patients to be entered in this protocol have metastatic melanoma which is refractory to standard therapy, and limited life expectancies.

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.

12.2 Participation of Children

The use of the nonmyeloablative and myeloablative 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.

12.3 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 **12.4**), all subjects ≥ age 18 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 for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 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.

12.4 Evaluation of Benefits and Risks

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. Although we have seen responses to this treatment, we do not know whether adding total body irradiation to the lymphodepleting chemotherapy regimen will result in a higher completer response rate or longer survival. 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. Side effect associated with TBI include mucositis, infections, cataracts, pulmonary insufficiency, liver toxicity and secondary late malignancies.

The risks in this treatment are further detailed in Section **6.4**. The goal is to compare survival and complete response rate in patients receiving adoptive cell therapy and aldesleukin with or without TBI. The success of this effort cannot be predicted at this time. Because all patients in this protocol have melanoma cancer and limited life expectancies the potential benefit of both arms is thought to outweigh the potential risks.

12.5 Consent Document

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.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, funding agency and the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer

Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 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 **Appendix 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**.

14.2 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 (2-8EC).

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.

14.3 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.

14.4 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.

14.5 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.

14.6 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 on the first Monday, Wednesday, or Friday on or after the first dose of chemotherapy 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.

14.6.1 Aerosolized Pentamidine in Place of TMP/SMX DS

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.

14.7 HERPES VIRUS PROPHYLAXIS

14.7.1 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 the day after the last dose of fludarabine 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.

14.7.2 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.

14.8 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.

14.9 CELL PREPARATION

The procedure for growing and expanding the autologous young CD8+ TIL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration and used at the NCI in protocol 07-C-0176. This product will be provided for investigational use only under a sponsor-investigator IND. The Certificate of Analysis is in **Appendix 1** and the Standard Operating Procedures for the growth of TIL and the purification of CD8+ cells are included in the IND.

14.10 OKT3

OKT3 will be obtained by the Surgery Branch Laboratory from commercial sources.

Formulation: Muromonab-CD3 (Ortho), NSC #618843, is provided as a sterile, clear, colorless solution at a concentration of 1 mg/mL in 5 mL ampoules. The solution may contain a few fine, translucent protein particles. The antibody is dissolved in a buffered solution at pH of 6.5 to 7.5. The solution contains 2.25 mg of monobasic sodium phosphate, 9 mg of dibasic sodium phosphate, 43 mg of sodium chloride and 1 mg of polysorbate 80 per 5 mL of water for injection.

Storage/Stability: Ampules should be stored in a refrigerator at 2-8°C. Solution should not be frozen or shaken. Each ampule bears an expiration date.

14.11 SUPPORT MEDICATIONS

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.

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.

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16 FIGURES, TABLES & APPENDICES

Table 1

Table 1. Characteristics of patients enrolled on lymphodepleting adoptive cell therapy protocols

		NMA		TBI-200		TBI-1200		TOTAL	
TOTAL	**Patients**	**43**	**(100%)**	**25**	**(100%)**	**25**	**(100%)**	**93**	**(100%)**
Sex	Male	26	(60%)	17	(68%)	18	(72%)	61	(66%)
	Female	17	(40%)	8	(32%)	7	(28%)	32	(34%)
Age	11 - 20	2	(5%)	0	(0%)	1	(4%)	3	(3%)
	21 - 30	3	(7%)	3	(12%)	2	(8%)	8	(9%)
	31 - 40	8	(19%)	5	(20%)	6	(24%)	19	(20%)
	41 - 50	16	(37%)	9	(36%)	4	(16%)	29	(31%)
	51 - 60	12	(28%)	8	(32%)	12	(48%)	32	(34%)
	61 - 70	2	(5%)	0	(0%)	0	(0%)	2	(2%)
ECOG	0	33	(77%)	20	(80%)	19	(76%)	72	(77%)
	1	10	(23%)	5	(20%)	6	(24%)	21	(23%)
Prior Rx	None	0	(0%)	0	(0%)	0	(0%)	0	(0%)
	Surgery	43	(100%)	25	(100%)	25	(100%)	93	(100%)
	Chemotherapy	22	(51%)	7	(28%)	5	(20%)	34	(37%)
	Radiotherapy	15	(35%)	6	(24%)	2	(8%)	23	(25%)
	Hormonal	1	(2%)	0	(0%)	1	(4%)	2	(2%)
	Immunotherapy	42	(98%)	24	(96%)	21	(84%)	87	(94%)
	Any 2 or more	43	(100%)	25	(100%)	22	(88%)	90	(97%)
	Any 3 or more	27	(63%)	9	(36%)	6	(24%)	42	(45%)

Table 2

Table 2: Transfusions and grade 3 and 4 non-hematologic toxicities associated with NMA plus TBI lymphodepleting preparative regimens.

	200cGy TBI	**1200cGy TBI**
Total patients	**25**	**25**
Transfusions administered (+SD)		
Platelets (6-10 units per transfusion)	3.8 (±3.4)	8.1 (± 4.4)
Packed RBCs	4.0 (±3.7)	6.2 (± 4.0)
Infection related toxicities		
CMV infection	1	1
Herpes zoster	1	2
Positive blood cultures	2	4
Other toxicities		
Intubated for somnolence	1	4
Pulmonary hypertension	1	0
Febrile neutropenia	12	16
Jugular venous thrombosis	1	0
Autoimmune uveitis and hearing loss (transient)	0	1
Thrombotic microangiopathy	0	4*
Death (bowel-perforation sepsis)	1	0

***Two of these patients have had biopsy evidence of microangiopathy and have stable renal function and are ongoing complete responders at 49+ months and 46+ months and are living normally. Two other patients were suspected to have this diagnosis. One is an ongoing complete responder at 52+ months and one had a partial response lasting 13 months but died of progressive metastatic melanoma.**

Table 3

Table 3: 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

Table 4

Table 4: Objective response rates in 3 ACT studies.

Cell Transfer Therapy (8/1/10)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (82+, 81+, 79+, 78+, 64+)	21 (49%)
200 TBI	25	8 (14, 9, 6, 6, 5, 4, 3, 3)	5 (68+, 64+, 60+, 57+, 54+)	13 (52%)
1200TBI	25	8 (21, 13, 7, 6, 6, 5, 3, 2)	10 (48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19)	18(72%)

(52 responding patients: 42 had prior IL-2; 22 had prior IL-2 + chemotherapy)
(20 complete responses: 19 ongoing at 37 to 82 months)

Table 5

Objective Responses in Patients with Metastatic Melanoma

	Total	CR	PR	OR
		number of patients (%)		
Dacarbazine[1]	149	4(2.7%)	14(9.4%)	18(12.1%)
Interleukin-2[2]	270	17(6.3%)	26(9.6%)	43(17.9%)
Ipilimumab[3]	540	3(0.6%)	35(6.4%)	38(7.0%)
PLX 4032[4]	48	3(6.3%)	34(70.8%)	37(77.1%)
Cell Transfer[5]	93	20(21.5%)	32(34.4%)	52(55.9%)

1) Middleton et al JCO, 18:158, 2000
2) Atkins et al JCO, 17:2105, 1999
3) Hodi et al NEJM, 363:711, 2010
4) Flaherty et al NEJM, 363:809, 2010
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Figure 1

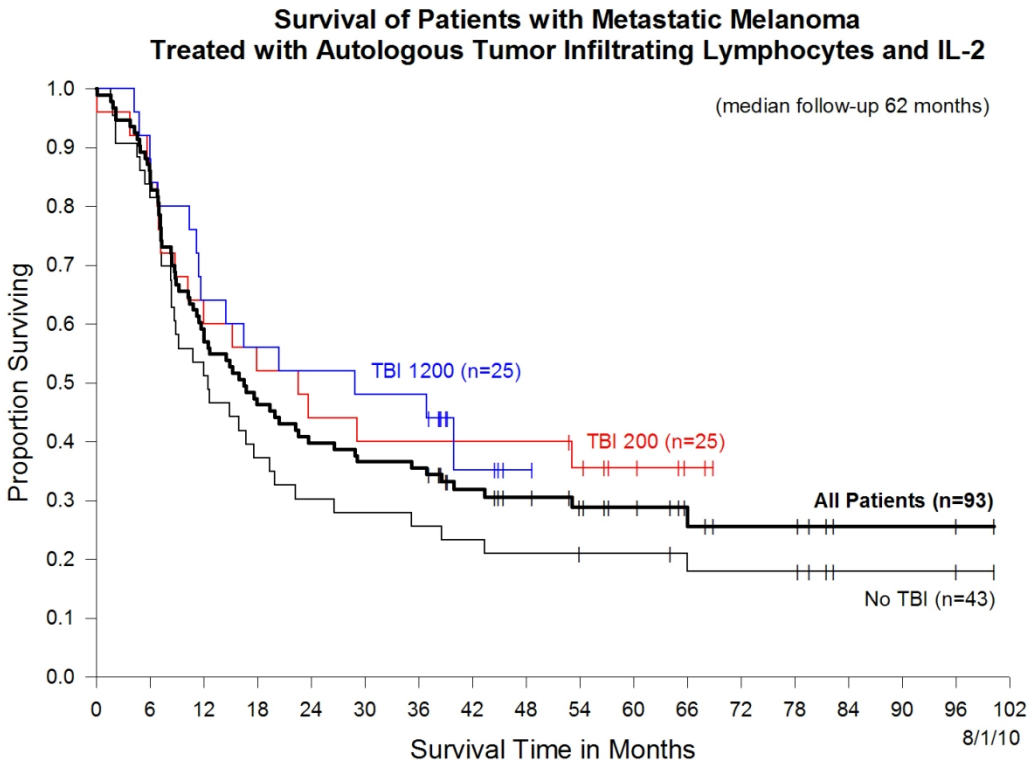
Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(median follow-up 62 months)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TBI 1200 (n=25)
TBI 200 (n=25)
All Patients (n=93)
No TBI (n=43)
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Survival Time in Months
8/1/10

Figure 2

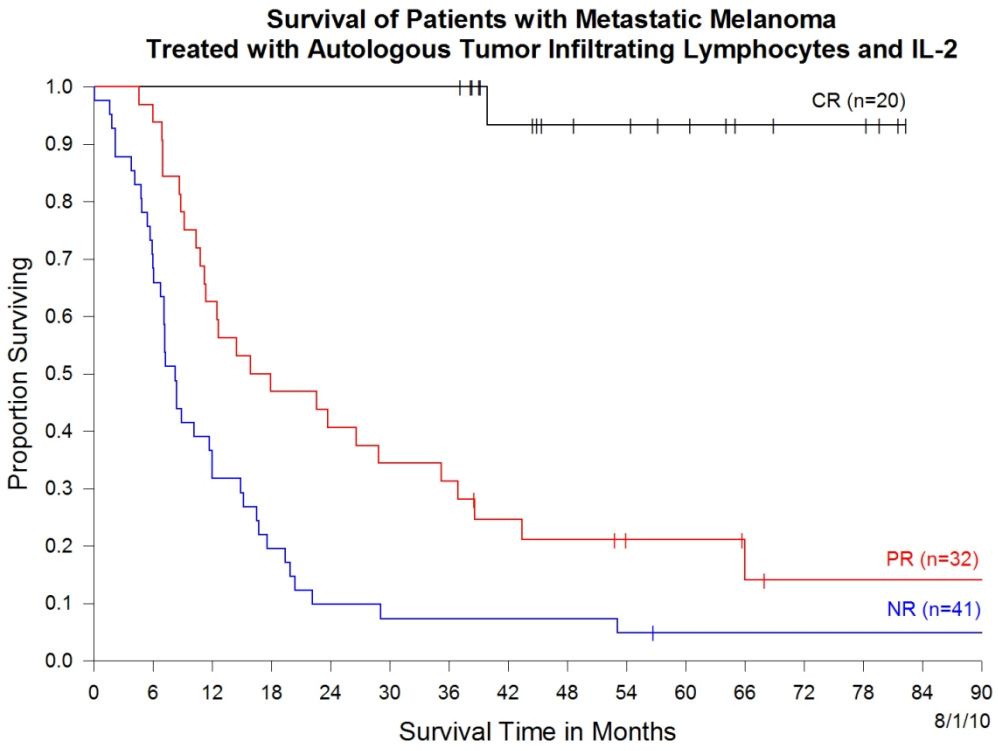
Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
CR (n=20)
PR (n=32)
NR (n=41)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
Survival Time in Months
8/1/10

Figure 3

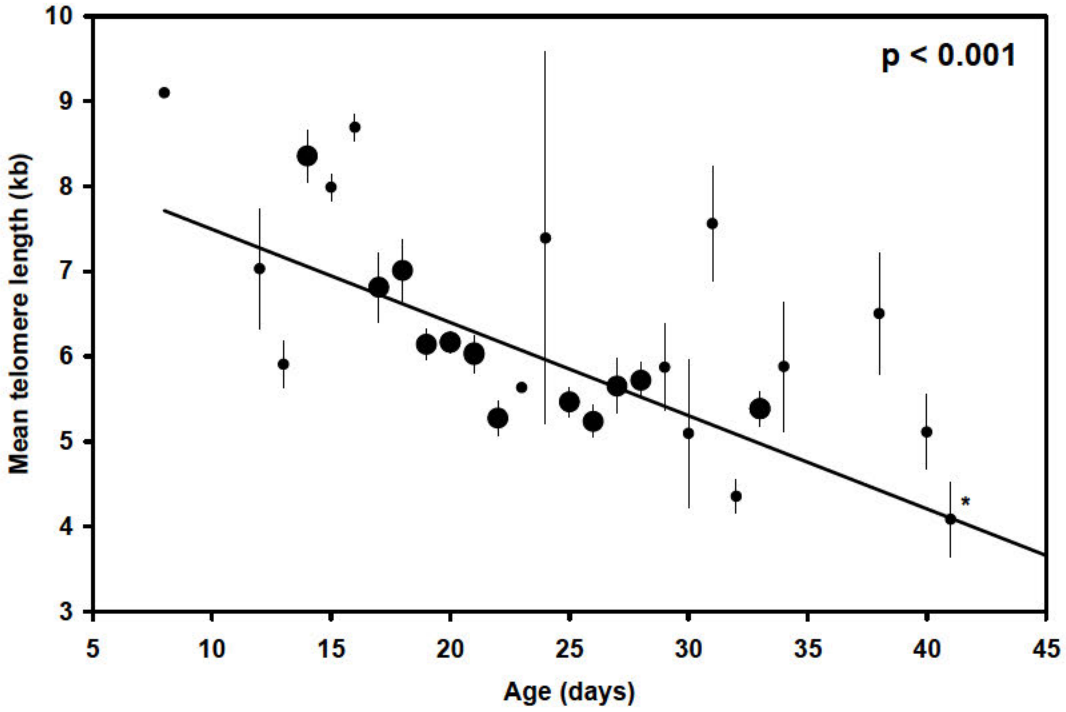

Figure 3. Telomere length decreases over time as TIL are maintained in culture. Telomere lengths of 495 TIL from 48 consecutive patients were evaluated by quantitative fluorescent in-situ hybridization. Mean telomere lengths calculated from 10 or more independent samples are indicated by large data points and from less than 10 samples are indicated by small data points. Error bars indicate standard errors. The trend line was calculated from all 495 individual data points. *Cells are from TIL 41 to 56 days old.

Figure 4

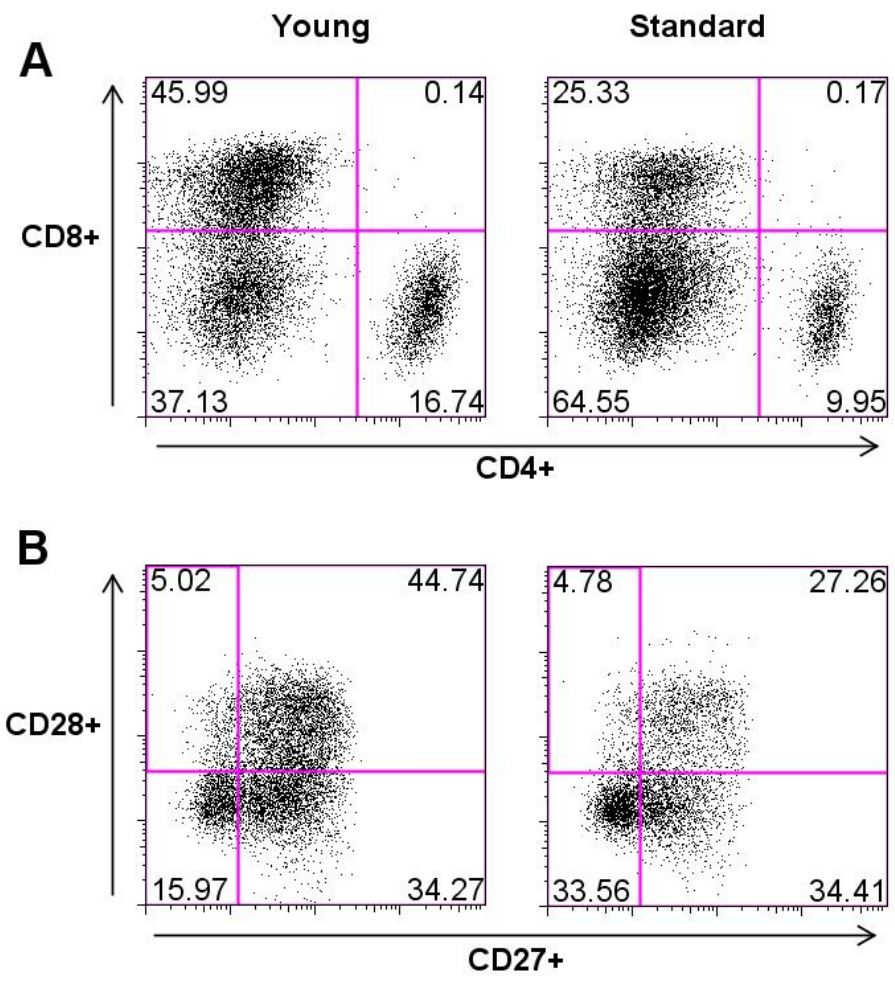

Figure 4: Expression of CD4, CD8, CD 27, and CD28 of TIL generated from one tumor specimen. Young TIL were 15 days old and standard TIL were 29 days old. Plots of CD27 and CD 28 expressions are gated for CD8+ lymphocytes.

Figure 5

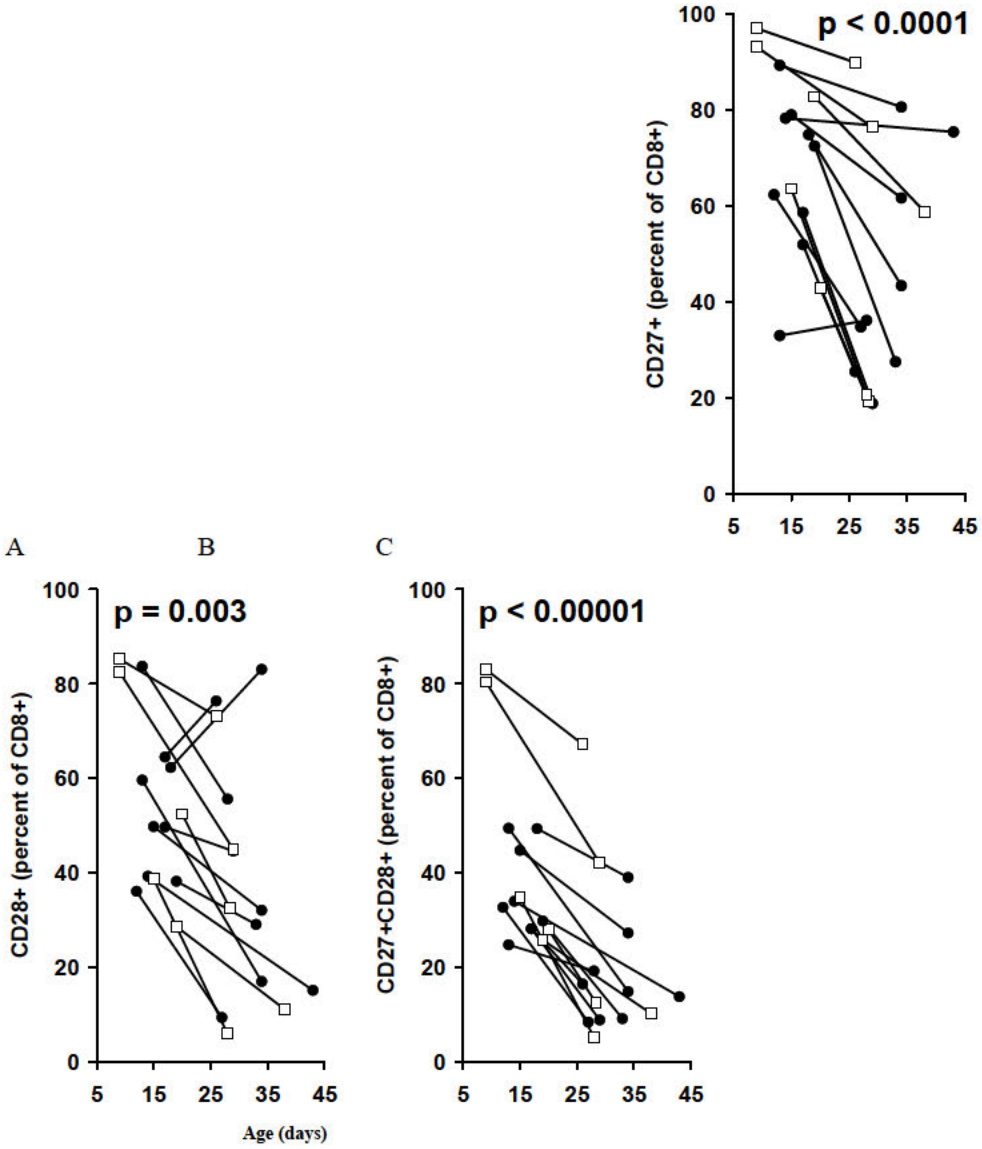

Figure 5. The phenotype of CD8+ lymphocytes changes over time as TIL are maintained in culture. Filled circles represent TIL generated from enzymatic digests of tumor and open square represent TIL from tumor fragments. CD8+ lymphocytes in young TIL (mean age of 15 days) express significantly more CD27 and CD28 than standard TIL (mean age of 31 days).

Appendix 1

Certificate of Analysis:

Young TIL

Patient:
Date of preparation of final product:
Unique TIL identifier (tumor and culture number):
Allogeneic PBMC
Donor Name(s):
Pheresis date:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*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: _____
James C. Yang, M.D. or designee

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.

Appendix 3

ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (n=525)[1]

Body System	% Patients	Body System	% Patients
Body as a Whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*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

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 supportive measures	No
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.