Amendment

Protocol Number: 13C0093-M **Reference Number:** 370214

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(NIH Employee Name, Institute/Branch, Telephone and e-mail)

Protocol Title: Phase II Study in Patients with Metastatic Ocular Melanoma Using a Non-Myeloablative Lymphocyte Depleting Regimen of Chemotherapy Followed by Infusion of Autologous Tumor-Infiltrating Lymphocytes with or without High Dose Aldesleukin

SIGNATURES

Principal Investigator (*):
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Branch Chief/CC Department Head (**):
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N/A

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N/A

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N/A

For Institute/Center Scientific Review Committee:
N/A

Other IC Clinical Director signatures:
N/A

APPROVALS

IRB Chair:
Michael Hamilton - applied signature on 06/30/2017 10:09 AM EDT

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N/A

CONCURRENCE

OPS Protocol Specialist:

Tonica Johnson	AM M	07/10/17
Signature	Print Name	Date

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: N/A

DEC Clearance Date: N/A

Protocol Version Date: 05/30/2017

Abbreviated Title: Ocular Melanoma TIL
CC Protocol Number: 13-C-0093 M
Version Date: 05/30/2017
NCT Number: NCT01814046

PROTOCOL TITLE

Phase II Study in Patients with Metastatic Ocular Melanoma Using a Non-Myeloablative Lymphocyte Depleting Regimen of Chemotherapy Followed by Infusion of Autologous Tumor-Infiltrating Lymphocytes with or without High Dose Aldesleukin

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Investigator Roles:

A. *Obtain information by intervening or interacting with living individuals for research purposes*
B. *Obtaining identifiable private information about living individuals*
C. *Obtaining the voluntary informed consent of individuals to be subjects*
D. *Makes decisions about subject eligibility*
E. *Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes*
F. *Studying, interpreting, or analyzing de-identified data or specimens for research purposes*
G. *Some/all research activities performed outside NIH*

Investigational Agents:

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

Commercial Agents: Cyclophosphamide, Fludarabine, and Aldesleukin

PRÉCIS

Background:

- Metastatic ocular melanoma (OM) carries a poor prognosis with estimated survival of 4-6 months. There are no known effective systemic therapies. Metastatic OM is classified as an "orphan" disease and there are currently few clinical trial options for these patients. Thus, novel systemic approaches are desperately needed.
- Administration of autologous tumor infiltrating lymphocytes (TIL) generated from resected metastatic cutaneous melanoma can induce objective long-term tumor responses.
- Minimally invasive, safe, and effective surgical approaches have been developed in the Surgery Branch to procure liver tumor tissue for TIL generation.

Objectives:

- To determine whether autologous Young TIL infused with or without the administration of high-dose aldesleukin may result in clinical tumor regression in patients with metastatic ocular melanoma receiving a non-myeloablative lymphoid depleting preparative regimen.

Eligibility:

- Patients with metastatic ocular melanoma who are ≥ 16 years of age, and are physically able to tolerate non-myeloablative chemotherapy. Patients who can tolerate high-dose aldesleukin will receive it following cell infusion; those who cannot tolerate high-dose aldesleukin due to medical comorbidities or refuse high-dose aldesleukin will receive cell infusion without aldesleukin.
- There is no requirement for prior systemic therapies, given the lack of known effective systemic treatments for metastatic OM.

Design:

- Patients will undergo biopsy or resection to obtain tumor for generation of autologous TIL cultures and autologous cancer cell lines.
- All patients will receive a non-myeloablative lymphocyte depleting preparative regimen of cyclophosphamide (60 mg/kg/day IV) on days -7 and -6 and fludarabine (25 mg/m2/day IV) on days -5 through -1.
- On day 0 patients will receive between $1x10^9$ to $2x10^{11}$ young TIL and then begin high dose aldesleukin (720,000 IU/kg IV every 8 hours for up to 15 doses) or no aldesleukin if they are not medically eligible to receive it.
- A complete evaluation of evaluable lesions will be conducted 4-6 weeks after the last dose of aldesleukin in the aldesleukin arm and 4-6 weeks after the cell administration in the no aldesleukin arm.
- Patients will be enrolled into two cohorts. The cohort receiving high-dose aldesleukin (cohort A) will be conducted using a small optimal two-stage Phase II design, initially 19 patients will be enrolled, and if 4 or more of the first 19 patients have a clinical response (PR or CR), accrual will continue to 33 patients, targeting a 35% goal for

objective response. For the cohort that will not receive aldesleukin (cohort B), the study will be conducted as a Minimax two-stage phase II trial. Initially 12 evaluable patients will be enrolled to this cohort, and if 1 or more the first 12 have a response, then accrual would continue until a total of 21 patients, targeting a 20% goal for objective response.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective:

- To determine whether autologous Young TIL infused with or without the administration of high-dose aldesleukin may result in clinical tumor regression in patients with metastatic ocular melanoma receiving a non-myeloablative lymphoid depleting preparative regimen.

1.1.2 Secondary Objective(s):

- To study immunologic correlates associated with Young TIL therapy for ocular melanoma.
- To determine the toxicity of this treatment regimen.

1.2 BACKGROUND AND RATIONALE:

This is a clinical protocol aimed at treating patients with metastatic ocular (uveal) melanoma (OM) with adoptive transfer of autologous young tumor infiltrating lymphocytes (young TIL). OM is the most common primary intraocular cancer in adults. Nearly half of primary uveal melanoma tumors will metastasize, but there are currently no effective therapies for metastatic OM. The potential of TIL to mediate complete and durable responses in metastatic cutaneous melanoma has been established by work from the Surgery Branch[1]. We are now seeking to determine if this therapeutic approach can mediate objective tumor regression in patients with metastatic OM.

1.2.1 Ocular (Uveal) melanoma (OM)

OM is the most frequent tumor of the eye. It arises from the pigmented uveal tract of the eye in 97% of the cases (choroid, ciliary body, iris) but can also rarely appear in the conjunctiva (3%). Its annual incidence is 5.1 per million and has remained stable over the past 30 years in the US[2]. Based on these statistics OM represents a "rare disease" as defined by The Rare Disease Act of 2002 (HR 4013) and the US Orphan Drug Act. Approximately half of patients diagnosed with primary OM will develop metastatic disease[3]. Although there are effective therapies to eradicate and prevent local recurrence of primary OM within the eye (radioplaque, proton beam, and enucleation), there are currently no effective therapies for metastatic uveal melanoma[3]. The most common sites of OM metastases metastasizes are liver (95%), lungs (24%), bone (16%), and skin (11%)[4]. The clinical course of patients with uveal melanoma is highly dependent on disease progression in the liver. The median survival after diagnosis of patients with liver metastases is approximately 4 to 6 months with a 1-year survival of approximately 10% to 15%[5].

Because the liver is the main site of metastases in up to 90% of the patients, efforts to develop loco-regional therapies for hepatic metastases, including surgical resection[6,7] chemo-embolization[8,9], immune-embolization[10,11], radioembolization[12], intra-arterial chemotherapy[13-15] and hepatic arterial perfusion[16,17] have been explored. Unfortunately, to date there is no clinical evidence that these loco-regional therapies improve overall survival for patients with metastatic OM.

The development of effective systemic therapies for patients with metastatic OM has similarly shown limited success. There are currently no standard systemic therapies for the treatment of metastatic OM. Various chemotherapy agents, alone or in combination, have been studied

without evidence of significant anti-tumor activity[18]. Promising small molecule inhibitors that have shown clinical responses in metastatic cutaneous melanoma[19,20] have not translated to the successful treatment of OM due to significant differences in the expression of common driver mutations (ie BRAF and NRAS) in OM[21]. Further recently approved immune modulating agents such as anti-CTLA-4 antibody have shown initial limited activity in patients with OM[22,23]. Given the poor prognosis and lack of effective therapies for patients with metastatic OM, novel effective systemic approaches are desperately needed.

1.2.2 Adoptive Cell Transfer experience at the Surgery Branch, NCI

The NCI-SB has pioneered novel T cell based cancer therapies for chemotherapy-refractory cancers and continues efforts to expand their application. This work has its foundation in the successful treatment of metastatic cutaneous melanoma with adoptive transfer of tumor infiltrating lymphocytes (TIL). We have reported the results of adoptive transfer therapy in 93 patients with metastatic melanoma who received TIL following a lymphodepleting regimen plus aldesleukin administration, with or without total body irradiation (**Figure 1**)[24]. Forty-three patients received a non-myeloablative chemotherapy consisting of 60 mg/kg cyclophosphamide q daily x 2 and 25mg/m^2 fludarabine q daily x 5 prior to cell transfer and aldesleukin administration. Twenty-five patients each also received the same chemotherapy agents in conjunction with either 200 or 1200 cGy total body irradiation (TBI) prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%. The clinical results in these three trials are shown in **Table 1**, 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 who had an undetected diverticular abscess prior to beginning therapy. Of the 52 responding patients in this trial, 42 had disease that was refractory to aldesleukin therapy and 22 had disease that was refractory to prior aldesleukin plus chemotherapy. Thus TIL therapy shows promise as an effective treatment for chemotherapy refractory metastatic cutaneous melanoma.

In the Surgery Branch, work is underway to extend TIL therapy to non-cutaneous melanoma cancers. In an ongoing protocol we are targeting digestive tract tumors with young TIL. Most of the toxicities observed in this study were expected toxicities of the non-myeloablative chemotherapy and the IL-2. TIL was grown successfully in 14 of 15 attempts from resected GI tumors (**Table 4**), demonstrating our ability to reliably generate a cell product from a variety of cancers. This work has been further supported by the successful generation of TIL from patients with cervical cancer (Table 5) in another ongoing clinical effort in the Surgery Branch. Based upon these findings, we believe that the generation of young TIL from OM metastases should be readily feasible with our current cell production methodologies.

A significant and unique challenge associated with this clinical trial is the ability to successfully obtain metastatic tumor tissue from patients with OM, given that the majority of patients will have only liver metastases. We recently reported on our series of cutaneous melanomas patients in which we performed minimally invasive laparoscopic liver metastasectomy to obtain tumor tissue for the generation of TIL. In this report we demonstrated the safety and feasibility of performing minimally invasive laparoscopic liver resection to obtain tumor tissue to generate TIL for therapy[25]. Median hospital stay was 3 days for patients undergoing laparoscopic liver resection. Objective tumor response was seen in 5 of 11 patients (45%) who received TIL procured in this manner, with one patient experiencing an ongoing complete response (32+

months). Thus, laparoscopic liver resection can be performed with minimal morbidity and serve as an effective means to procure tumor to generate therapeutic TIL for patients with metastatic cutaneous melanoma. We now aim to evaluate this therapeutic strategy in patients with metastatic OM.

In summary, we are uniquely qualified to procure tumor tissue from patients with ocular melanoma and to generate young TIL for experimental therapy. We believe that the current proposed clinical administration of TIL to patients with metastatic OM represents a novel and desperately needed treatment strategy for investigation in patients with this disease.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

a. Measurable metastatic ocular melanoma.
b. Confirmation of diagnosis of metastatic ocular melanoma by the Laboratory of Pathology of the NCI.
c. Patients with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for 1 month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.
d. Greater than or equal to 16 years of age and less than or equal to age 75
e. Able to understand and sign the Informed Consent Document
f. Willing to sign a durable power of attorney
g. Clinical performance status of ECOG 0 or 1
h. Life expectancy of greater than three months
i. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after receiving the treatment.
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, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.
k. Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.
l. Hematology
    - Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim
    - WBC $\geq 3000/mm^3$
    - Platelet count $\geq 100{,}000/mm^3$
    - Hemoglobin > 8.0 g/dl
m. Chemistry:
    - Serum ALT/AST $\leq$ to 3.5 times the upper limit of normal
    - Serum creatinine $\leq$ to 1.6 mg/dl

- Total bilirubin ≤ to 2.0 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

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

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**.

2.1.2 Exclusion Criteria

a. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.
b. 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.
c. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
d. Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).
e. Concurrent systemic steroid therapy.
f. History of severe immediate hypersensitivity reaction to any of the agents used in this study.
g. The following patients will be excluded from the high-dose aldesleukin arm (but may be eligible for cells alone arm):

- History of coronary revascularization or ischemic symptoms
- Documented LVEF of less than or equal to 45%. Testing is required in patients with:
  - Clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block
  - Age ≥ 60 years' old
- Clinically significant patient history which in the judgment of the Principal Investigator would compromise the patient's ability to tolerate aldesleukin

2.2 SCREENING EVALUATION

2.2.1 Within 4 weeks prior to starting the chemotherapy regimen:

a. Complete history physical examination, including weight, ECOG, and vital signs noting in detail the exact size and location of any lesions that exist and any allergies/sensitivities to antibiotics. (**Note**: patient history may be obtained within 8 weeks.)
b. Chest x-ray
c. EKG
d. Baseline imaging (CT, MRI, and/or PET) to evaluate the status of disease.
e. Patients with a prolonged history of cigarette smoking or symptoms of respiratory dysfunction will undergo Pulmonary Function Testing. Patients with an FEV1 of less

than or equal to 60% predicted for age will not be eligible to receive high-dose aldesleukin, but may be eligible to receive cells without aldesleukin (may be performed within 8 weeks of treatment). ***Note**: Pulmonary function testing will not be required for patients who are not candidates to receive high-dose aldesleukin due to medical judgment or patient refusal.*

f. Patients who are greater than or equal to age 60, or have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart block, will undergo cardiac evaluation (stress thallium, echocardiogram, MUGA etc.,). Patients with a LEVF of less than or equal to 45% will not be eligible to receive high-dose aldesleukin but, may be eligible to receive cells without aldesleukin (may be performed within 8 weeks of treatment). Patients under the age of 60 who have cardiac risk factors may also undergo cardiac evaluations as noted above (e.g., diabetes, hypertension, obesity) *Note: Cardiac evaluation will not be required for patients who are not candidates to receive high-dose aldesleukin due to medical judgment or patient refusal.*
g. HIV antibody titer and Hb_sAG determination, anti HCV (may be performed within 3 months of chemotherapy start date).
h. Anti CMV antibody titer, HSV serology, and EBV panel. (**Note**: Patients who are known to be positive for any of the above do not need to be retested - may be performed within 3 months of chemotherapy start date.)
i. Verification that HLA typing is completed (testing is permitted to be conducted at any time prior to this point)

2.2.2 Within 14 days prior to starting the chemotherapy regimen:

a. Baseline blood tests
    - Chemistries: (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)
    - Thyroid panel
    - CBC with differential and platelet count
    - PT/PTT
b. Urinalysis; urine culture, if indicated

2.2.3 Within 7 days prior to starting the chemotherapy regimen:

- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- ECOG performance status of 0 or 1

2.3 REGISTRATION PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to tumor resection for young TIL generation, by the clinical fellow or research nurse within 24 hours of the patient signing the consent and sending the completed Eligibility Checklist via encrypted email to: NCI Central Registration

Office (HOIS) ncicentralregistration-l@mail.nih.gov. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (COA), patients will sign the consent document for this protocol.

2.3.2 Registration Procedure

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

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The study will be conducted using a Phase II design. Young TIL will be generated from tumor fragments and/or digests according to standard operating procedure.

There will be two cohorts of patients depending on eligibility to receive high-dose aldesleukin (see 2.1.2 **g**)

- Cohort A - Patients who are eligible will receive high-dose aldesleukin following the cell product;
- Cohort B - Patients who are not eligible to receive high-dose aldesleukin will receive cells alone.

Each cohort will accrue independently of the other. Patients will receive no other experimental agents while on this protocol.

Patients will receive the standard NCI Surgery Branch non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed by IV infusion of Young TIL +/- aldesleukin. All patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation. Patients may undergo a second treatment as described in Section **3.4**.

3.1.1 Cell Preparation:

Patients with evaluable metastatic ocular melanoma who have lesions that can be resected with minimum morbidity will undergo resection of tumor. TIL will be obtained while enrolled on the Surgery Branch protocol 03-C-0277, "Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols". Separate tumor biopsies may be performed under protocol 03-C-0277 to obtain TIL if initial tumor biopsy could not successfully generate TIL. Young TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. Young TIL will be assessed for potency by interferon-gamma release as specified in the Certificate of Analysis shown in **Appendix 4**. Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the COA, the patient will be registered on this study and receive the lymphocyte depleting preparative regimen consisting of fludarabine and cyclophosphamide, followed by infusion of cells and administration of high-dose

aldesleukin. It is anticipated that Young TIL that meet the COA will not be achievable in approximately 20% of patients who undergo resection. These patients may undergo a second resection to grow Young TIL, if another suitable lesion exists.

3.1.2 Protocol Stopping Rules:

The study will be temporarily halted pending discussions with the FDA and NCI IRB regarding safety and the need for protocol revisions if any of the following conditions are met:

- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen or aldesleukin (or circumstances unrelated to the study).
- If one of the first three patients (or 2 of the first 6 patients, or 3 of the first 9 patients, or 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or *equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.*
- If one of the first four patients (or 2 of the first 6 patients, or 3 of the first 9 patients, or 4 of the first 12 patients) develops a grade 3 or greater non-hematologic toxicity due to the prior conditioning regimen, aldesleukin or the cell product that does not resolve in 72 hours, or experiences a toxicity requiring intubation, pressors or CVVH.
- If one or more treatment related deaths occur due to the cell infusion, we will promptly discuss this with the NCI IRB and FDA.

3.2 DRUG ADMINISTRATION

3.2.1 Preparative Regimen with Cyclophosphamide and Fludarabine:

(Times are offered as examples and may be changed as long as a similar time relationship between administration of the drugs is maintained. Study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. All other medications should be given +/- one hour of the scheduled time; the length of administration is all +/- 15 minutes. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated – the times noted below are offered only as examples. Chemotherapy infusions maybe slowed or delayed as medically indicated)

DAYS -7 and -6

6 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 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.

4 PM

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

5 PM

Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with Mesna 15 mg/kg/day X 2 days over 1 hr. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

6 PM

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section **11**) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

DAYS -5 to -1

Fludarabine 25 mg/m^2/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

3.2.2 Cell Infusion and Aldesleukin Administration:

The patient's Young TIL are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Cells will be administered at a dose of between 1x10^9 to 2x10^{11} lymphocytes over 20-30 minutes. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Aldesleukin Administration (Cohort A): Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15-minute period beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Dosing will be delayed or stopped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix 1**. Toxicities will be managed as outlined in **Appendix 2**. In addition, dosing may be held or stopped at the discretion of the treating investigator. (**Appendix 3** lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center[26]). Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

DAY 0 (one to four days after the last dose of fludarabine):

- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes (between one and four days after the last dose of fludarabine).
- Aldesleukin as described in Section **3.2.2**above (only for cohort A).

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

- Beginning on day 1 or 2, filgrastim may be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0 x10^9/L X 3 days or > 5.0 x10^9/L.
- Aldesleukin as described in section **3.2.2**above (only for cohort A).

3.2.3 Study Calendar

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

[1]One to four days after the last dose of fludarabine
[2]Initiate within approximately 24 hours after cell infusion
[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive 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

3.3 ON-STUDY EVALUATIONS:

3.3.1 Prior to starting the preparative regimen

- Apheresis as indicated
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, liver function tests, and TBNK. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.3.2 During the preparative regimen: DAILY

- Complete Blood Count
- Chemistries: 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 as needed.

3.3.3 After Cell Infusion:

- Vital signs will be monitored hourly x4 and then routinely (every 4-6 hours) unless otherwise clinically indicated
- Once total lymphocytes count is greater than 200/mm3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). **Refer to section 5 for additional post cell infusion evaluations.**

3.3.4 During Hospitalization:

Every 1-2 days

- A review of systems and physical exam as clinically indicated
- CBC
- Chemistries: 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
- Other tests will be performed as clinically indicated.

3.4 RETREATMENT

Patients experiencing a sustained stable disease, partial or complete response may receive a second treatment when progression by RECIST criteria is documented after evaluation by the principal investigator. Retreatment will consist of the same regimen that they had been given safely previously. Patients who develop grade 3 or grade 4 toxicity due to cell infusion will not be retreated. Patients must continue to meet the original eligibility criteria to be considered for retreatment. Toxicity related to cyclophosphamide, fludarabine, or aldesleukin should be stable and resolved to less than grade 1 prior to retreatment. Retreatment benefits and risks will be carefully explained to the patient. A maximum of 1 retreatment course may occur.

3.5 POST STUDY EVALUATION (FOLLOW-UP)

- All patients will return to the NIH Clinical Center for evaluation 6 weeks (+/- 2 weeks) following the administration of the cell product.

- Patients who have received multiple transfusions during the treatment phase or have been discharged with grade 3 or greater significant adverse events should be evaluated by the referring physician within 2 weeks of discharge and repeat labs drawn as appropriate to be faxed to the Research Nurse. Patients will receive appropriate treatment as determined by their treating physician.

- 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 2
  - As per PI discretion for subsequent years

  Note: Patients may be seen more frequently as clinically indicated

- At each scheduled evaluation patients will undergo:
  - Physical examination
  - Chemistries: 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
- TBNK, until CD4 > 200 x 2
- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis. 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. If the patient is unable to undergo apheresis, approximately 96 ml of blood may be obtained at the first follow up visit. Subsequently, 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 are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contacts. Patients may be asked to send laboratory, imaging and physician exam reports performed be their treating physician.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

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

3.6.1 Criteria for removal from protocol therapy

Patients will be taken off treatment (and followed until progression of disease) for the following:

- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.6.2 Off Study Criteria

Patients will be taken off study for the following:

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Progressive disease, unless the patient is eligible for a second treatment
- Death

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

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

3.6.3 Off Protocol Therapy and Off-study procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site **(**http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS:

Note: Appropriate anti-infective agents may be substituted at the discretion of the treating physician.

4.1.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/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 between days -5 and -8.

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 monthly thereafter.

4.1.2 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 every 12 hrs if the patient is not able to take medication by mouth. 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 Pneumocystitis 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.

4.1.3 Fungal Prophylaxis

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.

4.1.4 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/mm3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications

4.2 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 >10,000/mm^3. All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg g12h). If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

Correlative Studies for Research: 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.

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).

5.1 SAMPLES SENT TO DR. FIGG'S LAB

- Venous blood samples will be collected in either a 4ml or an 8ml SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube.
- For sample pickup, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.
- The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.2 SAMPLES SENT TO SURGERY BRANCH CELL PROCESSING LABORATORY

- Venous blood samples will be collected in 8ml CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube.
- Samples will be picked -up by the research nurse or designee and transported to the SB Cell Processing Laboratory.
- The samples will be processed, barcoded, and stored in SB Cell Processing Laboratory.

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

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

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

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

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

5.4.1 Sample Data Collection

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

a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

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

5.4.2 Sample Storage and Destruction

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

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

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

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

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

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

5.5 PRIOR TO CHEMOTHERAPY ADMINISTRATION

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

5.6 PRIOR TO CELL INFUSION

- Blood samples for cytokine analysis (one 8 ml tube) – Figg's lab

5.7 POST CELL INFUSION EVALUATIONS:

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

5.8 IMMUNOLOGICAL TESTING:

- Apheresis may be performed prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be cryopreserved for immunological monitoring of cell function.
- Lymphocytes will be tested directly and following in vitro culture using some or all of the following tests. Direct immunological monitoring will consist of quantifying T cells reactive with targets FACS analysis using tetramer staining. Ex vivo immunological assays will consist of cytokine release by bulk PBL (+/- peptide stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the TIL cryopreserved at the time of infusion. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences. Foxp3 levels will be analyzed by semiquantitative RT-PCR to evaluate for mRNA on PBL samples obtained prior to cell infusion and at the follow up time point.

Note: The collection and analysis of research labs will be monitored by the TIL lab and not by Harris.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal

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

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

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

6.1.1 Routine Adverse Event Reporting

Following registration through 30 days after cell infusion, adverse will be recorded in the source documents, reviewed by the designated data manager or research nurse or principle investigator and captured in Surgery Branch Immunotherapy database. 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.

6.1.2 Exclusions to Routine Adverse Event Reporting:

Patients will be receiving multiple agents which include commercially available agents (fludarabine, cyclophosphamide and supportive medications) in combination with the investigational agents. Therefore, Grade 2 adverse events 'unrelated' or 'unlikely related' to the investigational agent, and 'possibly', 'probably' or 'definitely' related to the commercially available agents as specified in the package inserts do not require reporting/recording. In addition, all grade 1 events and all expected grade 2 events unrelated to the cell product will not be reported/recorded.

6.1.3 Reporting of laboratory events

Laboratory results will be uploaded in C3D however only those events (including grade 3 and 4 events) that support the diagnosis of a reportable adverse event or that reflect major organ function will be considered adverse events. For example, grade 3 and 4, creatinine, liver function tests, hemoglobin, ANC, ALC, platelets, and lipase and amylase as indicated will be captured as adverse events; electrolytes, BUN, albumin, total protein, uric acid etc., and the remainder of the CBC differential will not be captured as adverse events.

For reportable adverse events: the adverse event start date will be the date the event reaches a grade 3; the event will be considered resolved once it reaches grade 2. The highest grade the event reaches in that period will be considered the grade of the event. For hematological toxicities, the event will not be considered resolved until it reaches grade 2 without the support of transfusions or growth factors.

6.1.4 Reporting of non-laboratory events

For reportable expected adverse events: the adverse event start date will be the date the event reaches a grade 3; the event will be considered resolved once it reaches grade 2. The highest grade the event reaches in that period will be considered the grade of the event.

For unexpected adverse events, the adverse event start date will be the date the event reaches a grade 2; the event will be considered resolved once it reaches grade 1 or baseline.

6.1.5 Reporting Infections

- Febrile neutropenia will be captured as follows: The start date will be the date the fever of 38.5 or greater was first recorded. The end date will be the date the patient has been afebrile greater than 48 hours or the date the patient develops a clinically significant infection.
- If a patient has a positive culture during the period of febrile neutropenia, the event will be captured as "infection with neutropenia" with the start date as the date the fever of 38.5 was first recorded.
- Infection will only be captured once in any given period regardless of the number of organisms cultured or sites involved.
- Positive cultures seen on routine surveillance cultures with no clinical symptoms will not be captured as infections regardless of whether anti-infective agents are given.

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

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

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[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."

6.3.3 Evaluation of best overall response

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

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

6.4 TOXICITY CRITERIA

This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE 3.0.

Over 150 patients have been treated in the Surgery Branch, NCI with tumor infiltrating lymphocytes. 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 cells can 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.

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 except those due to myelosuppression or IL-2.

Toxicities seen on protocols using this non-myeloablative regimen 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, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this nonmyeloablative 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 including sensory neuropathies and blindness, 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.

High-dose aldesleukin: a variety of side effects have been associated with high-dose aldesleukin administration. A listing of these side effects in 525 patients treated with high-dose aldesleukin is listed in **Appendix 1**.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section **6**.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2** and **7.3.**

An abnormal laboratory value will be considered an AE 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.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

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

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

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

7.1.5 Serious Adverse Event

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

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant 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.

7.1.6 Disability

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

7.1.7 Life-threatening adverse drug experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 Non-Compliance (NIH Definition)

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

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

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

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

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

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

- A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
- A summary of any instances of non-compliance.

A tabular summary of the following adverse events:

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

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

7.2.3 NCI-IRB Reporting of IND Safety Reports

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

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

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

7.3.1 Reporting Pregnancy

7.3.1.1 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. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

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

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

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

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

The same timelines apply when outcome information is available.

7.3.1.2 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.

7.4 DATA AND SAFETY MONITORING PLAN

7.4.1 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 reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's 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 an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

8 STATISTICAL CONSIDERATIONS

The primary objective of this pilot trial is to determine whether adoptive transfer of Young TIL will result in objective clinical responses. Patients may be enrolled on this protocol with insufficient cardiac or pulmonary capability to be able to receive high-dose aldesleukin, but in all other aspects are eligible for enrollment. These patients will be assigned to receive cells (PBL) alone. The results obtained, if suggestive of benefit, will provide direction for larger confirmatory studies to increase applicability of this potentially successful treatment modality.

For the cohort who will receive high dose aldesleukin, the study will be conducted as an optimal two-stage phase II trial (Simon R, Controlled Clinical Trials 10:1-10, 1989), in order to rule out an unacceptably low 15% clinical response rate (PR+CR; p0=0.15) in favor of a higher response rate of 35% (p1=0.35). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), the study will initially enroll 19 evaluable patients and if 0-3 of the 19 have a clinical response, then no further patients will be accrued. If 4 or more of the first 19 have a response, then accrual would continue until a total of 33 patients have enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 4-7 responses in 33 patients, this would be an uninterestingly low response rate, while if there were 8 or more responses in 33 patients, then this would be sufficiently interesting to warrant further study in

later trials. Under the null hypothesis (15% response rate), the probability of early termination is 68%.

For the cohort who will receive cells alone, the study will be conducted as a Minimax two-stage phase II trial (Simon R, Controlled Clinical Trials 10:1-10, 1989), in order to rule out an unacceptably low 5% clinical response rate (PR+CR; p0=0.05) in favor of a modest response rate of 20% (p1=0.20). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.20 (probability of rejecting a good treatment=0.20), the study will initially enroll 12 evaluable patients and if 0 of the 12 have a clinical response, then no further patients will be accrued in this category. If 1 or more the first 12 have a response, then accrual would continue until a total of 21 patients receiving cells alone have enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 1-2 responses in 21 patients, this would be an uninterestingly low response rate, while if there were 3 or more responses in 21 patients, then this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 54%.

If only the first stages of both cohorts are required to enroll patients, then a minimum of 31 patients will enroll. If both cohorts require accrual to a second stage, then accrual of up to 54 evaluable patients may be required. It is anticipated that up to 2 patients per month may be enrolled onto this trial, and thus approximately 3 years may be required to enroll the maximum number of required patients. To allow for the possibility of a small number of unevaluable patients, the accrual ceiling for the trial will be set at 57.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADA) with Lion Biotechnologies, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have metastatic or recurrent/refractory locally advanced ocular melanoma-associated cancer 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.

10.2 PARTICIPATION OF CHILDREN

The use of the nonmyeloablative 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 under the age of 16 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 younger pediatric population to evaluate potential benefit in that patient population.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **10.5**), all subjects ≥ 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.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though it is not known if it will do so. The risks of this treatment are detailed in section **6.4**. The success of this effort cannot be predicted at this time. Because all patients in this protocol have incurable metastatic or recurrent/refractory locally advanced ocular melanoma-associated cancers the potential benefit is thought to outweigh the risks.

10.5 RISKS/BENEFITS ANALYSIS

Because all patients in this protocol have metastatic or recurrent/refractory locally advanced ocular melanoma-associated cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

10.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Patients initially signs a consent when they agree to have TIL obtained for study and growth on protocol 03-C-0277, Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols. If the Young TIL can be generated for infusion and 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 as well as risks and potential benefits will be presented. The Informed Consent document is given to the patient, and the parents if applicable, who are 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 or the parents.

Informed consent of non-English speaking subjects:

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OSHRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

10.7 ADDITIONAL CONSIDERATIONS FOR PARTICIPATION OF SIXTEEN AND SEVENTEEN-YEAR-OLD PATIENTS

In addition to the specific requirements, risks and benefits noted above, any particular risks specific to this age group will be discussed with both the patient and their parents. The parents and the patient will be given the opportunity to ask questions; both parents will be asked to sign the informed consent document. Patients will be treated on 3NW.

11 PHARMACEUTICAL INFORMATION

Cyclophosphamide, fludarabine, and aldesleukin, the commercial drugs used in this study will not alter labelling of the FDA approved drugs. The investigation is not intended to support a new indication for use or any other significant changes to labeling or advertising in Cyclophosphamide, Fludarabine, or Aldesleukin. The investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug products.

11.1 INTERLEUKIN-2 (ALDESLEUKIN, PROLEUKIN, RECOMBINANT HUMAN INTERLEUKIN 2)

How Supplied: Interleukin-2 (aldesleukin) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, NSC #373364, 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 (2^0 - 8^0C) 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, 2^0 – 30^0C.

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 1** and **Appendix 2**. 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 1**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 2.**

11.2 FLUDARABINE:

(Please refer to package insert for complete product information)

Description: 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, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

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

Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.3 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 **Table 6**.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered,

patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

11.4 YOUNG TIL PREPARATION

The Certificate of Analysis is similar to those approved by the Food and Drug Administration and used in other Surgery Branch, NCI TIL clinical studies. The autologous Young TIL product will be provided for investigational use only under a sponsor-investigator IND. The Certificate of Analysis is in **Appendix 4** and the Standard Operating Procedures for the growth of the Young TIL are included in the IND. For all cohorts, cells will be administered at a dose of between $1x10^9$ to $2x10^{11}$ lymphocytes. Note: Penicillin, Streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

11.5 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 **Table 6**. Toxicities include nausea, vomiting and diarrhea.

11.6 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. G-CSF 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. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash,

myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

11.7 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 (MUST be on non-consecutive days) beginning on day -7 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.

11.8 AEROSOLIZED PENTAMIDINE IN PLACE OF TMP/SMX DS:

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

11.9 HERPES VIRUS PROPHYLAXIS:

11.9.1 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. Valcyclovir 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.

11.9.2 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. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

11.10 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.

Note: Appropriate anti-infective agents may be substituted at the discretion of the treating physician.

11.11 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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13 FIGURES, TABLES & APPENDICES

Table 1

Response rates and duration in patients with melanoma treated with tumor infiltrating lymphocytes plus high-dose aldesleukin following three different lymphoconditioning regimens.

Cell Transfer Therapy (3/1/11)

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 (88+, 86+, 85+, 82+, 71+)	21 (49%)
200 TBI	25	8 (14, 9, 6, 6, 5, 4, 3, 3)	5 (75+, 71+, 67+, 64+, 61+)	13 (52%)
1200 TBI	25	8 (21, 13, 7, 6, 6, 5, 3, 2)	10 (55+, 52+, 51+, 51+, 46+, 45+, 45+, 45+, 44+, 19)	18(72%)

(52 responding patients: 42 had prior IL-2; 22 had prior IL-2 + chemotherapy)
(20 complete responses: 19 ongoing at 44 to 88 months)

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

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

Patient characteristics and success at generating tumor infiltrating lymphocytes from non-cutaneous melanoma tumors (digestive tract cancers).

Patients		Age, gender	Prior chemo-therapy regimens	Metastatic adenocarcinoma (site of TIL harvest)	Technique for growing TIL	Successful first outgrowth	Total cell count, end of REP (x e9)
Preclinical studies							
1)	R. W.	63, M	2	Colon (liver)	GMACS, O/N digest	yes	47.5
2)	A. S.	44, F	3	Gastric (liver)	GMACS, O/N digest	yes	38.3
3)	T. H.	45, M	1	Colon (liver)	GMACS, O/N digest	yes	25.1
Clinical studies							
4)	M. L.	45, F	3	Colon (liver)	GMACS, O/N digest	yes	18.5
5)	D. A.	57, M	3	Biliary tract (omentum)	GMACS, O/N digest	yes	*no REP*
6)	J.F. L.	42, M	1	Colon (retro-peritoneum)	Fragments	yes	32.1
7)	J. M.	51, M	4	Rectal (lung)	Fragments, GMACS	yes	20.0
8)	M. M.	51, M	2	Colon (lung)	Fragments, GMACS	yes	*no REP*
9)	M. S.	53, F	2	Colon (abdominal wall)	Fragments, GMACS	no	-
11)	S.M.	57, F	3	Colon (liver)	Fragments, GMACS	yes	*no REP*
12)	J. R.	51, F	1	Colon (liver, omental nodule)	Fragments, GMACS	yes	30.3
13)	A. H.	52, F	6	Colon (lung)	Fragments, GMACS	yes	69.5
14)	B. D.	41, M	5	Colon (Axillary node)	Fragments, GMACS	yes	*no REP*
15)	M. W.	37, F	2	Colon (liver)	Fragments, GMACS	yes	75.0

Table 5

Characteristics of tumor infiltrating lymphocytes grown from non-cutaneous melanoma tumors (cervical cancer)

					Frequency of cells in the lymphocyte gate				
Patient	Prior therapy	Growth positive wells/total wells[1]	Autologous tumor reactivity[2]	Fragments for REP	CD3+	CD4+	CD8+	CD3-CD56+	REP fold expansion
1	none	8/24	n/a	F1	97	73	24	3	1216
				F2	96	9	85	2	1110
				F3	79	35	38	18	1326
				F4	62	9	93	6	1890
2	chemo/RT	5/24	positive	F1	80	10	42	19	344
				F2	78	31	36	19	1752
				F3	86	70	10	11	1224
				F4	78	8	71	21	816
3	none	17/26	negative	F1	99	75	24	0	2226
				F2	99	15	84	1	1632
				F3	93	35	32	3	2240
				F4	99	90	7	0	[3]
4	none	2/19	n/a	F1	95	69	8	3	2737
				F2	95	11	84	5	1968
5	none	13/13	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[1] Growth positive defined as lymphocyte confluence in 4 wells of a 24-well plate, approximately $10e^6$ cells.

[2]Autologous tumor reactivity was determined by IFN-γ production in overnight coculture with cryopreserved autologous tumor targets. Positive recognition was defined as two times the negative control and greater than 200 pg/mL of IFN-γ.

[3]Pooled with F3 for the REP.

n/a = not tested

Table 6

Modification of Dose Calculations* in patients whose BMI is greater than 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

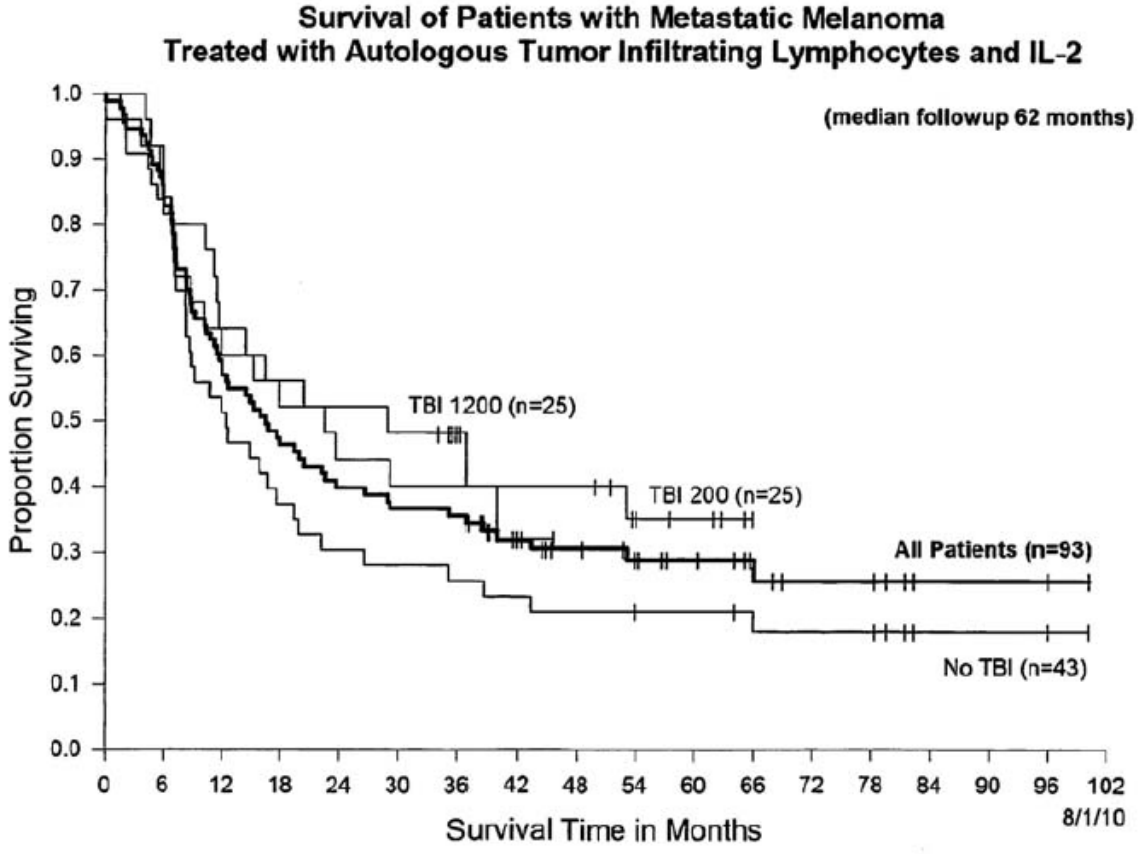
   45.5 + 2.3 (3) = 57 kg

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.

Figure 1

Survival of patients with metastatic melanoma treated with autologous tumor infiltration lymphocytes and IL-2 following three different lymphoconditioning regimens.



Appendix 1

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 2

Expected IL-2 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 pr, 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	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
Hyperbilirubinemia	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 treatment.

Appendix 3

Interleukin-2 toxicities observed in patients treated at the NIH Clinical Center

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

Appendix 4

Certificate of Analysis:

Young TIL from Ocular Melanoma
Patient:
Date of preparation of final product:
Unique TIL identifier (tumor and culture number):

Tests performed on final product:

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

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

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

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

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

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

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____

Qualified Laboratory or Clinical Supervisor