Amendment

Protocol Number: 12C0116-K **Reference Number:** 359203

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Protocol Title: A Phase II Study of Lymphodepletion followed by Autologous Tumor-Infiltrating Lymphocytes and High-Dose Aldesleukin for Human Papillomavirus-Associated Cancers

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CONCURRENCE

OPS Protocol Specialist:

Signature	Print Name	Date
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* 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: Expedited

DEC Clearance Date: NA

Protocol Version Date: 09/08/2016

NCI Protocol Number: 12-C-0116 K
Version Date: 09/08/2016

PROTOCOL TITLE

A Phase II Study of Lymphodepletion followed by Autologous Tumor-Infiltrating Lymphocytes and High-Dose Aldesleukin for Human Papillomavirus-Associated Cancers

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Chief Medical Officer
Lion Biotechnologies, Inc.

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

Investigational Agents:

Drug Name:	Young TIL
IND Number:	IND 14355
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Processing Facility

Commercial Agents: Cyclophosphamide, Fludarabine, and Aldesleukin

PRECIS

Background:

- Metastatic or locally advanced refractory/recurrent human papillomavirus (HPV)-associated malignancies (cervical, vulvar, vaginal, penile, anal, and oropharyngeal) are incurable and poorly palliated by standard therapies.
- Administration of autologous tumor infiltrating lymphocytes (TIL) generated from resected metastatic melanoma can induce objective long-term tumor responses.
- Young TIL can be generated from HPV-associated tumors.

Objectives:

- To determine if autologous Young TIL infused in conjunction with high dose aldesleukin following a non-myeloablative lymphodepleting preparative regimen can mediate tumor regression in patients with metastatic or locally advanced refractory/recurrent HPV-associated cancer.
- To study immunologic correlates associated with Young TIL therapy for HPV-associated cancers.
- To determine the toxicity of this treatment regimen.

Eligibility:

- Patients greater than or equal to 18 years old with a pathologically confirmed diagnosis of metastatic or locally advanced refractory/recurrent HPV-16+ or HPV-18+ human papillomavirus-associated cancer.

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/m^2/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).
- Clinical and immunologic response will be evaluated about 4-6 weeks after TIL infusion.
- Initially, 18 evaluable patients will be enrolled in two cohorts; patients with cervical cancer and those with non cervical cancer For each cohort, if 0 to 2 of the 18 patients experience a clinical response, then no further patients will be enrolled. If 3 or more of the first 18 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 35 evaluable patients have been enrolled in each cohort. Up to 73 patients may be enrolled over approximately 3-4 years.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. STUDY OBJECTIVES

1.1.1. Primary Objective:

- To determine the objective tumor response rate and duration in patients with metastatic or recurrent/refractory locally advanced HPV-associated cancers who receive autologous Young TIL plus aldesleukin following a lymphodepleting preparative regimen.

1.1.2. Secondary Objective(s):

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

1.2. BACKGROUND AND RATIONALE:

This is a clinical protocol to treat HPV-associated cancers with adoptive transfer of autologous young tumor infiltrating lymphocytes (young TIL). This approach seeks to direct the potent immunotherapy of TIL adoptive transfer at the attractive immunologic target of HPV-associated cancers. The potential of TIL to mediate complete and durable responses in metastatic melanoma has been established by work from the Surgery Branch[1]. We are now seeking to expand the application of young TIL to HPV-positive cancers, which are particularly appealing candidates for TIL therapy because *i)* better treatments are desperately needed, especially for patients that have previously received chemotherapy, *ii)* these cancers express the viral antigens E6 and E7, which are good immunologic targets and are important for malignant cell function and survival, *iii)* the presence of known tumor antigens permits preferential transfer of tumor specific T cells and enables novel scientific studies that can inform future clinical trials.

1.2.1. HPV-associated malignancies

HPV-associated cancers, particularly cervical cancer, are a major international health concern, but also remain problematic in the United States. In the United states annually, there are greater than 19,000 new cases of HPV-associated cancer and approximately 11,000 deaths from cancer at HPV-associated sites, which include cervix, vagina, vulva, anus, penis, and oropharynx (Table 1)[2-5]. Metastatic or recurrent/refractory locally advanced malignancies at these sites are incurable and difficult to palliate. Responses to chemotherapy (platinum-based) are variable but generally short-lived with median progression-free survival (PFS) around only 3 to 6 months[6-8]. In a recent Gynecologic Oncology Group randomized trial comparing four cisplatin-based doublets as first line therapy for cervical cancer the response rates were only 22 to 29 percent[9]. Median PFS was four to six months and median overall survival (OS) was 10 to 13 months[9]. Randomized trials of second line therapy are lacking but response rates for single agents are poor at around 3 to 12 percent[10]. For oropharyngeal cancer, HPV positive tumors have improved prognosis in patients receiving chemoradiation as first line treatment of the primary tumor; however the effect of HPV status on outcomes of metastatic or

recurrent/refractory disease is unknown. Furthermore HPV positive tumors have not been studied as distinct entities in trials of systemic therapy. The best estimates of the chemotherapy responsiveness of these tumors probably comes from looking at the oropharyngeal site (an HPV associated site) in subset analyses of broader head and neck cancer clinical trials. In a pivotal clinical trial that established platinum, 5-flurouracil, plus cetuximab as first line therapy in head and neck cancer, patients with oropharyngeal tumors experienced PFS of four to six months and OS of eight to 11 months[7]. As with cervical cancer, randomized trials of second line therapy following platinum treatment failure are lacking. A phase II trial of cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) reported a response rate of 13 percent with time to progression of 70 days; cetuximab is now considered to represent the most promising second line therapy for this disease[11]. Thus, second line therapies for HPV-associated tumors have low response rates and poor response duration, and novel treatments must be developed.

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 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)[12]. 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 2, and the toxicities shown in Table 3 for the TBI studies (04-C-0288, 06-C-0136) and Table 4 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 melanoma.

HPV-associated cancers are rational targets for adoptive immunotherapy with TIL because *i)* they express E6 and E7 oncoproteins which are non-self antigens and therefore good immunologic targets, *ii)* E6 and E7 are not only specific to tumor cells but important to their survival, *iii)* better clinical cancer outcomes correlate with T cell reactivity against E6 and E7, *iv*) polyclonal responses against E6 and E7 have been identified in TIL, and *v*) E6 and E7 have been successfully targeted by vaccine therapy in premalignant neoplasia. E6 and E7 are small (18 and 13 kDa respectively) oncoproteins that cooperate to induce malignant transformation of cells infected with high-risk HPV

types. These proteins are particularly attractive therapeutic targets because they continue to be expressed after transformation and their expression is required for survival of the transformed cells. The importance of ongoing E6 and E7 expression for cell survival has been demonstrated by studies showing that RNA silencing or expression of HPV E2 (which suppresses E6 and E7 transcription) causes cellular senescence of HPV-positive tumor lines[13-16]. Expression of E7 is routinely detected by immunohistochemistry for p16 (CDKN2A, p16Ink4A), which is increased by E7-mediated induction of KDM6B, a histone 3 lysine 27-specific demethylase[17].The clinical importance of immune responses against E6 and E7 is suggested by studies linking immune responses against E6 and E7 to improved clinical outcomes [18-23] (Table 5). A number of studies indicated that E6 and E7 could be targeted with TIL therapy [24,25]. In one study, reactivity against the E6 and E7 antigens could be detected in 23 of 54 TIL and tumor draining lymph node specimens from HPV16- and HPV18-positive cervical neoplastic lesions (Table 6)[25]. TIL could be generated from both adenocarcinomas and squamous cell carcionomas, and TIL cultures grew to a mean of 68 percent CD3-positive cells at three weeks, with a mix of CD4- and CD8-positive cells present ($CD3^+CD4^+$ 38% +/- 21 %, $CD3^+CD8^+$ 48% +/- 24%). Reactivity against HPV was determined by examining T cell proliferation in response to autologous monocytes pulsed with full length proteins as well as pools of long overlapping peptides spanning E6 and E7 of HPV16 and HPV18. In TIL samples from 19 of 51 patients HPV E6- and/or E7-specific T cells were detected. The HPV reactive cells were both CD4- and CD8-positive and in several cases responded to more than one E6 or E7 peptide, a finding consistent with other work showing a polyclonal TIL response against multiple epitopes[24]. MHC restriction analysis revealed frequent use of DP and DQ HLA molecules (13 of the 16 class II restricted TIL), although DR and class I restricted responses were also present. Thus, TIL from cervical tumors display diverse polyclonal reactivity against E6 and E7. While TIL from HPV associated cancers have not been tested in clinical trials, E6 and E7 have been targeted with therapeutic vaccines. In a trial of long-peptide vaccination for vulvar intraepithelial neoplasia (VIN)[26], an HPV-induced premalignant condition, 15 of 19 patients had clinical responses to vaccine therapy, and 9 of 19 had complete responses all of which were maintained at 24 months of follow up. Therapeutic vaccines for advanced HPV-associated cancers, as for melanoma, have been universally unsuccessful[27]. However, TIL therapy has been highly successful in treating melanoma, and the existing data indicate that HPV-associated cancers may also be excellent targets for TIL therapy.

In the Surgery Branch, work is underway to extend TIL therapy to non-melanoma cancers. In an ongoing protocol we are targeting digestive tract tumors with young TIL. We have treated five patients thus far; two patients were taken off study due to progressive disease, one patient was treated too recently to evaluate response, and two patients died on study and were not evaluable for clinical response. 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 7), demonstrating our ability to generate reliably a cell product from a variety of cancers. We have also successfully generated TIL from five of five cervical cancers (Table 8). These TIL cultures displayed high frequencies of CD3-positive cells, and they varied in CD4 and CD8 composition (Table 8). Both CD4- and CD8-predominant fragments displayed excellent growth in rapid expansion protocol (REP) expansions

(Table 8). Testing for HPV-specific reactivity was limited by lack of samples of peripheral blood mononuclear cells for use in recognition assays; however autologous tumor recognition was present in one of two samples that could be tested. As additional tissue becomes available through this protocol, we hope to develop tests for E6 and E7 reactivity. Such testing could be valuable in selecting the optimal TIL to expand for therapy. In conclusion, HPV-associated tumors are immunogenic and give rise to TIL targeting viral oncoproteins that are critical to malignant cells and that have been successfully targeted with cancer vaccine therapy. They are therefore promising targets for TIL therapy and a clinical trial of adoptive immunotherapy with TIL is warranted.

Studies of the first nine patients treated in the cervical cancer cohort of this protocol revealed a statistically significant positive association between the HPV reactivity of the infusion product and response to therapy. The three patients who received cells with the greatest HPV reactivity experienced clinical responses. Five patients with low or undetectable reactivity did not respond to treatment. We therefore are introducing Amendment I to the protocol, which will require testing for the presence of HPV E6 or E7 reactivity in the cell product after the initial TIL outgrowth. If reactivity is low or undetectable, a final cell product will not be generated and patients will not be treated. This change is intended to spare patients with a low probability of benefiting from the treatment the toxicities of the therapy.

2. ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

a. Measurable metastatic or locally advanced refractory/recurrent malignancies that are HPV-16 or HPV-18 HPV positive by in situ hybridization (ISH) or polymerase chain reaction (PCR) or any cancer from the uterine cervix..
b. All patients must have received at least one standard chemotherapy or chemoradiotherapy.
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.
d. Greater than or equal to 18 years of age and less than or equal to age 70.
e. Able to understand and sign the Informed Consent Document
f. Clinical performance status of ECOG 0 or 1.
g. Life expectancy of greater than three months
h. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after receiving treatment.
i. Serology:
    - Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus 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.

j. Women of child bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.
k. Hematology
   - Absolute neutrophil count greater than 1000/mm^3 without the support of filgrastim
   - WBC ≥ 3000/mm^3
   - Platelet count ≥ 100,000/mm^3
   - Hemoglobin > 8.0 g/dl
l. Chemistry:
   - Serum ALT/AST ≤ to 2.5 times the upper limit of normal
   - Serum creatinine ≤ to 1.6 mg/dl
   - Total bilirubin ≤ to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.
m. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo). **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.
n. More than four weeks must have elapsed since any prior radiation therapy.

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. History of coronary revascularization or ischemic symptoms.
h. Any patient known to have an LVEF less than or equal to 45%.
i. Documented LVEF of less than or equal to 45% tested in patients with *i*) clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial

fibrillation, ventricular tachycardia, second or third degree heart block or *ii*) age ≥ 60 years old.

j. Active Bleeding

2.2. SCREENING EVALUATION

2.2.1. Within 4 weeks prior to starting the chemotherapy regimen:

a. Complete history and physical examination, including weight, and vital signs noting in detail the exact size and location of any lesions that exist. (Note: patient history may be obtained within 8 weeks.)
b. Chest x-ray
c. EKG
d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms. (Note: brain MRI may be obtained within 8 weeks prior to starting the chemotherapy regimen.)
e. Pulmonary Function testing for patients with a prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years) or symptoms of respiratory dysfunction. (Note: may be performed within 8 weeks of treatment).
f. Cardiac Evaluation (stress thallium, echocardiogram, MUGA etc.) for 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, or heart block. Patients with a LVEF of less than or equal to 45% will not be eligible. Patients under the age of 60 who have cardiac risk factors may also undergo cardiac evaluation as noted above (e.g. diabetes, hypertension, obesity). (Note: may be performed within 8 weeks of treatment).
g. HIV antibody titer and HbsAG determination, anti HCV, (Note: 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. Medical history (may be conducted at any point prior to this time).
j. Verification that HLA typing is completed or in process. Testing may 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
   - Chem 20 equivalent: (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 and culture, if indicated

2.2.3. Within 7 days prior to starting the chemotherapy regimen:

a. β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
b. ECOG performance status of 0 or 1
c. Calculated creatinine clearance for patients with renal impairment
Please note: Patients with hydronephrosis or other renal impairment may be required to undergo additional testing in order to ensure adequate renal function, (e.g. renal ultrasound, renal scan).

2.3. REGISTRATION PROCEDURES

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 by faxing a completed Eligibility Checklist to the Central Registration Office (CRO) at 301-480-0757. 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. 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 faxed to 301-480-0757. 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. Patients will be stratified according to whether they have cervical or non-cervical cancer.

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. Assays for identification of E6- and E7-reactive cells will be developed and used to preferentially select wells of TIL for rapid expansion and administration. Patients whose TIL cultures display low or undetectable HPV E6 and E7 reactivity after initial outgrowth will not be treated. Patients will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine that has been used in prior Surgery Branch trials. The conditioning regimen will be followed by intravenous infusion of Young TIL plus aldesleukin.

3.1.1 Cell Preparation:

Patients with evaluable metastatic or locally advanced refractory/recurrent HPV-associated malignancies 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. After initial outgrowth, lymphocyte cultures will be tested for reactivity against HPV E6 and E7 as described in TIL Laboratory SOP 4.4, "Screening tumor infiltrating lymphocytes (TIL) from patients enrolled on the Human Papillomavirus (HPV) TIL clinical protocol (12-C-0116) for reactivity against HPV antigens". If three or more cultures demonstrate HPV E6 or E7 reactivity then production of the cell product will proceed. If two or fewer cultures show HPV E6 or E7 reactivity then production of the cell product will be terminated and the patient will not be treated. A positive test for HPV reactivity will be defined as interferon-γ production greater than or equal to twice the negative control (gp100) and greater than or equal to 200 pg/mL. 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. Cells will be administered at a dose of between $1x10^9$ to $2x10^{11}$ lymphocytes over 20-30 minutes. 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.

3.1.2 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 4 or greater toxicity at any point in the study only attributable to the cell infusion.
- 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 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. Chemotherapy infusions maybe slowed or delayed as medically indicated. 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.)

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 9.

6 PM

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) 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 9.

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 9.

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. 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.

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).
- Aldeskeukin will be given as described in section 3.2.3.

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 will be given as described in section 3.2.3.

Table 3.2

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 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

3.2.3 Aldesleukin Administration

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 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. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered by supportive measures within 24 hours of the last dose of aldesleukin. 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. 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).

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

3.3 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, and liver function tests. 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
- Chem 20 equivalent: 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.

3.3.3 After Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). **Please refer to section 5 for additional post cell infusion evaluations.**

3.3.4 During Hospitalization:

Every 1-2 days

- A review of systems and physical exam
- CBC
- Chem 20 equivalent: 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 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 aldeskeukin 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)

3.5.1 All patients will return to the NIH Clinical Center for evaluation 6 weeks (+/- 2 weeks) following the administration of the cell product. **Please note:** Patients discharged with grade 3 or greater significant adverse events should be evaluated by referring physician within 2 weeks of discharge.

3.5.2 Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Every month (+/- 2 weeks) x3
- 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

3.5.3 At each evaluation patients will undergo:

- Physical examination
- Chem 20 equivalent: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- Complete blood count
- Thyroid panel as clinically indicated
- TBNK, until CD4 > 200 X 2
- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis. This end of course evaluation will be used to determine tumor response. 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 aphersis, approximately 96 ml of blood may be obtained at the first follow up visit. Subsequently, approximately 60 ml of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed.

3.5.4 Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or email contacts. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.6.1 OFF TREATMENT CRITERIA

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

- Grade 3 autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a patient experiences a grade 3 or 4 toxicity due to cell infusion (reaction to cellular product or infusion reaction) the patient will receive no further treatment.
- Completion of treatment period

3.6.2 OFF STUDY CRITERIA

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant patient noncompliance
- Radiographic or clinical disease progression, unless the patient is eligible for second treatment.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Death

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

Note: Patients must be followed until all adverse events have resolved to grade 2 or less with the exception of lymphopenia and alopecia. If an adverse event is not expected to resolve to grade 2 or less this will be noted in the patient medical record and the patient may be taken off study.

4. CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS:

Note: Other 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 of chemotherapy start date 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 that 200 at 6 months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200 x2.

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.

4.2 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/mm3. 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. Empiric antibiotics

4.3 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.

4.4 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

5.1 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.

5.1.1 Prior to Chemotherapy Administration:

- 5 CPT tubes (8ml each)
- 1 SST tube (8ml)
- 1 SST tube (4ml) daily; starting day of chemotherapy

5.1.2 Prior To Cell Infusion:

- Blood samples for cytokine analysis (1-8ml tube)

5.1.3 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 x 5 days, then weekly (while the patient is hospitalized):
  - 5 CPT tubes (8 ml each)
  - 1 SST tube (8 ml)

5.1.4 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.
- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this

study, if the subject provides permission on the optional studies section of the consent document. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g., analysis of germ line genetic mutations) the principal investigator must amend the protocol and obtain informed consent from all research subjects. Any new use of samples will require prospective IRB review and approval.

- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study if the subject provides permission on the optional studies section of the consent document for protocol 03-C-0277.

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

5.2. SAMPLE STORAGE, TRACKING AND DISPOSITION

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 Clinical Pharmacology Program (CPP) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Data is entered and stored securely in the Patient Sample Data Management System (PSDMS) utilized by the CPP, and data will be updated to the Surgery Branch central computer database weekly. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

At the conclusion of this protocol, if additional studies are to be performed on any samples obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations, and guidelines of the Federal Government and the State of Maryland.

Any loss or unintentional destruction of the samples will be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6. DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The investigators will be responsible for the collection, maintenance, quality control of the study data. Clinical 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.

6.2 ROUTINE ADVERSE EVENT REPORTING

Following registration through 30 days after cell infusion, adverse events will be recorded in the source documents, reviewed by the designated data manager or research nurse or principal investigator and captured in the C3D 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 to the treatment will be captured in C3D.

6.2.1 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.

6.2.2 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.

6.2.3 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.

6.2.4 Reporting Infection

- 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.

6.3 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

6.3.4.1 Confirmation

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

6.3.4.2 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive

disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

6.3.4.3 Duration of Stable Disease

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

6.4 TOXICITY CRITERIA

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.

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-dos aldesleukin administration. A listing of these side effects in 525 patients treated with high-dose aldesleukin are 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, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactoryresolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

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
- 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**
- 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 REPORTING

7.2.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

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

Reports must be received by the NCI-IRB within 7 working 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:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. 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.

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 Dr. William Dahut, authorized representative for the IND Sponsor (CCR) at:

William Dahut, M.D.

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Copy all MedWatch forms to: nciprotocolsupportoffice@mail.nih.gov

7.4 FDA REPORTING CRITERIA

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

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 and violations 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

A detailed description of the clinical trial monitoring plan has been included in the initial IND submission as required.

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. 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.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, base on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

7.4.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8. STATISTICAL CONSIDERATION

The primary objective of this pilot trial is to determine whether adoptive transfer of Young TIL will result in objective clinical responses in patients with either cervical or non-cervical cancer who are eligible for this trial.

The study will be conducted using a two-stage optimal design[28]. Following amendment D, patients will be stratified according to whether they have cervical or non-cervical cancer. In each cohort, the objective will be to determine if the combination of lymphocyte depleting chemotherapy, Young TIL, and high-dose aldesleukin is able to be associated with a clinical response rate that can rule out 10% (p0=0.10) in favor of a modest 30% PR + CR rate (p1=0.30). The responses determined at 4 month evaluation time point will be the definitive results used for this assessment.

The following design will be used in each of the two cohorts. With alpha=0.05 (5% probability of accepting a poor therapy) and beta=0.10 (10% probability of rejecting a good therapy), initially 18 evaluable patients will be enrolled in a cohort. If 0 to 2 of the 18 patients experience a clinical response, then no further patients will be enrolled in that cohort. If 3 or more of the first 18 evaluable patients enrolled in a cohort have a clinical response, then accrual will continue until a total of 35 evaluable patients have been enrolled in that cohort. To reduce the likelihood of transient responses to chemotherapy leading to expansion of a cohort, patients must have a PR or CR at least 4 months after cell infusion to count toward the clinical responses required for cohort expansion. 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 3 to 6 of the 35 in a given cohort have a clinical response, then this will be considered inadequate for further investigation. If 7 or more of 35 patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration for those patients. Under the null hypothesis (10% response rate), the probability of early termination is 73%.

To complete the entire study in both cohorts, a total of up to 70 patients may be required. In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at 73 patients. Provided that about 2 patients per month will be able to be enrolled onto this trial, approximately 3-4 years may be needed to accrue the maximum number of required patients.

9. COLLABORATIVE AGREEMENTS

We have established a Materials Transfer Agreement (MTA) with Prometheus Laboratories, Inc., to supply Proleukin (aldesleukin) for use in this study.

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 HPV-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 to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3. 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 in 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 HPV-associated cancers the potential benefit is thought to outweigh the risks.

10.4. RISKS/BENEFITS ANALYSIS

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

10.5. 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 will be presented. The Informed Consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

11. PHARMACEUTICAL INFORMATION

11.1. 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 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 9.

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 9.

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 in a volume of 100-200 mL.

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 9. 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.

Fluconazole:

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

11.10. 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. Deaths from cancers at HPV-associated sites in the United States in 2010.

Site	Deaths
Oral cavity and pharynx (not tongue or mouth)	4,060
Cervix	4,210
Vulva	920
Female genital	780
Penis	310
Anus	720
Total	11,000

Adapted from: American Cancer Society. Cancer Facts & Figures 2010. Atlanta: American Cancer Society; 2010.

Table 2. 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 3. 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 4. 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 5. Studies reporting correlations between HPV-associated tumor immunological responses and clinical outcomes.

Site	Disease extent	Link to T cell immunity	Authors
Cervix	Large early-stage	$CD8^+$ T cell infiltration correlates with absence of lymph node metastases	Piersma, *et al*[21]
Cervix	Early stage	Tumor infiltration with T cells associated with improved survival	Bethwaite, *et al*[22]
Cervix	Resected tumors	TIL from 23 of 54 patients displayed HPV E6 or E7 specific responses	Piersma, *et al*[25]
Cervix	Deeply infiltrating	HPV-specific immune response correlated with improved disease free survival	Heusinkveld, *et al*[19]
Cervix	Resected tumors	Tumor-reactive TIL can be generated from cervical cancers	Ghosh, *et al*[29]
Cervix	Resected tumors	Tumor-reactive TIL can be generated from cervical cancers	Hilders, *et al*[30]
Head and Neck	All stages	Tumor infiltrating activated $CD4^+$ T cells associated with improved overall survival	Badoual, *et al*[31]
Oro- and hypopharynx	"Low-risk"	High numbers of intraepithelial $CD8^+$ TIL related to improved disease-free survival	Distel, *et al*[32]
Vulva	Intraepithelial neoplasia	Regression of vulvar intraepithelial neoplasia following HPV16 E6/E7 long-peptide vaccination	Kenter, *et al*[26]
Anal	All stages	Improved survival in patients with high number of tumoral intraepithelial lymphocytes	Rubio, *et al*[33]

Table 6. HPV16 and HPV 18 responses detected in infiltrating lymphocytes[25].

HPV Status	Origin	Patient	Age	Cell type	Stage of disease	Reactivity	SI[1]	Responding peptides	Responding T cell
HPV16	TIL								
		176	45	Squamous	FIGO 1B	E6	80	2	CD4/CD8
		178	40	Squamous	FIGO 1B	E7	11	1	CD4
		185	56	Squamous	FIGO 3B	E7	6	1	CD8
		192	37	Squamous	FIGO 1B				
		194	67	Adeno	FIGO 2A	E7	5		
		226	56	Squamous	FIGO 1B	E6	3	1	CD4
		229	42	Squamous	FIGO 1B				
		230	45	Squamous	FIGO 1A				
		246	31	Squamous	FIGO 1B				
		265	44	Squamous	FIGO 1B	E6	104	2	CD4/CD8
		267	49	Squamous	FIGO 1B	E6	109	2	CD4
		271	40	Squamous	FIGO 1B				
		281	35	Squamous	FIGO 1B				
		283	51	Squamous	FIGO 1B				
		308	39	Squamous	FIGO 1B				
		312	30	Adeno	FIGO 1B				
		331	65	Squamous	FIGO 1B	E6	3	2	CD4/CD8
		332	32	Squamous	FIGO 1B				
		334	41	Squamous	FIGO 1B	E6	5	1	CD8
		338	34	Squamous	FIGO 1B				
		340	29	Squamous	FIGO 1B				
		343	51	Unknown	FIGO 1B				
		344	43	Squamous	FIGO 2A				
		363	45	Squamous	FIGO 1B				
		369	33	Adeno	FIGO 1A				
		371	31	Squamous	FIGO 1B				
		372	72	Squamous	FIGO 1B				
		390	33	Adeno	FIGO 1B	E6/E7	4		
		398	48	Squamous	FIGO 1B				
		405	41	Squamous	FIGO 2B				
		418	34	Squamous	FIGO 1B				
		415	46	Squamous	FIGO 1B				
		424	35	Squamous	FIGO 1B				
		441	51	Squamous	FIGO 1B				
		446	29	Squamous	FIGO 1B	E6	4	4	CD4/CD8
	CIL								
		279	60	Unknown	CIN3				
		284	36	Squamous	CIN2	E7	13	1	CD4
		285	27	Squamous	CIN3				
		310	46	Squamous	CIN3				
		314	34	Squamous	CIN3	E7	11		
		355	47	Squamous	CIN3				
		356	26	Squamous	CIN3	E7	3.5	1	CD4
	LN								
		148	46	Squamous	FIGO 1B	E6/E7	9/3		CD4
		267	49	Squamous	FIGO 1B	E6	4		CD4
		271	40	Squamous	FIGO 1B	E6/E7	1.5/2		CD4
		427	28	Squamous	FIGO 1B	E6	9		CD4/CD8
HPV18	TIL								
		187	43	Squamous	FIGO 1B	E6	2	1	CD4
		196	48	Adenosquamous	FIGO 2A				
		209	55	Squamous	FIGO 1B				
		214	42	Adeno	FIGO 1B	E7	15	1	CD4
		228	37	Squamous	FIGO 2A	E7	18	1	CD4
		251	39	adenosquamous	FIGO 2A	E7	3		
		261	38	Squamous	FIGO 1B				
		335	33	Adeno	FIGO 1B				
		378	40	Adeno	FIGO 1B	E7	8	1	CD4
	LN								
		151	43	Squamous	FIGO 1B	E6/E7	2/3		CD4
HPV16-18-	TIL								
		181	40	Squamous	FIGO 1B				
		182	80	Squamous	FIGO 2B				
		215	31	Squamous	FIGO 1B				
		245	41	Squamous	FIGO 1B				
		248	46	Squamous	FIGO 2A				
		264	35	Adeno	FIGO 1B				
		280	31	Squamous	FIGO 1B				
		287	61	Carcinosarcoma	FIGO 2B				
		289	45	Adeno	FIGO 1B				
		292	32	Squamous	FIGO 1B				
		324	51	Squamous	FIGO 1B				
		353	35	Adeno	FIGO 1A				
		373	55	Squamous	FIGO 1B				
		377	85	Squamous	FIGO 1B				
		381	80	Adeno	FIGO 1B				
		384	75	Squamous	FIGO 1B				
		414	64	Squamous	FIGO 2A				
	CIL								
		348	35	Squamous	CIN3				
		354	39	Squamous	CIN3				
	LN								
		426	40	Squamous	FIGO 1B				

[1]SI, stimulation index.

Table 7. Patient characteristics and success at generating tumor infiltrating lymphocytes from 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 8. Characteristics of tumor infiltrating lymphocytes grown from five cervical cancer specimens.

					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 9:

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)

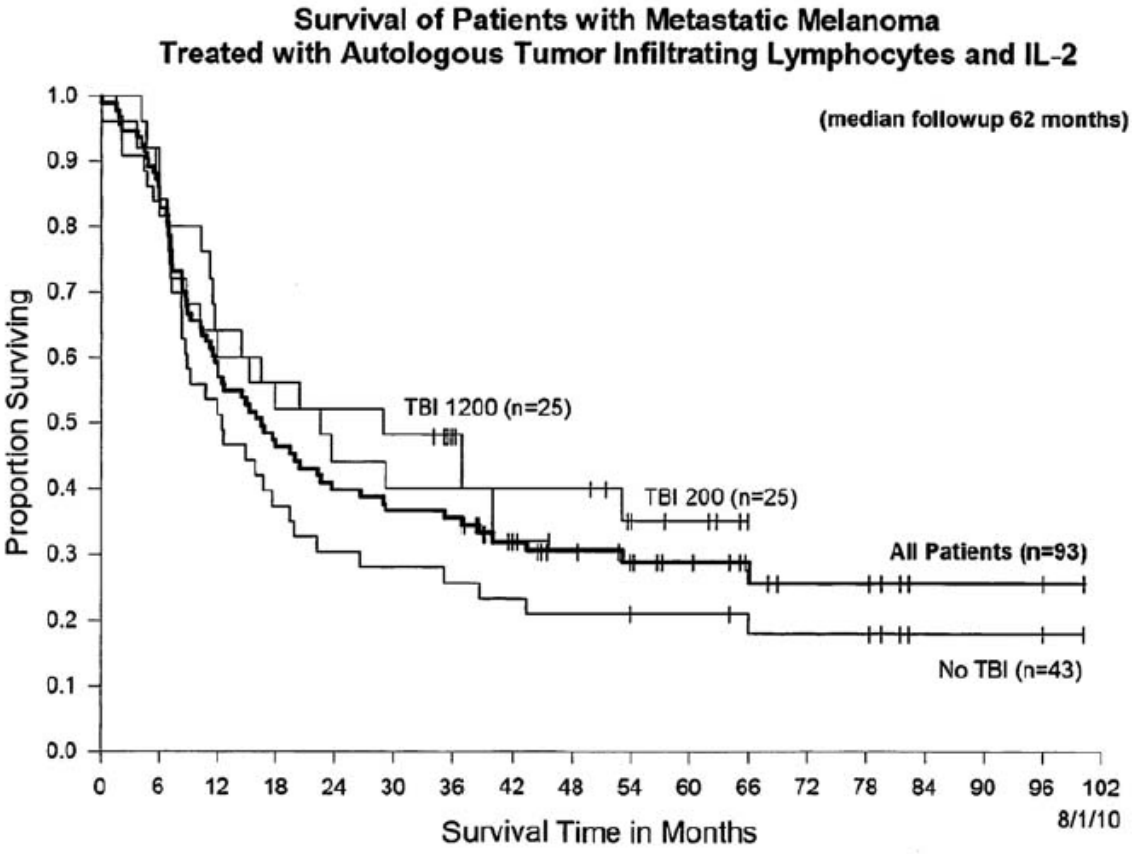
   Example: ideal body weight of 5'3'' female

   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 HPV+ Tumors

Patient:

Date of preparation of final product:

Unique TIL identifier (tumor and culture number):

Tests performed on final product:

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

[1] Performed on the final product prior to infusion. Results are available at the time of infusion.
[2] Performed 2 - 10 days prior to infusion (test performed prior to final manipulation). Results are available at the time of infusion.
[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.
[4] Sample for test collected on the final product prior to infusion. Results will not be available before cells are infused into the patient.
[5] Sample for test collected on the in process cells prior to the REP. Results will be available before cells are infused into the patient.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____

Qualified laboratory or Clinical Supervisor