Phase I Trial of Lymphodepletion followed by Adoptive Cell Transfer of Autologous Tumor Infiltrating Lymphocytes and High-Dose Interleukin 2 in Select Solid Tumors

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Study Agent: Autologous tumor-infiltrating lymphocytes **IND Holder Name:** Gregory A Daniels, MD, PhD

Commercial Sources:
Aldesleukin
Cyclophosphamide
Mesna
Fludarabine

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: Gregory Daniels, MD, PhD

PI Signature: _____

Date: _____

TABLE OF CONTENTS

STUDY SUMMARY

Title	**Phase I Trial of Lymphodepletion Followed by Adoptive Cell Transfer of Autologous Tumor Infiltrating Lymphocytes and High-Dose Interleukin 2 in Select Solid Tumors**
Short Title	Safety of Adoptive Cell Transfer of Autologous Tumor Infiltrating Lymphocytes and High-Dose Interleukin 2 in Select Solid Tumors
Phase	Phase I
Methodology	Single arm.
Study Duration	2-3 years
Study Center(s)	University of California, San Diego
Objectives	1. To establish a low rate of Dose Limiting Toxicity for this treatment regimen in metastatic or locally advanced refractory/recurrent head and neck cancer or melanoma. 2. To obtain preliminary evidence of efficacy 3. To assess the safety and tolerability of this treatment regimen 4. To study immunologic correlates associated with TIL therapy. 5. To determine if autologous TIL infused in conjunction with high dose aldesleukin following a non-myeloablative lymphodepleting preparative regimen can mediate tumor regression in patients with cancer.
Study Endpoints	The primary safety endpoint is the rate of Dose Limiting Toxicity, defined as • Any treatment related death • Neutropenia (grade 4 ANC) 4 weeks following treatment. • Thrombocytopenia (grade 3 or 4 platelets) 4 weeks following treatment. • Grade 4 infection. • Grade 3 creatinine level at 4 weeks post treatment. • Grade 4 acute respiratory distress syndrome. • Any grade 3 or higher AE, except as described above, at least possibly related to study treatment that does not resolve within 48 hours The primary efficacy endpoint is objective response rate using RECIST criteria
Number of Subjects	12 subjects with metastatic or locally advanced refractory/recurrent head and neck cancer 12 subjects with metastatic or locally advanced refractory/recurrent melanoma
Diagnosis and Main Inclusion Criteria	Patients greater than or equal to 18 years of age with a pathologically confirmed diagnosis of metastatic or locally advanced refractory/recurrent head and neck cancer or melanoma refractory to standard therapy.

Study Product(s), Dose, Route, Regimen	• Patients will undergo biopsy or resection to obtain tumor for generation of autologous TIL cultures and autologous cancer cell lines per HRPP 190787. • All patients will receive a non-myeloablative lymphocyte depleting preparative regimen of cyclophosphamide (60 mg/kg/day IV) on days -8 and -7 and fludarabine (25 mg/m2/day IV) on days -6 through -2. • On day 0 patients will receive between $1x10^9$ to $3x10^{11}$ 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 5 weeks after TIL infusion. A second confirmatory scan will be performed at 10 weeks post infusion.
Duration of administration	Hospital course of approximately 10 to 21 days
Reference therapy	Patients who have progressed following at least one prior systemic therapy and are not candidates for known curative therapy.
Statistical Methodology	This is a Phase I study with no dose escalation. There is a safety stopping rule based on cohorts of size 3, within each indication. In addition, there is a futility stopping rule after the first 6 patients, within each indication. • There will be up to 4 cohorts of size 3 in each indication (melanoma and HNSCC). Safety will be assessed at 4 weeks post treatment in each cohort prior to enrolling the next cohort. The trial will stop if one or more DLT's are observed in any cohort. • Within each indication, after the first 6 patients have been treated if one or fewer patients have achieved an objective response by RECIST criteria, the trial will stop for that indication. For this stopping rule, efficacy will be assessed at 8 weeks following infusion of TILs. Study will be suspended pending on further investigation and discussion with FDA if excessive rate of severe toxicities are observed in the enrolled subjects, e.g., grade 3 or greater toxicities related to study products are observed in enrolled subjects.

1.0 BACKGROUND AND RATIONALE

The aim of this study is to establish the safety of autologous cell therapy developed for melanoma and other solid tumor patients at the Surgery Branch of the National Cancer Institute here at the University of California, San Diego (UCSD)[1-3]. UCSD is both a high-dose interleukin 2 treatment center with >100 patients treated and a bone marrow transplant center. Tumor infiltrating lymphocytes (TILs) demonstrate activity in melanoma and other select solid tumors including metastatic HPV related head and neck mucosal squamous cell cancer. We seek to establish a protocol to deliver TILs in a safe and effective manner utilizing the Surgery Branch protocol as a model. We will examine as separate cohorts patients with melanoma and head and neck cancers. Subsequent projects will look to better optimize and expand this technique to a variety of tumor types.

2.0 CANCER IMMUNE THERAPY

Cancer immune therapy has transformed oncology practice with multiple agents from vaccines, cytokines, checkpoint inhibitors and cell products. Tumors responsive to immune modulation include but not limited to melanoma, renal cell cancer, lung cancer, cervical cancer, colorectal cancer and head and neck cancers.

The first successful solid tumor immune therapy was high-dose interleukin 2 (IL2)[4-7]. IL2 was clinically developed at the NCI as part of efforts of the surgery branch to develop cell therapy. Following completion of a series of phase 2 studies, high-dose IL2 was approved in the 1990s for the treatment of metastatic renal cell cancer and melanoma. The enthusiasm for IL2 was based upon the induction of complete and durable clinical responses in select patients. However, use was tempered due to the toxicity associated with therapy and the relatively low response rate.

The application of immune therapy has exploded with the development of additional immune modulators that includes targeting the CTLA4[8,9] and PD1[10-12] (see below melanoma) receptors. Ipilimumab (anti-CTLA4) was approved in 2011 for patients with metastatic melanoma. Similar to IL2, Ipilimumab delivers durable remission in a small number of patients but is administered as an outpatient. The immune dysregulation accompanying CTLA4 blockade also places the patient at risk for immune-related adverse events (irAEs) including colitis, hepatitis, dermatitis and other autoimmune reactions. Blocking either PD1 or PDL1 receptors demonstrates better response rates and improved toxicity profiles for some patients with not only melanoma but also other malignancies having an inflamed context. To date, combinations of immune modulators have increased both the response rate and toxicity for patients. In melanoma, the response rates are more than 80% when combining ipilimumab and Nivolumab for therapy[13-15]. However, the majority of patients experience grade 3 or 4 dose limiting toxicity and can not proceed with further therapy. Thus, there continues to be a need for additional treatment options and combination immune therapy trials are ongoing.

The tumor microenvironment (TME) offers a window to predict patient outcomes as well as a source of effector cells. Tumors rich in lymphocytic infiltration have a better prognosis. In fact, patients can be risk stratified based upon an “immunoscore” that out performs TNM staging in colorectal cancer patients[16]. This observation has been extended to a number of other solid tumors[17]. The cells in the TME are not only prognostic and predictive of outcomes and therapy response but can be utilized as a therapeutic tool. Ex vivo manipulation of TILs allows for the generation and activation of a high number of tumor specific cells. Durable responses have been observed in a variety of solid tumor patients including but not limited to melanoma, cervical

and head and neck cancer patients. The application of TILs may expand as we understand better the regulatory control and nature of the effector response.

2.1 Tumors Targeted by this Protocol

Melanoma

Melanoma incidence continues to rise accounting for approximately 10,000 deaths per year in the US. Interleukin 2 therapy was approved for treatment of select patients with advanced melanoma in 1998 due to long-term disease control in a subset of patients. It was not until 2011 that outcomes began to significantly change for the majority of patients with the approval of both targeted agents (BRAF inhibitors) and immune modulation (CLTA4). Additional targeted agents (cKit and MEK) along with immune checkpoint modulators (PD1/PDL1) has led to dramatic changes in overall survival in advanced disease. Unfortunately, there continues to remain a need for additional therapeutic options due to significant toxicities (immune related adverse events) and progression despite these new agents. We will examine patients who have undergone TIL harvest and have no standard therapy options.

Head and neck squamous cell cancers

Despite a decline in tobacco and alcohol related oropharyngeal cancers, the total rate of oropharyngeal cancers has increased due to HPV infections. HPV-associated cancers, including cervix, vagina, anus and oropharynx, represent a large and increasing health burden accounting for approximately 11,000 deaths per year. Viral-related cancers would appear to be an ideal target for immune-based therapy due to potentially unique tumor-associated viral antigens, a possible pre-existing natural immune response and dependency upon viral driven pathways for oncogenesis. However, response to immune modulation (anti-PD1 therapy) appeared similar between HPV-positive and HPV-negative oropharyngeal cancer patients. Preliminary response data from the NCI suggests responses to unselected TIL therapy in patients with metastatic HPV-related head and neck cancers. We propose to include both HPV-positive and HPV-negative patients.

2.2 Immune Therapy at UCSD

The expansion of immune therapy beyond a few select tumors (melanoma and renal cell cancer) in oncology has been reflected with the changing treatment patterns at UCSD. Immune therapeutics (cytokine therapy, cell therapy and immune checkpoints) are commonly utilized in standard cancer care. In addition, novel agent development and immune response monitoring continue to be an active area of research at UCSD.

2.3 High Dose IL-2 at UCSD

IL2 (Aldesleukin) therapy was FDA approved in the 1990s for metastatic renal cell and then metastatic melanoma patients following a series of phase 2 clinical trials demonstrating durable remission in a modest percentage of highly selected patients. Modeled after the therapy developed at the NCI, UCSD initiated a HD IL2 protocol in 2007. In 2010, a medication utilization evaluation was conducted on the first 32 patients. The evaluation focused on safety, benefit and cost. No treatment related deaths were found in a heavily pretreated population of melanoma (n=27) and renal cell (n=5) patients. The length of hospital stay was less than other University of California programs while the number of doses of IL2 delivered was higher. The overall response rate of 15% was similar to other published reports. Thus, the conclusion in

2010 was that the developed standard protocol for IL2 was safe and effective. An update to this analysis is ongoing and will include the first one hundred consecutive patients.

2.4 Fluorine labeling and imaging at UCSD

Cell Sense (CS-1000) is perfluorocarbon emulsion designed to enable cellular uptake and the monitoring of cellular distribution by MRI after administration. CS-1000 is the subject of a Drug Master File (BB-MF14062) that was submitted to the U.S. Food and Drug Administration in 2009. CS-1000 is biologically and chemically inert. When CS-1000 is added to a cellular product in vitro, it is rapidly absorbed. In 2018 UCSD initiated cellular labeling experiments to examine cellular uptake and verify that the TIL cellular properties (viability, phenotype, and IFN-gamma production) were maintained (cellular labeling data included in Appendix 1). Cellular labeling is achieved by coincubation of CS-1000 with the cellular product for at least 24h before the completion of culture. Imaging optimization has also been performed using UCSD MRI facilities to accommodate the examination of TIL pharmacokinetics and trafficking patterns.

CS-1000 has been used in one US FDA-approved Phase I clinical trial (NCT 01671592). The trial was completed in February 2014. To date, no adverse events have been reported by the sponsor. CS-1000 is currently being used in one FDA-authorized Phase I clinical trial (NCT 02035085). It is contemplated that the trial will involve 6 patients presenting with radiation induced fibrosis following treatment for breast cancer. The patients will be treated with CS-1000 labeled autologous stromal vascular fraction derived from adipose tissues that are administered subcutaneously at the site of disease. Neither of these trials are affiliated with UCSD.

3.0 ADOPTIVE CELL THERAPY

3.1 Summary of NCI TILS

The NCI has pioneered novel T cell based cancer therapies for chemotherapy-refractory cancers and forms the basis of our protocol development. The NCI demonstrated in three consecutive trials the successful treatment of metastatic melanoma with adoptive transfer of tumor infiltrating lymphocytes (TILs) who received autologous product following a lymphodepleting regimen plus IL2 administration, with or without total body irradiation[2]. Forty-three patients received a non-myeloablative chemotherapy consisting of 60 mg/kg cyclophosphamide daily for two doses followed by 25mg/m^2 fludarabine daily for five days prior to cell transfer and IL2 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 IL2 administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%. 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 IL2 therapy and 22 had disease that was refractory to prior IL2 plus chemotherapy. Thus, TIL therapy demonstrated immune mediated regression in a significant fraction of patients with refractory metastatic melanoma.

The NCI and others have expanded the use of the same TIL product to other solid tumors including HPV-associated malignancies and GI tumors[1]. Cell product has been successfully produced and delivered in an analogous manner to HPV-associated head and neck and cervical cancer patients in ongoing protocols. Current protocols are examining the selection of cells, optimizing culturing conditions and subsequent treatment strategies.

The surgery branch of the NCI has provided guidance and templates for the development of this cell delivery protocol based upon the current ongoing cell delivery trials.

3.2 Predictive Outcomes

This protocol will focus on the development of a standard delivery platform for cell therapy. Understanding how to select patients who may respond to therapy, the mechanism of response and barriers to response will be the focus of a companion protocol. Examining the cell product, subsequent immune changes and response to additional therapies will be the focus of other protocols.

3.3 Autologous Cell Isolation

Tumor infiltrating lymphocytes will be isolated and expanded per a companion protocol (HRPP 190787). Briefly, after obtaining consent, patients will undergo resection of tumors appropriate for TIL generation. Autologous cells cultures will be initiated from tumor fragments in media containing IL2. Multiple tumor cultures will be pooled and rapidly expanded.

4.0 RATIONALE FOR DEVELOPING TIL DELIVERY PROTOCOL

We hope to establish a safe autologous cell delivery protocol for patients with melanoma and head and neck cancers. Once established, we anticipate that this protocol will provide a backbone for subsequent clinical trials directed at questions of patient selection, optimal cell preparation and therapy sequencing or combination.

5.0 STUDY OBJECTIVES

5.1 Primary Objectives

To determine the rate of Dose Limiting Toxicity of adoptive cell transfer (ACT) using cultured autologous tumor infiltrating lymphocytes (TIL) following non-myeloablative chemotherapy plus IL-2 treatment for treating patients with cancer.

5.2 Secondary Objectives

1. To provide preliminary evidence of efficacy.

2. To describe treatment related Adverse Events and to assess tolerability.

3. To describe the response rate and progression free survival in head and neck cancer and melanoma patients receiving ACT plus IL-2.

4. To characterize immunologic changes during therapy including distribution of TILs after infusion.

5.3 Endpoints

Primary Endpoint:

Dose Limiting Toxicity defined as any one of the following:

- Any treatment related death
- Neutropenia (grade 4 ANC) 4 weeks following treatment.
- Thrombocytopenia (grade 3 or 4 platelets) 4 weeks following treatment.
- Grade 4 infection.
- Grade 3 creatinine level at 4 weeks post treatment.
- Grade 4 acute respiratory distress syndrome.
- Any grade 3 or higher AE, except as described above, at least possibly related to study treatment that does not resolve within 48 hours

The primary efficacy endpoint is objective response rate using RECIST criteria.

Secondary Endpoints:

- Adverse events related to ACT plus IL-2 treatment (description, timing, grade [CTCAE v5.0], severity, seriousness, and relatedness).
- Progression free survival, complete response rate and overall survival
- Immunologic subsets in the TIL product and tumor microenvironment if available.

6.0 ELIGIBILITY ASSESSMENT AND ENROLLMENT

6.1 Eligibility Criteria

6.1.1 Inclusion Criteria

Patients must meet all of the inclusion criteria to participate in this study.

1. Patient has the ability to understand and the willingness to sign a written informed consent.

2. Patients with a histologically confirmed diagnosis of head and neck squamous cell carcinoma OR metastatic cutaneous or mucosal melanoma measurable per RECIST.

3. Progressive squamous cell cancer of the head and neck or metastatic melanoma since prior systemic treatment and who are:

    a. Not candidates for known curative intent therapy.
    b. Progressed following at least one prior systemic therapy.
        i. Unresectable metastatic melanoma: must have progressed on pembrolizumab, Nivolumab, or the combination of Nivolumab and ipilimumab prior to enrollment.

ii. Unresectable BRAF V600E-mutated metastatic melanoma: must have progressed on pembrolizumab, Nivolumab, or the combination of Nivolumab and ipilimumab prior to enrollment. In addition, must have progressed on (or been intolerant to) prior BRAF-targeting therapy (with or without a MEK inhibitor) prior to enrollment.
iii. Recurrent/metastatic squamous cell cancer of the head and neck: must have received prior therapy with a platinum-containing regimen and with pembrolizumab or Nivolumab prior to enrollment.

c. Have advanced disease
   i. Melanoma=unresectable stage III or stage IV
   ii. Head and Neck=recurrent or metastatic disease

4. Patient has undergone resection and a successful generation of an autologous TIL culture (per HRPP 190787).

5. Patient with up to three brain lesions may be included if the lesions are < 1cm and demonstrate radiographic stability for at least three months following therapy. .

6. Patient is ≥ 18 years of age.

7. Patient has a life expectancy of greater than 3 months.

8. Patient has an ECOG Performance Status of 0 or 1.

ECOG Performance Status	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

9. Patient has adequate organ and marrow function as defined below:

Absolute Neutrophil Count	≥ 1.0 x 10^9/L
Platelet count	≥ 100 x 10^9/L
Hemoglobin	≥ 9.0 g/dL
AST/SGOT and ALT/SPGT	≤ 2.5 X institutional upper limit of normal
Calculated creatinine clearance (Cockcroft gault)	> 50 ml/min
Total bilirubin	≤ 1.5 mg/dl (or ≤ 3 mg/dl in patients with Gilbert's Syndrome)

10. Patient is seronegative for HIV antibody.

Note: The experimental treatment being evaluated depends on an intact immune system. Patients who are HIV seropositive can have decreased immune competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.

11. Patient is seronegative for Hepatitis B antigen, or Hepatitis C antibody or antigen.

12. Patient agrees to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of study treatment, and for *4 months* after receiving all protocol related therapy.

13. More than four weeks has elapsed since the patient received any prior systemic therapy at the time of enrollment. Patients must have stable or progressing disease after prior treatment.
    Note: Patient may have undergone minor surgical procedures within the past 3 weeks, as long as all toxicities from surgery have recovered to grade 1 or less.

14. Patient has recovered to ≤ Grade 1 or baseline from adverse events due to prior therapy.

15. Patient has stable or progressing disease after at least one prior treatment.

16. Six weeks or more have elapsed since the patient received any prior anti-CTLA4 antibody therapy to allow antibody levels to decline.
    Note: If patient has previously received ipilimumab or tremelimumab, anti- PD1 or anti-PD-L1 antibodies, and have documented GI toxicity, the patient must have completely recovered to baseline and more than six weeks off steroid therapy.

17. A subset of head and neck squamous cell carcinoma subjects (N ≤ 5) will have the TIL product labeled with CS-1000. Subjects in this subset must meet the additional eligibility criteria below:
    a. Patients with neck recurrence should have solid (rather than cystic) masses below the angle of the mandible (e.g. levels 3-5).
    b. No evidence of hepatic metastases

6.1.2 Exclusion Criteria

Patients meeting any of the exclusion criteria at baseline will be excluded from study participation.

1. Patient is currently using investigational agents.

2. Patient had prior cell transfer therapy which included a non-myeloablative or myeloablative chemotherapy regimen.

3. Patient is a female of child-bearing potential who is pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.

4. Patient requires immune suppressive therapy including but not limited to greater than physiologic steroid replacement.

5. 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.

6. Patient has any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease and AIDS).

7. Patient has opportunistic infections.

8. Patient has a history of severe immediate hypersensitivity reaction to any of the agents used in this study.

9. Patient has a history of coronary revascularization or ischemic symptoms.

10. Patient is known to have an LVEF ≤ 45%.

11. Patients with clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block.

12. Patient has documented FEV1 ≤ 60% predicted.

13. Patient with untreated brain metastases.

6.2 Eligibility Evaluation

Prior to initiation of study treatment (within 2 Months prior to chemotherapy):

a. HIV antibody titer and HB_sAG determination, anti HCV (must be performed within 2 months of chemotherapy start date).

b. Anti CMV antibody titer, HSV serology, and EBV panel. (must be performed within 2 months of chemotherapy start; patients who are known to be positive for any of the above do not need to be retested.)

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

d. Stress cardiac (MUGA or echocardiogram)

e. Pulmonary evaluation (PFTs) (must be performed within 2 months of chemotherapy start)

f. Histologic confirmation of metastatic cancer. This can be done anytime prior to study participation on archived samples OR sample collected for TIL production.

g. For melanoma patients, limited molecular profiling will be documented including but not limited to mutation analysis for BRAF. Head and neck cancer patients will have HPV status confirmed utilizing either p16 immune histochemistry stains or HPV DNA analysis. The BRAF status (melanoma patients) and HPV status (HNSCC patients) will be recorded during screening.

Within 2 weeks prior to initiation of study treatment:
Note: the following evaluations should be repeated for safety if treatment will not begin within 3 weeks of eligibility confirmation.

a. Complete physical examination including height, weight, vital signs and eye exam, noting in detail the exact size and location of any lesions that exist. (Patient medical history may be obtained within 8 weeks)
b. Chest x-ray.
c. ECG.
d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
e. Complete Metabolic Panel (CMP):
    - albumin
    - alkaline phosphatase
    - aspartate aminotransferase (ALT)
    - alanine aminotransferase (AST)
    - bicarbonate (CO_2)
    - blood urea nitrogen (BUN)
    - calcium
    - chloride
    - creatinine
    - glucose
    - potassium
    - sodium
    - total protein
    - total bilirubin
f. Magnesium, phosphorus
g. CK, LDH, Uric acid
h. Thyroid stimulating hormone (TSH), free thyroxine (fT4)
i. Complete Blood Count (CBC) with differential:
    - hemoglobin,
    - hematocrit,

- platelet count,
- white blood cell count,
- percentage or absolute differential count

j. PT/PTT

k. Urinalysis and culture, if indicated

Within 7 days prior to treatment:

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

b. ECOG

7.0 STUDY IMPLEMENTATION

7.1 Overall Schema

Patients will have TIL generated under protocol HRPP 190787. TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. TIL cultures will be monitored regularly and when approximately $5x10^8$ cells are available the cells will undergo a rapid expansion. Prior to the start of the preparative regimen (approximately one week prior to infusion) TIL will be assessed for potency by interferon gamma release as specified in the Certificate of Analysis (COA) shown in the Laboratory Manual. TIL for a subset of HNSCC subjects will be labeled with CS-1000 before final harvest.

Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the COA (approximately 7 days after the rapid expansion protocol (REP) procedure has been initiated), the patient will receive the lymphocyte depleting preparative regimen, followed by infusion of between $1x10^9$ to $3x10^{11}$ lymphocytes and the administration of high-dose IL-2. The non-myeloablative chemotherapy (NMA) consists of 60 mg/kg/day cyclophosphamide for 2 days followed by 5 days of 25 mg/m^2/day fludarabine. On the day following the preparative regimen, patients will receive a bolus intravenous infusion of TIL and start high-dose IL-2 therapy. Patients will receive the FDA approved standard regimen of IL-2 at 720,000 IU/kg every eight hours to tolerance starting within 24 hours of cell infusion. Patients will be evaluated for response approximately 4-6 weeks following the completion of IL-2.

7.2 Drug Administration

Drug Administration Treatment schedule will be according to the following schedule (Table 1). Administration of diuretics, electrolyte monitoring and replacement, and hydration should all be performed as clinically indicated. Communication between the ACTL principal investigator and treatment team will occur per Appendix 5.

7.2.1 Preparative Regimen

A preparative regimen with cyclophosphamide, mesna and fludrabine will be provided on Days -8 through Day -2. Additional information can be found in the Pharmacy Manual.

Day -8 and -7:

Cyclophosphamide 60 mg/kg/day X 2 days IV per institutional standard or per MD discretion. .

Mesna therapy will also be provided per institutional standard or MD discretion.

Day -6 to Day --2:

Fludarabine 25 mg/m2/day IVPB daily per institutional standard or MD discretion.

7.2.2 Cell Infusion and IL-2 Administration

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

Autologous TIL infusion will be administered intravenously over 20 to 30 minutes (minimum 1 X 10^9 and up to a maximum of 3 X 10^{11} lymphocytes). Administer at an initial rate of 60mL/hr for 5 minutes. If well tolerated, increase and continue at a rate of 600mL/hr. Administer peripherally via gravity.

After at least 3 hours but within 24 hours of cell infusion administration of IL-2 will be initiated.

Day 0-5 (Day 0 is the day of cell infusion):

- Standard IL-2 given at approximately 720,000 IU/kg IV (based on total body weight) over 15 minute every eight hours (+/- 1 hour) for up to 5 days (max 15 doses). Dosing guidelines and supportive medications will be followed as outlined in Section 7.5 and per package insert.
- Beginning on Day 1: Filgrastim will be started at 300 mcg per day.

Table 1. Schedule of Study Treatment Administration

	Preparative Regimen								Cell Infusion and IL-2					
Day	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0[1]**	**1**	**2**	**3**	**4-5**	**21**
Therapy														
Cyclophosphamide 60 mg/kg	X	X												
Mesna	X	X												
Fludarabine 25 mg/m^2			X	X	X	X	X							
TIL Cells									X					
IL-2									X[2]	X	X	X	X	
Filgrastim[3] 300mcg/day										X	X	X	X	
TMP/SMX[4] 160mg/800mg (example)														X
Fluconazole[5] 100 mg po										X	X	X	X	
Valacyclovir or Acyclovir [6]							X	X	X	X	X	X	X	

1. One to three days after the last dose of agent in the preparative regimen.
2. Initiate within 24 hours after cell infusion.
3. Continue until neutrophils count > 1000 x 2 consecutive days. Zarxio may be used
4. The TMP/SMX schedule should be adjusted to BID, twice a week. Until ALC >1000 for at least 60 days.
5. Continue until ANC > 1000/mm^3.
6. Continue until Day +100 and ALC > 1000.

7.3 Infection Prophylaxis

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

7.3.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim [TMP] and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) p.o. twice daily twice weekly on non-consecutive days starting Day +21 and stopping when the absolute lymphocyte count is >1000/mm^3 for at least 60 days.

Pentamidine or Atovaquone will be substituted for TMP/SMX-DS in patients with sulfa allergies. Pentamidine will be administered aerosolized at 300 mg per nebulizer within one week prior to admission and continued monthly until the absolute lymphocyte count is >1000/mm^3 and for at least 60 days. Atovaquone will be administered at 1500mg by mouth daily after the first dose of chemotherapy on or after Day +21 and stopping when absolute lymphocyte count is >1000/mm^3 for at least 60 days.

7.3.2 Herpes Virus Prophylaxis

Patients with positive HSV serology will be given valacyclovir or acyclovir. Prophylaxis will be provided per institutional standard until absolute lymphocyte count count is greater than 1000/mm^3. 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.

7.3.3 Fungal Prophylaxis (Fluconazole)

Patients will start Fluconazole 100 mg orally after TIL administration on Day +1 and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 100mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

7.3.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th^h generation cephalosporin or a quinolone for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm^3. Aminoglycosides should be avoided unless clear evidence of sepsis.

7.4 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 Hgb >7gm/dl, and platelets >20,000/mm^3. All blood products with the exception of the stem cell product will be irradiated and leukocyte reduced.

7.5 IL-2: Intravenous Administration

IL-2 will be administered at a dose of approximately 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15 minute period every eight hours (+/- 1 hour) beginning within 24 hours after the cell infusion and continuing for up to 5 days (maximum 15 doses) per institutional practice. Doses may be delayed or skipped depending on patient tolerance if this is determined to be clinically indicated in the judgment of the attending physician. Doses will be skipped if patients reach Grade 3 or 4 toxicity due to IL-2 except for the reversible Grade 3 toxicities common to IL-2 such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 2. Toxicities will be managed as outlined in Appendix 3 and adapted from the experience of the cytokine working group[18]. If these toxicities can be easily reversed within 24 hours by supportive measures then additional doses may be given. If greater than two doses of IL-2 are skipped, IL-2 administration will be stopped. In addition, dosing may be held or stopped at the discretion of the treating investigator.

Table 2. Schedule of Study Assessments

Procedure	**Prior to treatment**	**Within 2 weeks (+/-1 week) prior to treatment**	**Preparative Regimen**	**During and After Infusion and IL-2 Until Hospital Discharge**	**Post-Discharge**	**Following Administration of Cell Product**			
	Eligibility		Days -8 to -1	Days 0 to 4 through Day of Discharge	Day 7 (+7 days) after discharge	Week 6 (+/- 2 wks)	Week 12 (+/- 2 wks)	Every 3 Months (+/- 1 MO) X 3 visits	Every 6 Months (+/- 1 MO) X 5 years
Physical Exam		X	X (at least 3 times/ week)	X	X	X	X	X	X
Vital Signs		X	X	X					
Review of Systems			X (at least 3 times/ week)	X	X	X	X	X	X
HLA Typing	X								
Antibody Titers and Serology	X+		X++						
Stress Cardiac	X^^								
Pulmonary Evaluation	X^^								
Histologic Confirmation	X								
BRAF Status (melanoma only)	X								
HPV Status (HNSCC only)	X								
Chest X-Ray		X							
ECG		X							
Imaging, as clinically indicated		X*			X	X	X	X	X
CMP		X	X	X	X	X	X	X	X
CBC		X	X	X					
Magnesium, Phosphorus		X	X	X	X	X	X	X	X
Calcium				X					
CK, Uric Acid		X	X		X	X	X	X	X
LDH		X			X	X	X	X	X
TSH, T4		X			X-	X-	X-	X-	X-
PT/PTT		X							
ECOG		X**							

Procedure	**Prior to treatment**	**Within 2 weeks (+/-1 week) prior to treatment**	**Preparative Regimen**	**During and After Infusion and IL-2 Until Hospital Discharge**	**Post-Discharge**	**Following Administration of Cell Product**			
	Eligibility		Days -8 to -1	Days 0 to 4 through Day of Discharge	Day 7 (+7 days) after discharge	Week 6 (+/- 2 wks)	Week 12 (+/- 2 wks)	Every 3 Months (+/- 1 MO) X 3 visits	Every 6 Months (+/- 1 MO) X 5 years
Pregnancy Test		X**							
Urinalysis		X@	X						
Blood Culture				X^					
Optional MRI***					X				

+ HIV antibody titer, HB_sAG determination, HCV, CMV antibody titer, HSV serology, and EBV panel. Must be performed within 2 months of chemotherapy start date.
++ CMV antigen assay only, if clinically indicated.
* Baseline CT of chest, abdomen, and pelvis and brain MRI. Other imaging scans and x-rays as clinically indicated.
** Within 7 days prior to treatment.
*** Optional for subset of HNSCC participants who received CS-1000 labeled cellular product. A repeat MRI may be performed 1-2 weeks after the first MRI.
@ If clinically indicated.
^ Up to 48 hours following the last IL2 infusion
^^ Must be performed within 2 months of chemotherapy start.
Note: Patients may be seen more frequently, as clinically indicated.

7.6 On study Evaluation

7.6.1 Prior to starting the Preparative Regimen (before Day -8)

Within 2 weeks prior to starting the preparative regimen (day -8) and 3 weeks from the cell infusion (day 0), patients will have the following:

- CBC with differential
- CMP
- Magnesium, phosphorus
- CK, LDH, Uric acid

If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

7.6.2 During the Preparative Regimen (daily)

- CBC with differential
- CMP
- Magnesium, phosphorus
- CK, Uric acid
- Urinalysis
- CMV antigen assay will be assessed if clinically indicated (e.g. unexplained fevers, pulmonary changes)
- Review of systems and physical exam at least 3 times/week
- Vital signs as per routine

7.6.3 During and After Cell Infusion

Vital signs (including heart rate, blood pressure, pulse oximetry, and respiratory rate) will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4 -8 hours) unless otherwise clinically indicated until discharged. The subject will be monitored for acute infusion reactions during and after autologous TIL infusion. Supportive care, including appropriate emergency medications (eg, epinephrine and diphenhydramine), will be available at bedside and institutional emergency guidelines will be followed as necessary.

7.6.4 During and after IL-2 administration until hospital discharge (daily)

- CBC with differential
- CMP
- Magnesium, phosphorus, calcium
- Review of systems and physical exam
- Vital signs as per routine

- Routine blood culture from central line up to 48 hours following the last IL2 infusion.

After the last dose of IL-2, patients will be discharged from the hospital once they are considered medically stable. The exact day will be dependent on their tolerance of the study procedures. The laboratory results and vitals above will be collected until hospital discharge. These results must be within a clinically acceptable limit to proceed with discharge.

7.7 Post Treatment Evaluation

The initial post treatment evaluation will be conducted approximately 7-14 days post-discharge.

Following administration of the cell product, patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Week 6 (+/- 2 weeks)
- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x3
- Every 6 months (+/- 1 month) x 5 years
- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated

At each scheduled evaluation patients will undergo:

- Physical examination
- CMP
- Magnesium, phosphorus
- CK, LDH, Uric acid
- TSH, free T4 if clinically indicated
- Toxicity assessment, including review of systems
- CT of the chest, abdomen and pelvis as clinically indicated. If clinically indicated, other scans or x-rays may be performed (e.g., brain MRI, bone scan).
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.

  Immune monitoring per separate protocol and consent (HRPP #)
- Patients who develop renal failure will undergo a nephrology consultation and a renal biopsy, if indicated.

  Note: Patients who develop progressive disease or patients who are unwilling or unable to return for follow up evaluation will not be required to undergo the above but will be contacted by phone approximately every 3-6 months for the first two years, and yearly thereafter.

The following information may be requested from the patient:

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

- Optional MRI. A subset of HNSCC subjects (≤ 5) will be offered CS-1000 labled cellular product followed by MRI scans of known metastases and the liver after discharge (estimated Day 14 to 21). MRIs will be performed per research protocol at the Center for Functional MRI, WM Keck Building, Osler Lane, UCSD School of Medicine Campus

7.7.1 Off Treatment Criteria

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

- Completion of treatment.
- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a patient experiences grade 3 or 4 toxicity due to cell infusion as referred to under section 7.5 and appendix 2 occurring within 24 hours post cell infusion that does not reverse to grade 2 or less within 8 hours with 2 doses of 650 mg p.o. of acetaminophen or two dose of 50 mg p.o. of diphenhydramine the patient will receive no further cells but may continue to receive IL-2.
- Grade 2 or greater allergic reaction including bronchospasm or generalized urticaria.
- Grade 3 or greater toxicity due to cytokines (as referred to under Appendix 3) that does not decrease to grade 2 or less within 96 hours of management according to study site standard institutional practices.
- Patients with progressive disease who elect not to undergo further active treatment.
- If the PI determines that continued treatment is not in the best interest of the patient.

7.7.2 Off Study Criteria

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Patient withdraws consent.
- There is significant noncompliance.
- General or specific changes in the patient's condition render continued participation unacceptable for further follow up in the judgment of the investigator.
- Patient becomes pregnant.
- Death.
- Lost to follow-up.

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.

If a patient becomes pregnant, treatment must be discontinued as soon as possible. Pregnant patients will be withdrawn from the study entirely. Patients who become pregnant will be monitored until the outcome of the pregnancy is known. Any adverse events or serious adverse events related to the pregnancy will be reported as described in Section 11.

8.0 SUPPORTIVE CARE

Patients will have blood transfusions in order to maintain a minimum hemoglobin of greater than 7 g/dL. Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chills develop. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), ibuprofen (600 mg q8h) and ranitidine (150 mg q12h) or equivalent per MD discretion. Patients who require transfusions will receive irradiated blood products. Additional antiemetic therapy will be administered for breakthrough nausea and vomiting. Patients will receive supportive care as indicated for IL-2 toxicities as listed in Appendix 3.

9.0 MEASUREMENT OF EFFECT

9.1 Toxicity Criteria

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of the treatment regimens will be performed. The study will use the CTCAE version 5.0 (http://ctep.cancer.gov/reporting/ctc.html) for reporting of toxicity and adverse events.

Over 100 patients have been treated in the Surgery Branch, NCI with TIL. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of IL-2 but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients. In 93 patients treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGy TBI) due to an unexpected but preexisting diverticular abscess.

The standard approach to the administration of IL-2 in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

Toxicities seen on protocols using this non-myeloablative regimen and IL-2 that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine.

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

- Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.
- Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.
- Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression, photosensitivity.
- High-dose IL-2 administration: A listing of these side effects in 652 patients who received 1,039 treatment courses are listed in Appendix 2.

9.2 Response Criteria

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST v1.1 criteria. See Appendix 4. A minimum of 1 and up to 5 lesions in total will be considered target lesions that fulfill the criteria for reproducibility and not previously treated with radiation. No more than 2 lesions per organ system (e lung or liver or skin or LN). Target lesions will be identified, recorded and measured at baseline.

10.0 STATISTICAL SECTION

10.1 Study Design

This is a Phase I trial of safety and activity of autologous cell therapy in metastatic melanoma and head and neck cancer patients. Up to 12 patients will be enrolled in each cancer type (a melanoma study arm and a head and neck cancer study arm). Patients will be enrolled in cohorts of size 3. There will be a safety assessment after each cohort, prior to enrollment of the next cohort. There will be an efficacy assessment after the first 6 patients in each cancer type.

The primary objective is to establish a low rate of Dose Limiting Toxicity of adoptive cell transfer (ACT) using cultured autologous tumor infiltrating lymphocytes (TIL) following non-myeloablative chemotherapy plus IL-2 treatment for treating patients with cancer.

The secondary objectives are to determine safety and tolerability, and the overall and complete response rate in head and neck cancer and melanoma patients receiving ACT plus IL-2. We will also assess progression free survival and overall survival in cancer patients treated with ACT plus IL-2.

10.2 Endpoints

The primary safety endpoint is the rate of Dose Limiting Toxicity, defined as

- Any treatment related death
- Neutropenia (grade 4 ANC) 4 weeks following treatment.
- Thrombocytopenia (grade 3 or 4 platelets) 4 weeks following treatment.
- Grade 4 infection.
- Grade 3 creatinine level at 4 weeks post treatment.
- Grade 4 acute respiratory distress syndrome.
- Any grade 3 or higher AE, except as described above, at least possibly related to study treatment that does not resolve within 48 hours

The primary efficacy endpoint is objective response rate using RECIST criteria.

Secondary Endpoints:

- Adverse events related to ACT plus IL-2 treatment (description, timing, grade [CTCAE v5.0], severity, seriousness, and relatedness).
- Overall and complete response rate assessed according to RECIST v1.1.
- Progression free survival and overall survival.

10.3 Sample size

There will be up to 4 cohorts of size 3 in each indication (melanoma and HNSCC). The total sample size will be 24 patients or less.

10.4 Safety stopping rule and safety reporting

For each of the first 3 patients in cohort 1, we will generate a safety report listing AEs by attribution at 30 days post TIL infusion and enrollment will be suspended. The trial will not enroll the next patient prior to receiving DSMB approval. After the first 3 patients, this safety report will be generated for each cohort of size 3 at 30 days post TIL infusion, prior to enrolling the next cohort. Each safety report will be filed with the DSMB and the FDA. The DSMB will approve enrollment of the next patient or cohort, as appropriate, or will stop the trial. The trial will suspend within 48 hours if one or more DLT's are observed in any cohort, and a safety report will be forwarded for discussion with the DSMB and the FDA.

10.5 Futility stopping rule

There will be 2 study arms: a melanoma arm and a head and neck cancer arm. After the first 6 patients have been enrolled in a study arm, enrollment will pause in that arm until response has been assessed. If there are one or fewer responses at 8 weeks post TILs, enrollment will cease in that arm due to a low response rate.

In the NCI experience the overall objective response rate using RECIST criteria in 93 patients with metastatic melanoma was 56%. Thus we expect the true response rate to be at least 50%. With this futility stopping rule if the true response rate is 50% the chance of stopping early is only 11%. However if the response rate is only 30%, there is a 42% chance of stopping for futility. If the response rate is 20%, or 10%, the chance of early stopping is 66% and 88% respectively. Thus there is a high chance of stopping early if the response rate is low. The intention is to protect the patients from the considerable toxicity of this treatment regime in the absence of the expected level of efficacy.

10.6 Evaluable populations

Efficacy analyses will be intent to treat, and will use all subjects who have received any study related treatment. Safety analysis will use subjects who have undergone at least one day of preparative regimen.

10.7 Analysis plan

Safety and efficacy will be summarized overall and by study arm. Safety analyses will include Adverse events by attribution including description, timing, grade [CTCAE v5.0], severity, seriousness, and relatedness. Efficacy results will be descriptive and will include overall and complete response rate assessed according to RECIST v1.1. and progression free survival and overall survival.

11.0 ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the investigational agent, is also an AE.

Pregnancy, although reportable, will not be considered an AE or SAE unless the pregnant patient experiences signs or symptoms of pregnancy complications. A follow up pregnancy report form will be completed to document the outcome of the pregnancy. If there is a complication, which qualifies as an SAE, the event will be reported as described in section 11.6.

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.

Interleukin-2:
Expected toxicities of aldesleukin are listed in the product labeling. No expedited reporting of expected grade 3 toxicities or grade 4 laboratory toxicities clearly related to aldesleukin, expected, easily managed, and reversed to less than grade 3 within 48 hours is required. Aldesleukin toxicities that must be reported in an expedited fashion include: need for endotracheal intubation, renal dialysis, coma, myocardial infarction, myocarditis, bowel perforation, sustained ventricular tachycardia, death or any grade 4 toxicity that is not listed in the package insert.

Reporting of laboratory events
Laboratory results (including grade 3 and 4) 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.

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.

Reporting Infection
Febrile neutropenia will be captured as follows: The start date will be the date the fever of 38.5C 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 38.5C 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.

Progression of the cancer under study or events which are unequivocally due to disease progression should not be reported as an AE during the study (unless it is considered to be drug related by the investigator).

11.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

As far as possible, each adverse event should be evaluated to determine:

- duration (start and end dates)
- severity (grade)
- seriousness
- relationship to study agent
- action taken (i.e., none, study agent modification, medical intervention)
- outcome (i.e., resolved without sequelae, resolved with sequelae, ongoing)

Adverse events monitoring begins at the time of initiating study treatment through 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier.

All patients experiencing an adverse event at least possibly related to study treatment will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any clinically significant abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

11.2 Severity

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE v5.0 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

Mild (grade 1): the event causes discomfort without disruption of normal daily activities.

Moderate (grade 2): the event causes discomfort that affects normal daily activities.

Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.

Life-threatening (grade 4): the patient was at risk of death at the time of the event.

Fatal (grade 5): the event caused death.

11.3 Seriousness

A "serious" adverse event (SAE) is defined in regulatory terminology as any untoward medical occurrence that:

1. **Results in death.**
   If death results from (recurrence of) the disease, the disease should be reported as event (SAE) itself.

2. **Is life-threatening.**
   (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

3. **Requires in-patient hospitalization or prolongation of existing hospitalization**.
   Note: Hospitalization (including hospitalization for an elective procedure) for a preexisting condition which has not worsened does not constitute a serious adverse event.
4.
5. **Results in persistent or significant disability or incapacity**. Substantial disruption of one's ability to conduct normal life functions.
6.
7. **Is a congenital anomaly/birth defect.**
8.
9. **Is an important medical event.**
   Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".

   For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

11.4 Relationship

Attribution categories for adverse events in relationship to protocol therapy are as follows:

Definite – The AE *is clearly related* to the study treatment.

Probable – The AE *is likely related* to the study treatment.

Possible – The AE *may be related* to the study treatment.

Unlikely – The AE *is doubtfully related* to the study treatment.

Unrelated – The AE *is clearly NOT related* to the study treatment.

11.5 Prior experience

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered "unexpected", for expedited reporting purposes only, when either the type of event or the severity of the event is <u>not</u> listed as known adverse events in this protocol or the current Investigator's Brochure.

11.6 Reporting Requirements for Adverse Events

11.6.1 Expedited Reporting

- The **Principal Investigator** must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.
- The **UCSD Human Research Protections Program (HRPP)** and **Moores Cancer Center Data and Safety Monitoring Board (DSMB)** must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others" (UPR).

  The following events meet the definition of UPR:

  1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was *unanticipated, involved risk to subjects or others, and was possibly related to the research procedures*.
  2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
  3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
  4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
  5. Any breach in confidentiality that may involve risk to the subject or others.
  6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

11.6.2 Routine Reporting Requirements

- The **UCSD HRPP** must be notified of any adverse events that are not unanticipated problems involving risk to subjects or others (non-UPRs) at the time of the annual Continuing Review.

FDA REPORTING CRITERIA

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 will also notify FDA within 15 calendar days of:

- 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's Brochure.
- 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.

FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report of the progress of the trial within 60 days of the anniversary date that the IND went into effect, then annually as indicated.

12.0 PHARMACEUTICAL INFORMATION

12.1 IL-2 (Aldesleukin, Interleukin-2, Proleukin, Recombinant Human Interleukin 2)

Please refer to Product Label for more comprehensive information.

How supplied: IL-2 is available from commercial sources.

Formulation/Reconstitution: Aldesleukin is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

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

Dilution/Stability: Reconstituted IL-2 should be further diluted with 50 mL of dextrose 5% water. Avoid diluting to a concentration <0.03mg/mL or >0.07mg/mL. IL-2 is chemically stable for 48 hours when refrigerated, 2° –8°C. Use immediately or within one hour when stored at room temperature.

Administration: The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. IL-2 will be administered as an inpatient. Do not use in-line filter during administration. Infusion will be performed per standard inpatient infusion treatment plan established at UCSD.

Toxicities: Expected toxicities of IL-2 are listed in the product label and in Appendix 2 and 3. 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 2. Additional grade 3 and 4 toxicities seen with IL2 are detailed in Appendix 2..

12.2 Fludarabine

Please refer to Product Label for more comprehensive 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: Fludarabine is available from commercial sources and is supplied in a 50 mg vial as 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. Administer finished dose within 8 hours of preparation. Store finished doses at room temperature.

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 30 minutes. The doses will be based on body surface area (BSA).

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 NCI Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

12.3 Cyclophosphamide

Please refer to Product Label for more comprehensive 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.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 36 hourswhen kept at 2-8°C.

Administration: It will be diluted in D5W (concentration </=2mg/mL) and infused over one hour. The dose will be based on the patient's body weight.

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-mercaptoethanesulfonate; 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.

12.4 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex,)

Please refer to Product Label for more comprehensive information.

How Supplied: Mesna will be obtained from commercial sources and is supplied as a 100 mg/ml solution.

Storage: Intact vials are stored at room temperature.

Stability: Mesna is chemically stable at room temperature for 24hours when diluted in dextrose 5% water or 0.9% sodium chloride. See package insert for additional information.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl. Administer intravenously as a continuous infusion.

Toxicities: Nausea, vomiting and diarrhea.

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

How Supplied: Filgrastim and filgrastim-sndz will be obtain from commercial sources. See approved labeling for availability, storage and dosing.

Toxicities: Side effects of filgrastim are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

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

TMP/SMX DS will be obtained from commercial sources and used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO twice daily two times a week (on NON-consecutive days) beginning on Day +21and continuing until the ALC is greater than 1000 for at 60 days. 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.

12.6.1 Aerosolized Pentamidine or Oral Atovaquone in Place of TMP/SMX DS

Pentamidine or Atovaquone will be substituted for TMP/SMX-DS in patients with sulfa allergies. Pentamidine 300 mg per nebulizer within one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and and for at least 60 days. Pentamidine Isethionate will be obtained 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. Atovaquone will be administered at 1500mg by mouth daily after the first dose of chemotherapy on or after Day +21 and stopping when absolute lymphocyte count is >1000/mm3 for at least 60 days. Atovaquone is commercially available and supplied as 750mg/5mL suspension. Known side effects include rash, diarrhea, nausea, vomiting, headache, insomnia and, in rare cases, increase in liver enzymes, Stevens-Johnson and methhemoglobinemia. See FDA approved package insert for additional information. .

12.7 Herpes Virus Prohpylaxis

12.7.1 Valacyclovir (Valtrex)

Valocyclovir will be obtained from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. See package insert for dosing, storage and additional information. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

12.7.2 Acyclovir

Acyclovir will be obtained from commercial sources. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. gancyclovir. In renal disease, the dose is adjusted as per product labeling. Prepare and administer per institutional standard. See package insert for additional information.

12.8 Fluconazole

Fluconazole will be obtained from commercial sources. It will be used to prophylax against fungal infections. Prepare and administer per institutional standard. See package insert for more information.

12.9 Cell Preparation

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

12.10 Support Medication

Odansetron hydrochloride

Odansetron hydrochloride will be obtained 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 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.

13.0 STUDY MANAGEMENT

13.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed according to UCSD conflict of interest policy.

13.2 Institutional Review Board (IRB) Approval and Consent

The IRB should approve the consent form and protocol prior to any study-related activities. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state

regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

13.3 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), subjects who have provided written informed consent must also sign a subject authorization to release medical information to the study Sponsor and allow a regulatory authority, or Institutional Review Board access to subject's medical information relevant to the study.

13.4 Data and Safety Monitoring/Auditing

In addition to adverse event monitoring and clinical oversight by the principal investigator and co-investigators, quality assurance of the study may be performed by the Moores Cancer Center Clinical Trials Office internal monitor. Monitoring intervals will be dependent upon the number of patients enrolled and the complexity of the study.

This study will also use the UCSD Moores Cancer Center Data Safety and Monitoring Board (DSMB) to provide oversight in the event that this treatment approach leads to unforeseen toxicities. Data from this study will be reported every six months and will include:

1) The protocol title, IRB protocol number, and the activation date of the study.
2) The number of patients enrolled to date.
3) The date and site of patients' enrollment.
4) A summary of all adverse events regardless of grade and attribution, as well as toxicities 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.

13.5 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

13.6 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

14.0 APPENDICES

Appendix 1. CS-1000 labeling of TIL

1 Summary

TIL were cultured and expanded according to clinical TIL protocols in the UCSD Advanced Cell Therapy Lab. 4 flasks of TIL were labeled at 10mg/mL of CS-1000 for the final 24 hours of culture (days 13-14). A fifth flask was maintained as a control. Individual flasks were sampled and a pool of the 4 labeled flasks was also analyzed. Labeled TIL, both individual flasks and pooled sample, had comparable viability to the unlabeled control. The labeling across the individual flasks was comparable to the pooled sample. All TIL, labeled and unlabeled, met the clinical release criteria of viability, yield, and IFN ɣ potency.

2 Viability

Sample	Viability (%)
Labeled 1	85
Labeled 2	93
Labeled 3	91
Labeled 4	88
Pooled Sample	93
Control	94

*all samples met release parameters

3 Label uptake

Sample	Cell count	NMR value	F/c	Average
Labeled 1	5.0×10^6	30.7	1.49×10^{13}	5.34×10^{13}
Labeled 2	4.5×10^6	4.77	2.58×10^{12}	
Labeled 3	$4.5x\ 10^6$	3.80	2.06×10^{12}	
Labeled 4	$4.5x\ 10^6$	3.37	1.82×10^{12}	
Pooled Sample	$4.0x\ 10^6$	11.44	6.96×10^{12}	-

Labeling efficiency will be measured for each batch of TIL. F/c = Fluorine atoms per cell, this number is used to quantify the number of cells in a MRI region of interest.

4 Phenotype

Population/marker	Control	CS-1000 labeled
Lymphocyte (%)	85.14	84.28
CD3+ (%)	84.29	83.26
CD4+ (%)	50.72	49.52
CD8+ (%)	42.48	43.98

Phenotype was assessed on a pooled sample of the labeled TIL compared to unlabeled control TIL. Phenotyping is not measured for release, but was assessed along with the IFNɣ potency testing.

5 IFN ɣ potency

Criteria	CS-1000 Labeled Cells	Unlabeled Cells
Frequency of IFNg+ T cells exceeds 3% of CD3+ Lymphocytes	3.65%	8.68%
Frequency of IFNg+ T Cells is greater than 2X the negative control	3.65 / 0.65 = 5.6X	8.68 / 2.56= 3.4X
Criteria Met	Yes	Yes

IFNɣ potency performed on a pooled sample of the labeled TIL compared to unlabeled control TIL.

Appendix 2. Adverse Events Occurring in ≥ 10% of Patients Treated with IL-2

Source: Proleukin® Prescribing Information – (n=525)

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
Arrythmia	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	Pruritis	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.

Appendix 3. Expected IL-2 Toxicities and their Suggested Management

Expected Toxicity	Expected Grade	Supportive Measures	Stop Cycle*	Stop Treatment**
Chills	3	IV Meperidine 25-50 mg, IV q15min prn chills or rigors	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethacin 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 8mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg q4h p.r., prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest interspersed with activity	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/ Weight gain	3	Diuretics prn	No	No

Expected Toxicity	**Expected Grade**	**Supportive Measures**	**Stop Cycle***	**Stop Treatment****
Hypotenstion	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	Yes
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours
** Unless the toxicity is not reversed to grade 2 or less by next re-treatment.

Appendix 4. Response Evaluation Criteria in Solid Tumors (RECIST)

Tumor assessments will be made according to the schedule of assessments. Response and progression will be evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines [Eisenhauer et al. 2009].

1 Definitions

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

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

1.1 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

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

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be

those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

1.2 Methods for Evaluation of Measurable Disease

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

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

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

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI . This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-

PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

1.3 Response Criteria

1.3.1 Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

1.3.2 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

1.3.3 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.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

1.3.4 Duration of overall response

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

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

1.3.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Appendix 5 Communication Plan

Purpose: The purpose of this plan is to define communication practices at the time patients initiate lymphodepletion chemotherapy and final product sterility testing.

Scope: The Advanced Cell Therapy Lab (ACTL) is a Good Manufacturing Process (GMP) facility providing the autologous cell product and testing as outlined by the standard operating protocols (SOPs) detailed in the Chemistry, Manufacturing and Control (CMC) section of the IND. The principle investigator (PI) is the holder of the IND and responsible for the safe and faithful implementation of the IND. The treating physician is the attending physician for the patient and responsible for writing orders for patient care. This plan formalizes the communication during the time patients begin preparative chemotherapy to the time of final product sterility testing between the ACTL, PI and the attending physician.

Methods of communication:

1. List Serve email list. The email listserv will be updated and acknowledged by the PI with the start of each product initiation. The listserv will be maintained by the project manager for the Cutaneous team. The updating and use will be documented on the eligibility check list. The email list will minimally include the treating physician, principal investigator, investigational pharmacy, the ACTL, the primary study coordinator and the project manager
2. Medical record
3. Treatment plan orders

Communication plan:

1. An initial “list serve email” will be sent when a consented patient in deemed eligible. Confirmation of receipt from investigational pharmacy, the principal investigator, treating physician and ACTL will be requested and documented by the research coordinator.

2. A second email will be sent when product expansion is confirmed and a date for lymphodepletion has been tentatively set. This email will include the cell phones for the principal investigator (Dr. Daniels), Dr. Cohen (back up to Dr. Daniels) and the attending physician (if different).

3. Prior to initiating lymphodepletion, the principal investigator or sub-investigator will verify with ACTL that there is no concern for infection and document that the patient is ready to proceed in the medical record. The infusion nurse and pharmacy will verify this documentation prior to infusion of chemotherapy per treatment plan order for that day.

4. The principal investigator or sub-investigator will verify daily with the ACTL that there is no concern for infection during lymphodepletion and document this communication in the medical record. The infusion nurse will verify this documentation prior to infusion of chemotherapy per treatment plan order for that day.

5. Prior to cell product release from the ACTL and patient infusion, the preliminary release certificate will be signed by the principal investigator (approx. 1 week after lymphodepletion begins). The final release certificate may take up to 28 days after product infusion. The investigator will confirm weekly with the ACTL until the final certificate is generated.

Notes: The product release form has a specific line indicating the communication time that product release was determined. If the product is deemed not meeting release parameters, a verbal notification will be documented and acknowledged by either Dr. Daniels or Dr. Cohen.

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