A Phase I Trial of Lymphodepletion plus Adoptive Cell Therapy with High-Dose IL-2 in Adolescent and Young Adult Patients with Soft Tissue Sarcoma

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1 STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the Supporting Agency Terms. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed of their obligation to meet the above commitments.

Principal Investigator: John E. Mullinax, MD Print/Type Name

Signed:_____ Date:

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Phase I Trial of Lymphodepletion plus Adoptive Cell Therapy with High-Dose IL-2 in Adolescent and Young Adult Patients with Soft Tissue Sarcoma

Study Description: Advanced sarcoma patients have few systemic treatment options, leading to a median survival expectation of only 1 year. Novel treatment approaches leveraging the adaptive immune response may offer some hope of improved outcomes. Preclinical evidence and results from this treatment approach with a similarly heterogenic solid tumor type, metastatic melanoma, are encouraging. Therefore, we hypothesize that use of tumor-infiltrating lymphocytes from within soft tissue sarcoma in an adoptive cell therapy approach will yield improved outcomes for advanced sarcoma patients. Before testing this hypothesis, the safety and tolerability of this treatment approach must be established.

Objectives:

Primary Objective: To determine the safety and feasibility of treatment with adoptively transferred tumor-specific T cells following nonmyeloablative lymphodepleting chemotherapy followed by high-dose IL-2 for patients with advanced sarcoma.

Secondary Objectives:

1. To observe the objective antitumor responses per RECIST v1.1 criteria

2. To evaluate the degree of sustained persistence of infused T cells

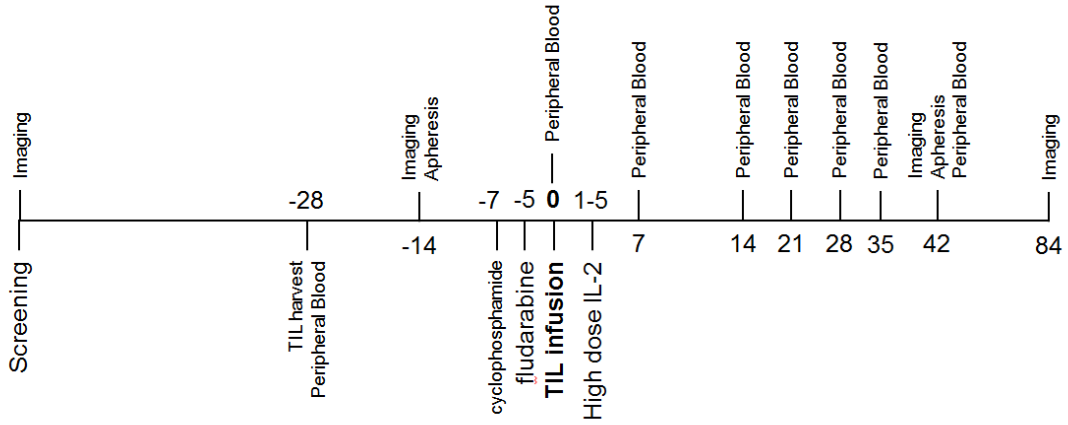
Endpoints:

Primary Endpoint: Patients able to safely tolerate infusion of TIL and subsequent IL-2, as measured by adverse event rate

Secondary Endpoints:

	1. Objective response (CR + PR) rate at 12 weeks following TIL infusion, as measured by RECIST v1.1 2. Persistence of TIL infusion product at 6 weeks following treatment, as measured by TCR repertoire comparison between the infusion product and circulating PBMC
Study Population:	Up to 15 patients, male and female, aged 18 to 39 years
Phase:	Phase I
Description of Sites/Facilities Enrolling Participants:	The H. Lee Moffitt Cancer Center and Research Institute, a high-volume, NCI-designated Comprehensive Cancer Center in Tampa, FL.
Description of Study Intervention:	Patients will undergo resection of a soft tissue sarcoma from which tumor-infiltrating lymphocytes will be cultured and expanded. This expanded TIL product will be infused following, nonmyeloablative lymphodepleting chemotherapy. High-dose IL-2 will be given after TIL infusion to support the cell product expansion.
Study Duration:	22 months
Participant Duration:	12 months

1.2 SCHEMA



Treatment

Days -7 and -6:

Cyclophosphamide 60 mg/kg/day intravenously (IV) in 250 mL normal saline (NS) over approximately 2 hours. Mesna 20 mg/kg with D5W or NS at 125 mL/hour, infused IV over 24 hours.

Days -5 to Day -1:

Fludarabine 25 mg/m^2 IV piggyback daily over approximately 30 minutes for 5 days.

Day 0:

T-cell infusion in 250 to 1000 mL NS over approximately 15 to 60 minutes depending on the volume to be infused. The rate of infusion may be adjusted based upon criteria listed in the adoptive cell therapy standard operating procedure (SOP). Vital signs (temperature, blood pressure, pulse, and respiratory rate) and pulse oximetry will be monitored per inpatient unit protocol following cell infusion and IL-2 administration.

Days 1 to 5:

High-dose IL-2, 600 000 IU/kg IV bolus (about 15 minutes) every 8 to 16 hours for up to 15 doses, beginning approximately 8 to 16 hours after T-cell infusion.

1.3 SCHEDULE OF ACTIVITIES (SOA)

				Hospital Stay			Hospital Stay					
Assessment	Screen	D -28*	D -14	D -7	D -6	D -5	D 0	D 1	Day 21 (+/7 days)	D 42 (+/- 7 days)	D 84 (+/- 7 days)	Every 3 mos for 12 mos (+/- 14 days)
Informed consent	●											
Medical history	●		●				●		●	●	●	●
Current medications	●		●	●			●		●			●
Physical Exam	●		●	●			●		●	●	●	●
Vital Signs	●		●	●	●	●	●	●	●	●	●	●
Performance Status	●		●				●		●			●
Baseline Symptom Assessment	●											
MUGA and PFT (1, 4)	●											
EKG (3)	●		●							●(3)	●(3)	●(3)
Blood Tests (1)												
CBC with diff, CMP	●		●	●	●	●	●	●	●	●	●	●
LDH	●		●							●	●	●
Blood clotting tests (PT/PTT)	●		●									
HLA typing (if not done before) (2)	●											
EBV ab	●											
Hepatitis B and C, HTLV-1 & 2	●											
HIV, RPR (FTA if necessary) (7)	●											
Thyroid Tests (Free T4, TSH) (8)	●		●				●		●			●
Pregnancy Test (5)	●		●	●					●			●
PBMC Collection (14)		●					●(q 1 wk x 5)		●		●	●
Immunodeficiency Panel											●	
Radiography Tests: CT Chest-Abdomen-Pelvis,(1, 6)	●		●							●	●	●
Tumor sample/biopsy for TIL		●										
Adverse Event Assessment (9)		●	●	●	●	●	●	●	●	●	●	●
Urinalysis (10)	●		●			●	●		●			●
Leukapheresis (16)			●							●		
Lymphodepletion:												
Cyclophosphamide (hospital x 2) (11, 12)				●	●							
Fludarabine (outpatient x 5) (11, 12)						●						
Adoptive Transfer of TIL (13)							●					
High dose IL-2 (hospital 7-10 days)								●				

*Study treatment dates are approximate; precise days will depend on rate of TIL growth and patient's clinical status.

Footnotes to the Treatment Schema

1. At screening, all laboratory and imaging studies must be complete and satisfactory within 30 days of signing the consent document with the exceptions of:
   a. HLA-typing will not be repeated if performed previously.
   b. Serum pregnancy test for women of child-bearing potential (see section 5.1.1) will be done within 7 days of screening for the trial, within 7 days of screening for chemotherapy.
   c. Pulmonary function tests (PFTs) and/or cardiac MUGA whose results are valid for 6 months if performed previously unless there is an interval change in the patient's clinical status determined by the Moffitt treating physician.
   d. CT imaging of the chest, abdomen and pelvis completed within 30 days prior to signing the consent may be used to confirm measurable disease per RESIST V1.1 criteria, and may be used for screening eligibility.
2. HLA typing will be sent at screening only if not done previously.
3. EKGs will be obtained at screening for the trial, at screening for chemotherapy, at 6 and 12 weeks after adoptive transfer of TIL, and then every three months for 18 months.
4. PFTs are required at screening. A cardiac MUGA scan will be done at screening in all patients in consideration of prior Adriamycin-containing chemotherapy regimens, considering the safe use of high dose IL-2. If PFTs and/or a cardiac MUGA scan have been done within 6 months of screening, they will not be repeated unless there is an interval change in the patient's clinical status determined by the Moffitt treating physician.
5. Serum or urine pregnancy test will be performed on women of child-bearing potential (see 5.1.2) during screening and again prior to initiation of chemotherapy.
6. CT scans of chest, abdomen, pelvis, and other areas will be done as outlined in the schedule of activities, or as judged appropriate by the treating physician or PI. For patients with CT IV contrast allergy, appropriate premedication, use of MRI or CT without IV contrast will be undertaken at the discretion and judgment of the ordering physician. Clinical visits after each of the scans will also include documentation of size and location of palpable lesions.
7. FTA only if RPR is positive
8. If T4 and TSH are abnormal, thyroid workup will be done.
9. Adverse events will be collected per section 8.4 throughout the study protocol beginning at initiation of treatment (day -28) and continuing at each treatment visit detailed in the study treatment schema until any one of the following: progression, loss to follow-up, withdrawal of consent, or death. Beyond 100 days from study treatment, subjects will continued to be followed for ongoing drug-related adverse events until resolved, return to baseline, deemed irreversible by the Moffitt treating physician, or until the subject is lost to follow-up, there is withdrawal of study consent, removal of the subject from the trial by the Moffitt treating physician, or start of a subsequent anti-cancer therapy.
10. Urinalysis will be done as indicated in the study calendar with the day -14 result used for Chemotherapy/Cell Infusion inclusion criteria.
11. Cyclophosphamide will be administered on Days -7 and -6, fludarabine will be administered on Days -5 to -1. CBC with differential and CMP will be performed every day of treatment during preparative cyclophosphamide/fludarabine and TIL/high dose IL-2 therapy per section 8.1.1.
12. Within 4 weeks prior to the initiation of chemotherapy, patients will undergo physical examination and documentation of size and location of measurable lesions where applicable. The timing of the start of the lymphodepleting regimen of cyclophosphamide and fludarabine is subject to change depending upon the rate of the TIL growth.

13. Adoptive transfer will take place at day 0.
14. 60 mL of PBMC will be collected as defined in the schedule of activities. On day 0, only 10mL of PBMC will be collected. Of note, if apheresis sample cannot be collected on day 42 due to a technical issue, then an additional venipuncture for 60 mL peripheral blood mononuclear cells will be collected and used as a substitute. Samples will be used for immune monitoring and determination of TIL persistence with an informal comparison to results generated from our previous adoptive cell therapy trials that utilized the same collection schedule. Blood draws will be omitted for patients with symptomatic anemia or deemed not feasible by the PI and/or treating physician.
15. Follow-up visits with disease evaluation will be conducted at days 42 and 84 (+/- 7 days) after TIL infusion, then every 90 days (+/- 14 days) for 12 months thereafter or until disease progression, withdrawal of consent, loss to follow-up, or death, whichever occurs first. After confirmed disease progression subjects will be followed every 3 months (+/- 4 weeks) for overall survival and report of any subsequent anti-cancer treatments. This can be accomplished by visit, phone or email contact.
16. Apheresis will be performed at day -14 and at day 42 (+/- 7 days). These samples will be used for immune monitoring purposes and determination of TIL persistence by comparing TCR clonality to the TIL infusion product.

2 INTRODUCTION

2.1 STUDY RATIONALE

Patients with advanced sarcoma have limited effective systemic treatment options available to them and are often young, falling into either the pediatric or young adult age groups. With current treatment options, patients can expect a median response duration of only 9 months and median survival of only 1 year. Because of the youth of those affected, death from sarcoma results in a significant loss of productive life years.

Novel approaches leveraging the adaptive immune response have been developed for several malignancies. Collectively termed “immunotherapy,” there are two broad approaches. First, monoclonal antibody therapy is used to reverse tumor-specific immune suppression directly. This has demonstrated efficacy for melanoma, renal cell carcinoma, and non-small cell lung cancer, and a plethora of open protocols have accrued for other disease states. Second, cellular immunotherapy strategies exist that seek to actively transfer a complement of tumor-specific T-cells into a patient, with the goal of enacting a living therapy that produces a very durable response.

Within the cellular immunotherapy approaches, some investigators have chosen to genetically modify T-cells towards a tumor-specific antigen target. This approach has been dramatically effective for hematologic malignancies but not solid tumors, possibly because of the general clonal nature of hematologic malignancies, in contrast to the incredible heterogeneity of solid tumors. Another approach is to use tumor-infiltrating lymphocytes (TILs) from within solid tumors as cellular therapy. The goal of this treatment approach, called adoptive cell therapy (ACT), is to circumvent suppressive

or tolerogenic influences *in vivo* through expansion of TILs *ex vivo*, followed by adoptive transfer. This has been shown to be effective for patients with metastatic melanoma, and there are currently open trials using this approach for squamous cell carcinoma of the cervix, squamous cell carcinoma of the head and neck, and non-small cell lung cancer. We believe that the ACT approach is superior due to the polyclonal nature of the cell product which better matches the heterogeneity of solid tumors. This has been borne out in clinical studies in which the TILs approached a response rate of nearly 40% for metastatic melanoma patients[1-4], whereas the success rate for gene-modified T-cell approaches in solid tumor patients has been much lower [5, 6].

We recently completed a pilot project that has generated preclinical data supporting the use ACT for patients with sarcoma. This project was undertaken using protocols developed in and validated by our significant melanoma ACT experience. In this project, we were able to successfully grow TILs from resected adult primary sarcoma specimens. The TILs grown from these specimens were expanded to a clinically meaningful number, and there was antitumor activity when the TILs were co-cultured with autologous tumor digest or human leukocyte antigen- (HLA-) matched sarcoma cell lines.

We hypothesize that ACT for patients with advanced soft tissue sarcoma will be safe and well-tolerated. Furthermore, we expect that the culture and expansion of this product is feasible based on preclinical laboratory work. We also hypothesize that the infusion of this TIL product will yield improved outcomes for advanced sarcoma patients.

2.2 BACKGROUND

2.2.1 INITIAL STUDIES OF THE IMMUNE INFILTRATE IN SOFT TISSUE SARCOMA

We recently completed a pilot protocol, funded by the Chotiner Foundation, which focused on the immune infiltrate of 37 adult (> 18 years old) patient-derived sarcoma specimens acquired from 3/2015 to 6/2017. Eleven different subtypes of sarcoma were identified, 78% of the tumors were high grade, 27% were recurrent, and 38% of patients had had preoperative therapy. An average of 48% (range, 3.6-76) cells in the tumor digest from the lymphocyte gate were CD3+ and TILs were grown from at least one fragment of all specimens. The phenotype of the CD3+ subpopulations from TIL cultures included an average 58% (range, 7-97) CD8+ and 19% (range 2-79) CD4+ cells. There was dramatic heterogeneity among patient TIL cultures. The function of expanded TILs has been tested, and an ELISA assay has identified tumor-specific function, as measured by interferon-gamma release after co-culture with an autologous tumor digest.

2.2.2 INITIAL STUDIES OF ADOPTIVE T-CELL TRANSFER FOR THE TREATMENT OF HUMAN CANCER

The identification of T cells with the ability to specifically recognize melanoma antigens, along with the technological capability to expand these tumor-reactive T cells to large numbers in the laboratory, has

led to the development of adoptive transfer protocols for patients with metastatic melanoma. TILs derived from resected tumors that were expanded in vitro were shown to be capable of specifically recognizing tumor antigens, particularly MART-1, in over two-thirds of melanoma patients[7, 8]. Such TILs, when expanded to large numbers (greater than 10 billion) and adoptively transferred intravenously to patients along with IL-2, resulted in an objective response rate of 35% [9, 10]. This response rate was nearly twice that observed with IL-2 alone and was also seen in patients that were refractory to IL-2 treatment.

In the first description of ACT, published in *Science* by the NCI Surgery Branch, 6 out of 13 patients demonstrated objective tumor regression, and 4 additional patients showed mixed responses, with substantial shrinkage of some lesions after lymphoid depletion and adoptive transfer of highly selected expanded tumor-reactive TILs and IL-2 [10]. Significant levels of tumor regression were observed in metastatic deposits in the liver, lungs, cutaneous and subcutaneous tissues, and lymph nodes. One patient had dramatic regression of axillary, pelvic and intraabdominal metastases, on-going at 17 months, and was rendered free of disease by a surgical removal of one residual intraperitoneal lesion. Two other patients had marked, persistent lymphocytosis up to 3 weeks after the TIL infusion. Molecular and immunological analyses confirmed that these lymphocytes from their peripheral blood were the progeny of the infused TIL [10]. In particular, in one patient, it was shown that one specific clone repopulated this patient's peripheral blood lymphocytes (PBL) up to 2 months after infusion. Immunohistochemistry studies revealed that specific clones from infused bulk oligoclonal TIL cells infiltrated the regressing tumor nodules. Although the mechanism for the continued proliferation in vivo of these rapidly expanded bulk TIL cells and their antitumor effects remain to be determined, it appears that some cells in the expanded bulk TIL might have provided necessary cytokines (such as IL-2) for cytotoxic T lymphocytes to persist and survive in vivo and eventually kill the tumor. Alternatively, the chemotherapy regimen used in this protocol might have depleted endogenous suppressive lymphocytes.

Our institution has published 2 reports of our experience with ACT in patients with metastatic melanoma. The first experience described the infusion of TILs followed by high-dose IL-2 after a nonmyeloablative lymphodepleting chemotherapy regimen administered in a manner identical to the NCI experience. There were 5 objective responses among the 13 patients treated (38%), which was a similar rate of efficacy to that uncovered by the NCI experience [4]; however, progression during cell preparation (6/19, 21%) was a limiting factor and the response rate by intention-to-treat analysis was 26%. A strategy was developed that added ipilimumab, a monoclonal antibody against cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4) to the treatment before TIL harvest and during cell preparation. In this experience, the attrition due to progression was decreased to 7% and the response, by intention-to-treat analysis, was increased to 38.5% from 26% in the first experience [3].

2.2.3 ADDITION OF NONMYELOABLATIVE LYMPHODEPLETION TO ADOPTIVE CELL TRANSFER

These collective results suggest that the nonmyeoablative, lymphodepleting chemopreparative regimen identical to that which is proposed in this current study can be tolerated for up to 2 full cycles and is potentially efficacious for the treatment of advanced metastatic disease. This may be due to the

homeostatic vacuum created by the chemotherapy regimen. After ablation of the endogenous lymphocyte compartment, the infused TIL cells may expand better in vivo without competition from endogenous lymphocytes. At the NIH, marked proliferation of the transferred TIL cells was not observed without this course of chemotherapy. It also appears that patients treated with the preparative regimen sustained lesser toxicities and tolerated more cycles of intravenous high-dose IL-2 that patients receiving IL-2 without non-myeoablative lymphodepleting chemotherapy. Thus, in the proposed study, the plan is to administer this preparative lymphodepleting chemotherapy regimen to patients prior to TIL cell infusion.

Mounting evidence suggests that the host immune environment can significantly impact the efficacy of adoptive cell transfer therapy. Data from mouse tumor models have demonstrated that sublethal doses of irradiation prior to adoptive transfer of tumor antigen-specific lymphocytes substantially increases the persistence and antitumor activity of the transferred cells [11]. While the mechanisms leading to this enhanced T-cell activity have not been precisely delineated, 2 non-mutually exclusive hypotheses may provide explanation. A subset of CD4+ T cells, expressing high levels of CD25 and the molecule FOXp3 and known as regulatory or suppressor T cells, is thought to have a negative impact on the activity of cytotoxic T cells *in vivo* [12, 13]. It has been hypothesized that increased numbers of suppressor T cells in cancer patients may correlate with an unfavorable prognoses and that elimination of these cells may result in an improved efficacy of adoptive immunotherapy [14-17]. Alternatively, prior depletion of lymphocytes may create ‘space’ for the adoptively transferred cells within the lymphocyte compartment [18]. Under this model, homeostatic lymphocyte survival may result in increased proliferation and enhanced survival of transferred T cells, perhaps through a mechanism involving increased access to endogenous cytokines like IL-7 and IL-15 [19].

Traffic of TILs to tumor sites was evaluated by labeling TIL with indium-111 and performing sequential radionuclide scans. Of 26 patients who received cyclophosphamide, TIL trafficking to tumor was seen in 21 patients (81%), which was greater than the TIL trafficking to tumor that was seen in the 42% of patients who did not receive cyclophosphamide (p = .026). No differences were seen in tumor regression rates. Thus, even with a mild and very transient leukopenia (about 5 days), evidence of increased lymphocyte trafficking to tumor was observed [20].

The animal and clinical studies cited above strongly suggest that the clinical effectiveness of these cells and their abilities to survive and repopulate the host would be enhanced if patients were significantly immunosuppressed by the depletion of lymphocytes prior to the adoptive transfer of lymphocytes.

A recent clinical trial investigated the addition of a lymphodepleting conditioning regimen to adoptive cell transfer therapy in patients with metastatic melanoma. Patients received a lymphodepleting chemotherapy regimen consisting of high-dose cyclophosphamide and standard doses of fludarabine before administration of highly selected, expanded, tumor-reactive TILs and IL-2 [1, 2]. The lymphodepletion step resulted in a transient myelosuppression and the elimination of all circulating lymphocytes for approximately 1 week, after which time patients recovered endogenous marrow function and had reconstituted their lymphocyte compartments towards normal levels within 2 to 3 weeks [21].

Because of the immunosuppression of fludarabine, one of the patients who had clonal repopulation from infused TIL cells and a complete response of metastatic melanoma, developed Epstein-Barr virus- (EBV-) associated B cell lymphoma. This patient was EBV-naïve prior to the treatments. The potential source of EBV was thought to be multiple blood product transfusions after chemotherapy. The patient later died of complications from the treatment of his lymphoma. Another patient developed polyneuropathy manifested in vision blindness, and motor and sensory defects approximately 2 months after chemotherapy. The etiology of this complication is unknown, but was possibly related to the fludarabine [1].

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The resection of tumor followed by non-myeloablative chemotherapy, adoptive TIL therapy, and high dose IL-2 regimens in this protocol entail serious potential discomforts and hazards for the patient. Side effects of common drugs used in this non-myeloablative regimen include:

Surgical Resection: Risks of resection are specific to the operation required for each patient to acquire tumor from which to culture the TIL product. General risks include infection, bleeding, injury to neurovascular structures or viscera, and venous thromboembolism. Resections on this protocol are designed with the lowest possible morbidity and lesions are chosen that will yield tissue without undue risk and for this reason superficial soft tissue lesions will be the preferred resection site. There are no specific inclusion or exclusion sites of harvest given that soft tissue sarcoma has a varied metastatic pattern between subtype histologies. Prior research has shown that the acquisition of visceral tumors for the initiation of TIL culture is safe and feasible[22]. Data from our own institution on other adoptive cell therapy clinical trial protocols further supports the safety of this approach. Superficial soft tissue sites will be prioritized over deep lesions to limit morbidity and lung resections will be considered in patients without a focus of disease in the soft tissue. Visceral and cavitary lesions will be obtained using a minimally invasive approach (i.e. video-assisted thoracoscopic surgery or laparoscopy) and open laparotomy or thoracotomy will not be used as a method to obtain tissue.

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, and Syndrome of Inappropriate Antidiuretic Hormone release.

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.

High Dose IL-2: A variety of side effects have been associated with high-dose IL-2 administration in our experience at the NCI and a listing of these side effects in 652 patients who received 1,039 treatment courses are listed in the Appendix D.

Adoptive cell therapy with TIL: a variety of side effects that potentially overlap with high dose IL2 have been associated with adoptive cell therapy in our experience and at the NCI. Prominent long-term side effects include: vitiligo, high frequency hearing loss, anemia and uveitis.

2.3.2 KNOWN POTENTIAL BENEFITS

While the design of this study is not to evaluate primarily the efficacy of this treatment, there is potential for response to ACT in patients with advanced soft tissue sarcoma. Extrapolating from the experience with melanoma patients (which has been recapitulated in the laboratory setting), ACT can yield meaningful responses in approximately 40% of patients. Notable among these responses is the durability of response compared to other treatments.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The patients enrolled on this trial have no other treatment options that are known to be effective. Any treatment course pursued outside of this trial will entail risks generally equal to those on this trial with generally less benefit since most of the trial alternatives are first in human trials of novel compounds. The treatment strategy of this protocol is not new and therefore some expectation regarding efficacy can be extrapolated from the prior ACT trials in patients with advanced cancer. Of note, the primary toxicity of this treatment is related to the IL-2 given after TIL infusion. The end organ reversible toxicities seen in prior trials were demonstrated in patients much older than the cohort enrolled on this trial. With superior end organ function of this patient cohort, the toxicity profile should be diminished.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the safety and feasibility of treatment with adoptively transferred tumor-specific T cells following nonmyeloablative lymphodepleting chemotherapy followed by high-dose IL-2 for patients with advanced sarcoma.	Patients able to safely tolerate study treatment dosage and procedures.	While this treatment strategy has been used in other advanced malignancies, this trial represents the first use in soft tissue sarcoma. Prior treatment of these patients and the starting material (metastatic tumor vs. tumor bearing LN) is unique, justifying a phase I approach.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
1. To observe the objective antitumor responses per RECIST v1.1 criteria	1. Objective response (CR + PR) rate at 12 weeks following TIL infusion, as measured by RECIST v1.1	Evaluate the efficacy of the infusion product and possible justification for subsequent phase 2 trial
2. To evaluate the degree of sustained persistence of infused T cells	2. Persistence of TIL infusion product at 6 weeks following treatment, as measured by TCR repertoire comparison between the infusion product and circulating PBMC	Evaluate the biologic capacity of the infusion product and association between persistence and response
Tertiary/Exploratory		
1. Evaluate the properties of the TIL culture and expansion including degree of expansion and immunologic phenotype of the infusion product.	1. Success of primary TIL culture 2. Degree of expansion during REP 3. Frequency of lymphocyte subpopulations in the preREP and infusion product	Data regarding the cell product will help to design future trials of ACT in soft tissue sarcoma and other solid tumor malignancies

4 STUDY DESIGN

4.1 OVERALL DESIGN

The current study will be a single-arm exploratory trial to evaluate prospectively the feasibility and toxicities of the TIL treatment protocol specified and the persistence of TIL survival in vivo following treatment.

Survival of infused TIL will be monitored by peripheral blood samples obtained as described in the Schedule of Activities (Section 1.3). Up to fifteen patients will be enrolled to obtain five eligible patients and enrollment will be sequential.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

A phase I design was chosen since this trial represents the first experience of ACT in patients with advanced soft tissue sarcoma. Patients will have vastly different prior chemotherapy regimen experience than patients enrolled on other ACT trials for solid tumors and therefore the safety of this schema is important to evaluate. Specifically, the immune reconstitution following lymphodepletion in a patient population with prior cytotoxic chemotherapy treatment is a concern as patients with metastatic melanoma have not been typically treated in this manner. The established immune reconstitution kinetics are unknown and therefore represent a concern regarding safety. Additionally, nearly all patients will have received prior Adriamycin-containing regimens and therefore the cardiac function may be impaired. While the function will be assessed and those with depressed function excluded, cardiac dysfunction would exacerbate the toxicity of IL-2.

In addition to safety, the feasibility of this regimen is important to consider in this trial design. Prior ACT trials have generally used tumor-bearing lymph nodes as the starting material from which the TIL product is generated. Soft tissue sarcoma does not typically metastasize via the lymphatic system and therefore a recurrent tumor or metastatic deposit will be used for the starting tissue in this trial. Extensive prior laboratory experience has generated positive results regarding the expansion of a TIL product from soft tissue sarcoma after several technical manipulations from the prior melanoma methods. It is important to establish the feasibility of consistently generating a TIL infusion product for patients with soft tissue sarcoma.

4.3 JUSTIFICATION FOR DOSE

As mentioned above, the lymphodepleting chemotherapy regimen and IL-2 infusion schedule are identical to the schema used in prior ACT trials at this institution and within the NCI Surgery Branch. The number of TIL in the final infusion product has been reported in the 1-10 billion range for prior ACT trials. Preclinical laboratory experience with soft tissue sarcoma samples has shown similar expansion profile and therefore the expectation is that a similar number of TIL will be in the final infusion product.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA: 2-STEP DESIGN

A 2-step design will be used to evaluate patients for eligibility:

5.1.1 STEP 1: RESECTION OF TUMOR & INITIATION OF TIL EXPANSION

Patients must fulfill all of the following criteria to be eligible for the study at the time of tumor resection and initiation of TIL expansion.

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 18 to 39 years
4. Patients must have metastatic, high-grade soft tissue sarcoma, all subtypes will be eligible
5. Residual measurable disease after resection of target lesion(s) for TIL growth
6. Clinical performance status of Eastern Cooperative Oncology Group (ECOG) 0 to 1. ECOG performance status of 0 to 1 will be inferred if the patient's level of energy is ≥ 50% of baseline.
7. Patients must have progressed on at least one prior standard of care treatment regimen for metastatic disease.
8. A negative pregnancy test (urine or serum) must be documented at screening for women of childbearing potential.
9. A MUGA scan (ejection fraction > 50% is required) ≤ 6 months prior to lymphodepletion.
10. Pulmonary function tests should be completed ≤ 6 months prior to lymphodepletion and forced expiratory volume (FEV1) > 65% or FVC > 65% of predicted are required.
11. Adequate renal, hepatic, and hematologic function, including creatinine of ≤ 1.7 gm/dL, total bilirubin ≤ 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL, AST and ALT of less than 3 X institutional upper limit of normal, hemoglobin of 8 gm/dL or more, white blood cells of 3000 per mm^3 and total granulocytes of 1000 per mm^3 or more, and platelets of 100 000 per mm^3 or more.
12. Patients must have a positive screening EBV antibody titre on screening test.
13. Patients that had previously grown sterile, validated TILs under good manufacturing practices (GMP) conditions meeting the above criteria are eligible using the previously established TIL product stored in the Cell Therapies Core facility for up to 2 years after harvesting.
14. All laboratory and imaging studies must be completed and satisfactory within 30 days of signing the consent document.

5.1.2 STEP 2: CHEMOTHERAPY/CELL INFUSION INCLUSION CRITERIA

To be eligible for chemotherapy/cell infusion, patients must fulfil the following criteria:

1. Patients must have adequate TILs available, as described in Appendix B.
2. Patients of both sexes must practice birth control for 4 months after receiving the preparative regimen.
3. Unless surgically sterile by bilateral tubal ligation or vasectomy of partner(s), the patient agrees to continue to use a method of contraception throughout the study and for 90 days after your last treatment such as: barrier (i.e. condom, diaphragm), hormonal, IUD, or sponge plus spermicide.
4. For women who have menstruated within the past 12 months and have not had a surgical procedure to accomplish sterilization, pregnancy testing (urine or serum) will be performed within 7 days prior to treatment.
5. Clinical performance status of ECOG 0 to 1 at the time of chemotherapy infusion. ECOG performance status of 0 to 1 will be inferred if the patient's level of energy is ≥ 50% of baseline.
6. Absolute neutrophil count greater than or equal to 750/mm^3.
7. Platelet count greater than or equal to 100 000/mm^3.
8. Hemoglobin greater than or equal to 8.0 g/dL.
9. Serum ALT and AST less than 3 times the institutional upper limit of normal.
10. Serum creatinine less than or equal to 1.7 mg/dL.
11. Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
12. Prothrombin time (PT) and partial thromboplastin time (PTT) within 1.5 times the institutional upper limit of normal
13. Patients with echocardiogram (EKG) within 14 days of initiation of chemotherapy demonstrating no new rhythm, axis, or ST segment changes will be included. If new ST changes are present, patients may be included if cardiac stress test indicates no evidence of inducible cardiac ischemia.
14. Urinalysis within 14 days demonstrating no evidence of a urinary tract infection.
15. Patients with evidence of ongoing disease regression that is attributed to a therapy that is not part of the trial and that was administered after TIL harvest and expansion but prior to adoptive transfer of TILs should continue on prior therapy and may be treated with TIL only if their disease is stable or there is evidence of progressive disease. In this event as described above, the TIL will be frozen and stored for future use, in the event of progression, prior to the rapid expansion step.

5.2 EXCLUSION CRITERIA

5.2.1 EXCLUSION CRITERIA STEP 1: RESECTION OF TUMOR & INITIATION OF TIL EXPANSION

Patients who meet the following criteria will be excluded from study participation:

1. Patients with active systemic infections requiring intravenous antibiotics, coagulation disorders, or other major medical illnesses of the cardiovascular, respiratory, or immune system are excluded.
2. Patients that have completed a chemotherapy regimen given with the intent of lymphodepletion or cellular immunotherapy which included a non-myeloablative lymphodepletion strategy.
3. Patients testing positive for HIV titer, hepatitis B surface antigen, human T-cell leukemia-lymphoma virus (HTLV) I or II antibody, or both rapid plasma regain (RPR) and fluorescent treponemal antibody (FTA) are excluded. Patients with hepatitis C antibody must have a negative (undetectable) viral load by polymerase chain reaction (PCR).
4. Patients who are pregnant or nursing are excluded.
5. Patients needing chronic immunosuppressive systemic steroids are excluded
6. Patients with autoimmune diseases that require immunosuppressive medications are excluded
7. Presence of a significant psychiatric disease, which in the opinion of the principal investigator or his designee, would prevent adequate informed consent or render immunotherapy unsafe or contraindicated
8. Patients with central nervous system metastases will be excluded.
9. Inability to comprehend and give informed consent

5.3 LIFESTYLE CONSIDERATIONS

N/A

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but do not subsequently receive the study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a laboratory abnormality or pregnancy may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Both men and women and members of all races and ethnic groups are eligible for this trial. It is anticipated that one patient per month will be accrued. The source of patients will be from the outpatient clinics and general public. The study will be advertised through patient advocacy groups, social media, and flyers in the clinic to increase participation. Given the

participation over one year, we will use multiple methods to contact patients including phone, email, and EHR patient portal to ensure continued participation.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

6.1.1.1 INTERLEUKIN-2 (ALDESLEUKIN, PROLEUKIN)

Interleukin-2 (IL-2) will be provided from commercial sources via the study sponsor (Iovance). IL-2 is a 133 amino acid long peptide primarily secreted by T-cells in response to various antigenic stimuli. The cytokine acts through a specific IL-2 receptor consisting of α, β, γ subunits. In addition to T-cell proliferation, IL-2 leads to activation and proliferation of natural killer (NK) cells, increasing their tumoricidal activity. Other actions of IL-2 include augmentation of B-cell growth and immunoglobulin production, enhancement of IFN-gamma and tumor necrosis factor-β production from T-cells, IL-6 production by monocytes, modulation of histamine release by basophils, and upregulation of IL-2 receptors. This triggers the release of various other cytokines leading to the total immune/inflammatory reaction and resultant toxicity.

IL-2 will be administered as an inpatient treatment within the inpatient unit that has been specially designed to treat patients with cellular immunotherapy and bone marrow transplants. The physicians administering IL-2 and other chemotherapeutic agents described below will be appropriately credentialed in the delivery of these medications and management of side effects. During the treatment phase (preparative chemotherapy, TIL infusion, and IL-2 dosing) the patients will be primarily managed by the Immune Cellular Experimental Treatment (ICE-T) service which is staffed by medical oncologists and physicians experienced with delivery of standard and experimental cellular therapies. Grade III toxicities common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, constitutional symptoms, and laboratory changes. Additional Grade IV and V toxicities have been seen with IL-2 (See Appendix D).

6.1.1.2 FLUDARABINE

Fludarabine phosphate is a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A), that is relatively resistant to deamination. Fludarabine is a purine antagonist antimetabolite. Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase, and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

It will be purchased by the MCC pharmacy from commercial sources. Fludarabine is supplied as a fludarabine phosphate powder in the form of a white, lyophilized solid cake. The fludarabine powder is stable for at least 18 months at 2 to 8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly, and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride United States Pharmacopeia over approximately 15 to 30 minutes. 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 toxicities. Hemolytic anemia has been reported after 1 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, fatigue, anorexia, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is rare at the currently administered doses. 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, and allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of chronic lymphocytic leukemia (CLL).

6.1.1.3 CYCLOPHOSPHAMIDE

Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. It is biotransformed principally in the liver to active alkylating metabolites by a mixed-function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. The mechanism of action is thought to involve cross-linking of tumor cell DNA.

Cyclophosphamide is well absorbed after oral administration with a bioavailability greater than 75%. The unchanged drug has an elimination half-life of 3 to 12 hours. It is eliminated primarily in the form of metabolites, but from 5% to 25% of the dose is excreted in urine as unchanged drug. Several cytotoxic and noncytotoxic metabolites have been identified in urine and in plasma. Concentrations of metabolites reach a maximum in plasma 2 to 3 hours after an intravenous dose. Plasma protein binding of unchanged drug is low but some metabolites are bound to an extent greater than 60%. It has not been demonstrated than any single metabolite is responsible for either the therapeutic or toxic effects of cyclophosphamide. Although elevated levels of metabolites of cyclophosphamide have been observed in patients with renal failure, increased clinical toxicity in such patients has not been demonstrated.

Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent and possesses potent immunosuppressive activity. The serum half- life after intravenous administration ranges from 3 to 12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

Cyclophosphamide will be obtained from commercially available sources by the MCC pharmacy. It will be diluted in 250 mL NS and infused over approximately two hours. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company Height and Weight Tables. Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia.

Anorexia, nausea, and vomiting may occur, especially after high doses. Diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported in patients. 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 an uroprotective agent.

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 also result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs.

Cardiotoxicity has been observed at high doses of cyclophophamide. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from 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.

6.1.1.4 MESNA (SODIUM 2-MERCAPTOETHANESULFONATE, MESNUM, MESNEX, NSC-113891)

Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

Mesna was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis. Analogous to the physiological cysteine-cystine, mesna is rapidly oxidized to its major metabolite, mesna disulfide

(dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic metabolites, resulting in their detoxification.

Mesna will be obtained commercially and is supplied as a 100 mg/mL solution. Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48 to 72 hours in D5W, 48 to 72 hours in D5W/0.45% normal saline, or 24 hours in normal saline. It will be diluted up to 20 mg Mesna/mL fluid in D5W or normal saline and will be administered intravenously as a continuous infusion. Toxicities include nausea, vomiting and diarrhea.

6.1.1.5 G-CSF (GRANULOCYTE COLONY-STIMULATING FACTOR)

Granulocyte-colony stimulating factor (G-CSF) will be obtained commercially and is supplied in 300 µg/mL and 480 µg/mL vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given daily subcutaneously as a blood product support if needed. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

Levofloxacin (Levaquin) and Trimethoprim and Sulfamethoxazole double strength (TMP/SMX DS, Bactrim)

Levaquin is used to prevent infections caused by bacteria. It is a synthetic broad spectrum antibacterial agent. The mechanism of action of levofloxacin involves inhibition of bacterial topoisomerase IV and DNA gyrase, enzymes required for DNA replication, transcription, repair, and recombination. An alternative antibiotic by mouth for patients who are allergic to Levaquin will be Keflex.

TMP/SMX DS will be obtained by the MCC pharmacy from commercial sources. It will be used for the prevention of *Pneumocystis carinii* Pneumonia (PCP).

The oral dose is 1 tablet orally twice a week. Like other sulfa drugs, Bactrim (sulfamethoxazole-trimethoprim) can cause allergies, fever, 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.

6.1.1.6 ACYCLOVIR (ZOVIRAX) AND VALACYCLOVIR HYDROCHLORIDE (VALTREX)

Acyclovir and Valtrex will be obtained by the MCC pharmacy from commercial sources.

Valtrex is the hydrochloride salt of *L*-valyl ester of acyclovir. It is rapidly converted to acyclovir, which has demonstrated antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV). The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. Acyclovir triphosphate stops replication of herpes viral DNA.

Acyclovir will be used to prevent the occurrence of herpes virus infections. It is supplied as powder for injection in 500 mg vials. Reconstitute in 10 mL of sterile water for injection for bacteriostatic water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/mL or less and infused over 1 hour to avoid renal damage.

Oral tablets of 200 and 800 mg are available, if the patient is able to tolerate medication by mouth. Reversible renal insufficiency has been reported with intravenous but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal electroencephalography have been reported with higher doses of acyclovir.

Should this occur, a dosage adjustment will be made or the drug should be discontinued. Stomach upset, headache, nausea, rash, hives, diaphoresis, hematuria; hypotension, and thrombocytosis have been reported. Hair loss from prolonged use has also been documented. Acyclovir will not be used concomitantly with other nucleoside analogs that interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

6.1.1.7 FLUCONAZOLE (DIFLUCAN)

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

6.1.1.8 ONDANSETRON HYDROCHLORIDE (ZOFRAN)

Ondansetron hydrochloride will be obtained by the MCC pharmacy 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 a complete list of side effects and specific dose instructions.

6.1.1.9 FUROSEMIDE (LASIX)

Furosemide, a loop diuretic, will be obtained by the MCC pharmacy 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 side effects and specific dose instructions.

6.1.1.10 CELL PREPARATION

The procedures and reagents for expanding the human TIL cells are contained in Appendix B. The cell preparation will occur in the Cell Therapy Facility at MCC (Appendix C)

6.1.2 DOSING AND ADMINISTRATION

Patients will undergo tumor resection from which the tumor infiltrating lymphocyte (TIL) product will be generated. The TIL will be cultured, expanded, and tested for tumor reactivity in a state-of-the-art GMP cell growth facility at Moffitt that is compliant with all FDA regulations regarding investigational cell transfer products.

Apheresis will be performed via a 2-armed approach or via a temporary central venous catheter. Approximately a 7-liter exchange using a Gambro Spectra machine will be performed to generate cells for immune monitoring and research related testing. Specific details on the apheresis procedure are included in an appendix to the IND under which this trial is being conducted, BB IND 14013.

All patients will receive non-myeloablative lymphodepleting chemotherapy with cyclophosphamide and fludarabine to enhance T cell persistence and effectiveness *in vivo*. Cyclophosphamide will be administered at 60 mg/kg/day I.V. in 250 mL NS over approximately 2 hours on Days –7 and –6. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Table.

Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 30 minutes on Days –5 to –1. To prevent undue toxicity with fludarabine, the dose will be based on body surface area (BSA) but will not exceed a dose calculated on surface areas based on body weights greater than 140% of

the maximum ideal body weight per Metropolitan Life Insurance Company Height and Weight Tables.

On day 0, all patients will receive not less than 10^9, and up to $1x10^{12}$ T cells in ≥250 mL NS as an inpatient by I.V. TIL will be infused at a rate of 5 mL/minute or less. Infusion rate will be adjusted downward if necessary to maintain endotoxin infusion at 5 EU/Kg/hour or less (refer to the adoptive cell therapy SOP and Appendix B for T cell preparation).

Eight (8) to sixteen (16) hours after completing the T cell infusion, all patients will receive high dose interleukin-2 (IL-2) on an inpatient basis at the standard dose of 600,000 IU/kg as an intravenous bolus over an approximate 15 minute period every 8-16 hours for up to 15 doses on Days 1 to 5, as tolerated. Doses will be skipped if patients reach Grade III or IV toxicity due to high dose IL-2, except for the reversible Grade III toxicities common to high dose IL-2 such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes (i.e. platelets, creatinine, total bilirubin) as detailed in Appendix C. If the toxicity is easily reversed by supportive measures, then additional doses may be continued. There will be no dose modification for IL-2, except for morbid obesity per standard practice for the use of high dose IL-2.

As above, doses may be skipped for hypotension or low urine output of grade III or IV that is controlled with neosynephrine, but IL-2 may continue if that toxicity is reversed to grade II or less with supportive measures. Patients will discontinue high dose IL-2 in a given cycle of treatment for altered mental status, supra- ventricular arrhythmias that require medication, evidence of myocarditis, uncontrolled hypotension, urine output less than 600 mL per 24 hours and/or creatinine of 3.5 gm/dL in spite of maximal supportive measures, bilirubin of 8 gm/dL, positive blood culture, or evidence of non-life-threatening infection. IL-2 will be permanently discontinued and not given again in patients who develop a life threatening ventricular arrhythmia, creatinine chronically elevated above 1.8 gm/dL, bilirubin that does not return to baseline, life threatening infection, myocardial infarction, or permanently altered mental status.

6.1.3 DURATION OF THERAPY

Antitumor Effect – Solid Tumors

For the purposes of this study, the baseline tumor measurements used to assess response will be from the Day -14 CT scan and patients should be evaluated for response at 6 weeks after TIL infusion then every 12 weeks. Initial disease progression at 6 weeks requires confirmation of progression at 12 weeks to result in patient withdrawal from study.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (*Eur J Ca* 45:228-247, 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST 1.1 criteria.

Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their initiation on the lymphodepleting chemotherapy regimen.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received TIL infusion, 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 6-week post-TIL imaging 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.

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 (≥ 2 cm) by chest x-ray or as ≥ 10 mm (≥ 1 cm) with CT scan, MRI, or calipers by clinical examination. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm (≥ 1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [< 1 cm] or pathological lymph nodes with ≥ 10 to < 15 mm [≥ 1 to < 1.5 cm] short axis), are considered non-measurable disease. Leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, are inflammatory breast disease, 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 that 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.

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 (≥ 1 cm) 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 radiography. 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 (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness.

Use of MRI remains a complex issue. MRI has excellent contrast and spatial and temporal resolution; however, there are many image acquisition variables involved in MRI that 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 that 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 reading radiologist 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 that may bias an investigator if it is not routinely or serially performed.

Ultrasonography. Ultrasonography is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasonographic 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 ultrasonography in the course of the study, confirmation by CT or MRI is required. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy and 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.

Cytology and Histology. Gross and histological examination of the formalin-fixed and paraffin-embedded tissue following the standard sarcoma pathology protocol at Moffitt Cancer Center can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types 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.

Response Criteria

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 (< 1 cm).
- Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of the 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 (0.5 cm). (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.

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 (< 10 mm [< 1 cm] short axis).
    - **Note:** If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- 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).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the lymphodepleting chemotherapy 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. See table for further clarification.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response

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

Duration of 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 lymphodepleting chemotherapy until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

Progression-Free Survival**:** PFS is defined as the duration of time from start of lymphodepleting chemotherapy to time of progression or death, whichever occurs first.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

All medications used will be provided by the pharmacy. The TIL infusion product will be prepared according to the SOP developed and retained in the Cell Therapy Facility, described in Appendix B.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The formulation and packaging will be according to the SOP developed and retained in the Cell Therapy Facility.

6.2.3 PRODUCT STORAGE AND STABILITY

Medications will be stored consistent with the package insert provided by the manufacturer. The TIL infusion product will be kept at 4^{o}C during transfer from the Cell Therapy Facility following collection on Day 0 and brought to room temperature immediately prior to infusion.

6.2.4 PREPARATION

See Appendix B

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This is a non-blinded study and there is no randomization of treatment for the enrolled patients. Response will be assessed by an independent radiologist using criteria described in section 6.1.3.

6.4 STUDY INTERVENTION COMPLIANCE

For the purposes of this study at Moffitt Cancer Center, the Protocol Data Management System (OnCore) will be employed. All patients will be registered in OnCore before any study specific tests are performed.

This trial will be monitored continuously by the principal investigator who will be assisted by the assigned clinical trial coordinator.

To ensure adherence to the protocol, safety and monitoring reports will be submitted to the Moffitt Internal Protocol Data Safety Monitoring Committee (PDSMC) once two patients have been treated or once the early stopping rule threshold has been met (whichever comes first) or more frequently if requested by the PDSMC. A final safety and monitoring report will be submitted to the PDSMC within 3 months of the last subject having been enrolled.

Internal audits will be conducted by Moffitt regulatory personnel in accordance with applicable regulatory standards. The following elements will be reviewed:

1. Source documentation verification of eligibility and protocol compliance
2. Regulatory review of IRB compliance and external reporting requirements
3. Drug/device accountability and handling
4. Completeness and quality of data

6.5 CONCOMITANT THERAPY

N/A

6.5.1 RESCUE MEDICINE

N/A

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

There are only two phases of treatment which are continuous: the lymphodepleting chemotherapy phase and the post-TIL infusion IL-2 phase. Criteria for discontinuation of both are listed below.

Lymphodepleting Chemotherapy Phase

Patients that develop an acute bacterial or viral infection during the lymphopenic phase of treatment will not continue to receive further treatments (TIL infusion and/or IL-2 infusion). Treatment may resume when deemed safe upon resolution of the infection. Patients who experience any Grade 4 non-hematologic organ-specific adverse event (neurologic, pulmonary, cardiac, gastrointestinal, genitourinary, hepatic or dermatologic) that does not resolve prior to TIL infusion will not receive TIL infusion and subsequent IL-2 infusion.

Post-TIL Infusion IL-2 Phase

Patients who experience any Grade 4 non-hematologic organ-specific adverse event (neurologic, pulmonary, cardiac, gastrointestinal, genitourinary, hepatic or dermatologic) not felt due to sarcoma or a pre-existing condition will discontinue further IL-2 administration. Patients who develop a life-threatening Grade IV toxicity due to administration of high dose IL-2 such as: ventricular arrhythmia,

life threatening infection, myocardial infarction, or permanently altered mental status will have IL-2 permanently discontinued.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded within the patient's medical record and OnCore and/or the Clinical Trial Management System (CTMS). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, may be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 consecutive scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 3 business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SAFETY ASSESSMENTS

Safety assessments will be performed at time points according to the study calendar (section 1.3). Additionally, as stated above, there will be a separate assessment for eligibility prior to lymphodepleting chemotherapy.

8.1.1 TREATMENT – CHEMOTHERAPY/CELL INFUSION

Patients must fulfill all of the following criteria to be eligible. Laboratory testing must be complete and satisfactory within 14 days of initiation of chemotherapy. Imaging tests must be complete and satisfactory within 28 days of initiation of chemotherapy, and the identical criteria and management will be followed as discussed in sections 5.1.1 and 5.1.2.

Apheresis will be performed on all patients

a. Patients will be evaluated by a physician or advanced practice provider (advanced registered nurse practitioner or physician's assistant) for clearance and certification prior to the initiation of the apheresis regimen. Any measurable disease found on physical examination will be documented within 30 days prior to initiation of chemotherapy. However, if there are no measurable metastatic lesions present, then no physical measurements will be documented.
b. Complete blood count, differential, chemistry panel including liver functions, mineral, kidney, and electrolytes, PT/PTT, platelet count, type and screen, LDH, Free T4, TSH, urine or serum β-HCG pregnancy test (for women of child-bearing potential), EKG, and chest x-ray will be performed within 14 days before the apheresis.

8.1.2 TREATMENT PLAN

Patients will undergo tumor resection from which the tumor infiltrating lymphocyte (TIL) product will be generated. The TIL will be cultured, expanded, and tested for tumor reactivity in a state-of-the-art GMP cell growth facility at MCC that is compliant with all FDA regulations regarding investigational cell transfer products.

Apheresis will be performed via a 2-armed approach or via a temporary central venous catheter. Approximately a 7-liter exchange using a Gambro Spectra machine will be performed to generate

cells for immune monitoring and research-related testing. Specific details on the apheresis procedure are included in an appendix to the IND under which this trial is being conducted, BB IND -18646.

All patients will receive nonmyeloablative lymphodepleting chemotherapy with cyclophosphamide and fludarabine to enhance T-cell persistence and effectiveness *in vivo*. Cyclophosphamide will be administered at 60 mg/kg/day IV in 250 mL NS over approximately 2 hours on Days -7 and -6. The dose will be based on the patient's bodyweight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company Height and Weight Table.

Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 30 minutes on Days –5 to –1. To prevent undue toxicity with fludarabine, the dose will be based on body surface area (BSA) but will not exceed a dose calculated on surface areas based on body weights greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company Height and Weight Tables.

On day 0, all patients will receive not less than 10^9, and up to $1x10^{12}$ T cells in ≥250 mL NS as an inpatient by IV. TILs will be infused at a rate of 5 mL/minute or less. Infusion rate will be adjusted downward if necessary to maintain endotoxin infusion at 5 EU/Kg/hour or less (refer to the adoptive cell therapy SOP and Appendix B for T cell preparation).

Eight (8) to sixteen (16) hours after completing the T cell infusion, all patients will receive high-dose interleukin-2 (IL-2) on an inpatient basis at the standard dose of 600 000 IU/kg as an intravenous bolus over an approximate 15-minute period every 8 to 16 hours for up to 15 doses on days 1 to 5, as tolerated. Doses will be skipped if patients reach grade III or IV toxicity due to high-dose IL-2, except for the reversible grade III toxicities common to high-dose IL-2, such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes (i.e. platelets, creatinine, total bilirubin) as detailed in Appendix D. If the toxicity is easily reversed by supportive measures, then additional doses may be continued. There will be no dose modification for IL-2, except for morbid obesity per standard practice for the use of high-dose IL-2.

As above, doses may be skipped for hypotension or low urine output of grade III or IV that is controlled with neosynephrine, but IL-2 may continue if that toxicity is reversed to grade II or less with supportive measures. Patients will discontinue high-dose IL-2 in a given cycle of treatment for altered mental status, supraventricular arrhythmias that require medication, evidence of myocarditis, uncontrolled hypotension, urine output less than 600 mL per 24 hours, and/or creatinine of 3.5 gm/dL in spite of maximal supportive measures, bilirubin of 8 gm/dL, positive blood culture, or evidence of non–life-threatening infection. IL-2 will be permanently discontinued and not given again to patients who develop a life-threatening ventricular arrhythmia, creatinine chronically elevated above 1.8 gm/dL, bilirubin that does not return to baseline, life-threatening infection, myocardial infarction, or permanently altered mental status.

The phase of treatment including the non-myeloablative chemotherapy, TIL infusion, and IL-2 dosing present risk to the patient in terms of toxicity with the side effects of chemotherapy and IL-2 being the most prominent. For this reason, these portions of treatment will be administered as inpatient

treatment within the inpatient unit that has been specially designed to treat patients with cellular immunotherapy and bone marrow transplants. The physicians prescribing IL-2 and other chemotherapeutic agents will be appropriately credentialed in the delivery of these medications and management of side effects. During the treatment phase (preparative chemotherapy, TIL infusion, and IL-2 dosing) the patients will be primarily managed by the Immune Cellular Experimental Treatment (ICE-T) service which is staffed by medical oncologists and physicians experienced with delivery of standard and experimental cellular therapies.

For the evaluation of peripheral blood cells following T-cell transfer, 60 mL blood samples in cell preparation or heparin tubes will be obtained on Days -28, 7, 14, 21, 28, 35, 84 , 168, 252, 336, and 420, when feasible. A 10 mL blood sample will be collected on Day 0. This is in addition to apheresis that will be performed prior to lymphodepletion and at week 6.These time points were selected based on previous studies at the National Cancer Institute. During high dose IL-2 administration (days 1-5), peripheral blood mononuclear cell (PBMC) yields are low; therefore, day 7 has been selected as the earliest feasible time point to obtain cells and evaluate the immediate effects of immunization. The other days have been selected as later time points to evaluate T-cell persistence and function. Sera samples will be obtained from the apheresis sample prestudy and week 6. Prophylaxis Treatment

Infection Prevention and Pneumocystis carinii Pneumonia (PCP) Prophylaxis

Patients will receive Levaquin at 500 mg daily (or Keflex at 500 mg 3 times a day if allergic to Levaquin) ūntil absolute neutrophil count recovers to greater than 500/mm^3 and the fixed combination of trimethoprim (TMP) and sulfamethoxazole (SMX) as double strength (DS) tablet [DS tabs = TMP 160 mg/tab and SMX 800 mg/tab] 1 orally twice a week. TMP/SMX-DS will be taken by patients beginning on Day -7 and continuing for a minimum of 6 months post chemotherapy and until CD4 counts are > 200 cells/mm^3 on 2 consecutive follow-up lab studies. Patients with sulfa allergies will receive aerosolized pentamidine 300 mg per nebulizer within 1 week prior to admission and continued monthly until absolute neutrophil count is greater than 1000/uL.

Patients will be given antibiotics (Levaquin or Ceftazidine if allergic to Levaquin) IV during high-dose IL-2 therapy.

Herpes Virus Prophylaxis

At the time of the T-cell infusion, patients with positive HSV serology will be administered valcyclovir 500 mg orally daily if patient is able to take oral medications or acyclovir 5 mg/kg IV piggyback every 8 hours if patient needs intravenous medications, which will be continued until absolute neutrophil count is greater than 1000/uL. Reversible renal insufficiency has been reported with IV-administered acyclovir but not with oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal electroencephalography have been reported with higher doses of acyclovir. If symptoms occur, a dosage adjustment will be made or the drug will be discontinued.

Acyclovir will not be used concomitantly with other nucleoside analogs (e.g. ganciclovir), which interfere with DNA synthesis. In patients with renal disease, the dose is adjusted as per product labeling.

Fungal Prophylaxis

Patients will begin Fluconazole 200 mg orally daily with the T-cell infusion (Day 0) and continue until the absolute neutrophil count is > 1000/uL.

Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalsosporin or a quinolone for fevers $\geq 38.5^{o}C$ with an absolute neutrophil count less than $500/mm^3$. Aminoglycosides should be avoided unless there is clear evidence of sepsis. Infectious disease consultation will be obtained from all patients with unexplained fever or any infectious complications.

Blood Product Support

In order to reduce neutropenia following chemotherapy and T-cell infusion, G-CSF will be given, starting Day +1 (>24hours from TIL infusion) at 5 µg/kg/day daily subcutaneously until neutrophil counts reach >1.5 K/uL. The patient will also receive platelets and packed red blood cells (PRBC's) as needed, using daily complete blood counts as a guide. Attempts will be made to keep Hb > 7.0 gm/dL and platelets > 20 000/uL. Leukocyte filters will be used for all blood and platelet transfusions to decrease sensitization to transfused white blood cells and decrease the risk of cytomegalovirus infection. Irradiated blood and blood products should be used.

8.1.3 EVALUATION DURING STUDY

During the preparative regimen patients will have a complete blood count and comprehensive metabolic panel every day of treatment. During the IL-2 phase of treatment, patients will have a complete blood count and comprehensive metabolic panel prior to each infusion (8 hour intervals). Patients will be monitored with vital signs at baseline and after cell infusion per inpatient unit protocol. Phlebotomy will be performed prior to tumor harvest and/or excision on Day -28, lymphodepletion (as part of apheresis) on Day -14, and again on Days 0, 7, 14, 21, 28, 35, 42 (as a part of apheresis), 84 ,168, 252, 336, and 420 when feasible. These blood samples will be used to evaluate T-cell persistence and function.

Complete evaluation of evaluable lesions with physical examination and appropriate CT scans will be performed at approximately 6 weeks (+/- 7 days) and at 12 weeks (+/- 7 days) after the cell infusion, at which time patients will be restaged.

Follow-up visits with CT scans to evaluate disease will then be then be conducted every 3 months (+/- 14 days) for 12 months or until disease progression, withdrawal of consent, loss to follow-up, or death, whichever occurs first. After confirmed disease progression subjects will be followed every 3 months

for overall survival and report of any subsequent anticancer treatments. This can be accomplished by visit or phone contact.

Excess blood and tissue specimens collected in the course of this research project may be banked and provided in the future to investigators with MCC IRB-approved research protocols.

8.1.4 RETREATMENT

Patients will not be eligible for retreatment on this study.

8.2 OTHER ASSESSMENTS

8.3 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES OR OTHER ENDPOINTS

Phlebotomy will be performed prior to tumor harvest and/or excision on Day -28, lymphodepletion (as part of apheresis) on Day -14, and again on Days 0, 7, 14, 21, 28, 35, 42 (as a part of apheresis), 84 ,168, 252, 336, and 420 when feasible. These blood samples will be used to evaluate T-cell persistence and function. The PBMC will be isolated from these samples and flow cytometry will be used to assess the phenotypic distribution of immune cell subpopulations. The TCR repertoire of the infusion product and PBMC at each time point will be assessed to evaluate the persistence of the infusion product.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

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

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,

- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 SEVERITY OF EVENT

All AEs will be graded using the CTCAE 5.0 criteria.
For AEs not included in the protocol-defined grading system (grade 1 to 4), the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

For all collected AEs, the PI will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories shown below.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related – There is some evidence to suggest a causal relationship (eg, the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (eg, the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (eg, the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (eg, the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the PI.

All adverse events will be recorded according to this table:

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Not related			X	X	X
Unlikely			X	X	X
Possibly	X	X	X	X	X
Probably	X	X	X	X	X
Definitely	X	X	X	X	X

Exceptions to this rule will include events and laboratory abnormalities that represent common symptoms and abnormalities of sarcoma and chemotherapy and/or have no clinical significance:

- Abnormalities in hematologic parameters due to myelosuppressive therapeutic effect:

    i. Anemia, neutropenia, lymphopenia, thrombocytopenia
    ii. Epistaxis or bleeding except for catastrophic CNS or pulmonary hemorrhage

- Common symptoms of cancer (unless grade ≥ 3) including:

i. Fatigue
ii. Weakness
iii. Bone, joint or muscle pain
iv. Alopecia
v. Loss of appetite, nausea, vomiting
vi. Chemistry abnormalities (phosphorus, calcium, glucose)
vii. Coagulation abnormalities (shortened PT, PTT, increased fibrinogen)

- Laboratory abnormalities:

i. LDH (increased or decreased)
ii. Alkaline phosphatase (increased or decreased)
iii. Low levels of the following: AST, ALT, creatinine, BUN, uric acid, bilirubin, albumin, total protein
iv. Electrolyte abnormalities (sodium, potassium, bicarbonate, CO2, magnesium

- General therapy related events

i. Catheter related events
ii. Rash related to antibiotic use

8.4.3.3 EXPECTEDNESS

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.4.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a patient.

All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate case report form (CRF). Information to be collected will include event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

All events beginning with the TIL Harvest (Day -28) until each patient is discharged from the hospital following the initial IL-2 infusion will be reported. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 ADVERSE EVENT REPORTING

All adverse events will be monitored and documented using adverse event case report forms/worksheets beginning at day -28 (TIL Harvest) and continuing until each patient is discharged from the hospital following the initial IL-2 infusion. On-going adverse events will be followed for 84 days after TIL infusion or until progression, loss to follow-up, withdrawal of consent, death, or initiation of new cancer treatment.

8.4.6 SERIOUS ADVERSE EVENT REPORTING

The PI will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

It is the responsibility of the PI and the research team to ensure that serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (probably related, unknown relationship, definitely

not related), date and time of administration of test medications, all concomitant medications, and medical treatment provided. The PI is responsible for evaluating all adverse events to determine whether criteria for “serious” as defined above are present. Adverse drug reactions that are serious, unlisted/unexpected, and at least possibly associated to the drug, and that have not previously been reported in the Investigator’s Brochure, or reference safety information document should be reported promptly to the Food and Drug Administration (FDA) in writing by each investigator/physician engaged in clinical research. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

The Principal Investigator or designee shall notify the FDA by telephone or by fax of any unexpected fatal or life threatening experience associated with the use of the drug, as soon as possible, but no later than 7 calendar days after the sponsor’s initial receipt of the information. Each phone call or fax shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND if applicable.

The Principal Investigator is required to notify his Institutional Review Board (IRB) of a serious adverse event according to institutional policy.

Expedited reporting to the FDA on a MEDWatch 3500A form will be used for all unexpected serious adverse events as defined in 21 CFR 312/32, for all non- hematologic Grade IV-V organ adverse events, for all grades 3-5 infusion reactions attributed to TIL, and for all grades 3-5 autoimmune events. TIL infusion reactions will be readily identified as there is an 8-16 hour window between TIL infusion and initiation of high dose IL-2.

In the annual report to the FDA, we plan to list infusion reactions and autoimmune events separately. In addition, all grade 3-5 toxicities not deemed due to the sarcoma or preexisting disorders will be added to the summary reports

8.4.7 REPORTING EVENTS TO PARTICIPANTS

N/A

8.4.8 EVENTS OF SPECIAL INTEREST

The use of the nonmyeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient. Although it is anticipated that this protocol is relatively safe because of the expected recovery of the patients’ bone marrow within 2 to 4 weeks, fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. The major hazards are infection and disease progression. The major discomforts are nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this nonmyeloablative regimen include:

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, syndrome of inappropriate antidiuretic hormone secretion.

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.

High-Dose IL-2: A variety of side effects have been associated with high-dose IL-2 administration in our experience at the NCI and a listing of these side effects in 652 patients who received 1,039 treatment courses are listed in Appendix D.

Adoptive cell therapy with TIL: a variety of side effects that potentially overlap with high-dose IL2 have been associated with adoptive cell therapy in our experience and at the NCI. Prominent long-term side effects include: vitiligo, high frequency hearing loss, anemia and uveitis.

8.4.9 REPORTING OF PREGNANCY

Pregnancy, although not itself a serious adverse event, should also be reported on a serious adverse event form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

8.5 UNANTICIPATED PROBLEMS

8.5.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.5.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB, depending on their policy, and to the DCC/study sponsor within 2 business days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 4 business days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 2 business days of the IRB's receipt of the report of the problem from the investigator.

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

N/A

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Feasibility is the primary endpoint of this trial, which is defined as a patient who can complete trial related therapy. If it is feasible for at least 3 patients out of 5 eligible patients, then this will be considered successful. Upon successful completion, a larger trial with efficacy endpoints will be designed. As the completion of treatment requires successful growth and expansion of T-cells, we will track causes of production failure to improve methods in the ultimate design of a trial with efficacy endpoints.

9.2 SAMPLE SIZE DETERMINATION

As this is a phase I design with safety/feasibility as the endpoint, there are no sample size calculations applicable. The sample size of 5 patients was chosen as a number that would yield sufficient safety data and opportunity to identify any possible efficacy signal from which to design a subsequent phase 2 trial.

9.3 POPULATIONS FOR ANALYSES

For the secondary endpoint of objective response, data will be analyzed in an intention-to-treat manner and also in a modified intention-to-treat manner (including only those who received the TIL infusion plus at least one dose of IL-2).

Safety analyses will be conducted on all participants that have received lymphodepleting chemotherapy.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

This is a feasibility pilot trial of lymphodepleting chemotherapy followed by autologous tumor-derived T cells for patients with advanced sarcoma. All patients will receive intravenous (I.V.) chemotherapy consisting of cyclophosphamide on Days – 7 and –6, fludarabine on Days –5 to -1, I.V. T cells on Day 0, and high dose IL-2 on Days 1 to 5.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoints include feasibility and safety. These will be analyzed as follows:

- Feasibility is defined as a patient who can grow and expand T-cells. If it is feasible for at least 3 patients out of 5 eligible patients, then this will be considered successful. Upon successful

completion, a subsequent, larger trial with efficacy endpoints will be designed. We will track causes of failure to improve methods in the ultimate design of a trial with efficacy endpoints.

- Safety will be analyzed by the record of adverse events as described in section 8.4. AEs will be coded by CTCAE v5.0 and calculated counted once only for a given participant. The data will be presented by System Organ Class (SOC), severity and frequency. The start date, stop date, severity, relationship, expectedness, outcome, and duration will be reported for any grade ≥4 AE that does not resolve within 14 days of the last dose of IL-2. There are two cutoff periods where AE would preclude continuation of therapy which are described in section 7.1 and again below.

    - Lymphodepleting Chemotherapy Phase

    - Patients that develop an acute bacterial or viral infection during the lymphopenic phase of treatment will not continue to receive further treatments (TIL infusion and/or IL-2 infusion). Patients who experience any Grade 4 non-hematologic organ-specific adverse event (neurologic, pulmonary, cardiac, gastrointestinal, genitourinary, hepatic or dermatologic) that does not resolve prior to TIL infusion will not receive TIL infusion and subsequent IL-2 infusion.

    - Post-TIL Infusion IL-2 Phase

    - Patients who experience any Grade 4 non-hematologic organ-specific adverse event (neurologic, pulmonary, cardiac, gastrointestinal, genitourinary, hepatic or dermatologic) not felt due to sarcoma or a pre-existing condition will discontinue further IL-2 administration. Patients who develop a life-threatening Grade IV toxicity due to administration of high dose IL-2 such as: ventricular arrhythmia, life threatening infection, myocardial infarction, or permanently altered mental status will have IL-2 permanently discontinued.

We anticipate that up to 15 patients will be screened to obtain 5 eligible patients for the study. The eligibility criteria are defined as patients that meet criteria defined in section 5. Anticipated rate of patients to be screened is 1 patient per month, thus this study will need at most 10 months to accrue. The primary expected reason for screen fail is depressed cardiac function found on MUGA after consent due to prior Adriamycin exposure for this patient population.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

There are two secondary endpoints for this trial, clinical response and persistence of the TIL infusion product. These will be analyzed as follows

- Objective response (OR) is defined as the patient being alive at Day 70 and tumor size evaluated using the RECIST 1.1 criteria to be a complete response (CR) or partial response (PR). Evaluations will be made by CT at 12 weeks after the cell infusion. This will be reported as a proportion using both the intent-to-treat population and the treatment

population. The ITT population is defined as any patient that underwent a resection for TIL culture and the treatment population is defined as any patient that completes lymphodepletion, TIL infusion, and at least one dose of IL-2.

- Persistence of the TIL infusion product will be reported individually per patient and collectively by responders and non-responders. For this analysis, we will record the overlap in the top 50 TCR clones between the infusion product (week 0) and the PBMC at the 1, 2, 3, 4, 5, and 6 week time point, using the ImmunoSEQ™ (Adaptive Biotechnologies) platform. We will construct a correlation table for the 21 unique pairwise comparisons for each patient and summarize the data. Analysis will include the number and sequence of each productive unique Vβ and Jβ genes identified within each sample and the degree of clone sharing between samples of the same patient, using the TIL infusion product (week 0) as a reference point.

9.4.4 SAFETY ANALYSES

See section 9.4.2 as the safety analysis is a component of the primary endpoint analyses.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Basic demographic data will be obtained on the enrolled patients, including reason for screen fail in those patients that do not meet eligibility criteria.

9.4.6 PLANNED INTERIM ANALYSES

There are no planned interim analyses.

9.4.7 SUB-GROUP ANALYSES

There are no planned sub-group analyses.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual data will be listed by measure and time point according to the study calendar.

9.4.9 EXPLORATORY ANALYSES

Exploratory analyses will be as follows:

- The phenotype of the preREP culture and infusion product relative to response will be reported. We will report the frequency of T-cell subpopulation as CD3+, CD3+CD8+, CD3+CD4+, and CD3-CD56+ in the preREP and final infusion product.

- Oncologic outcome

    - Progression-free survival (PFS), defined as the time from study entry to disease progression, relapse or death due to any cause, whichever is earlier, will be summarized with the Kaplan-Meier curve. Confidence intervals for the median and survival rates at different time points will be constructed if needed and appropriate. This secondary endpoint will be reported descriptively.

    - Overall survival (OS) is defined as the time from initiation of the study protocol to date of last patient contact or death due to any cause, whichever is latest. OS data will be analyzed and reported in the manner described for PFS. Additional survival follow-up for subjects who have progressed may continue until the patient is lost to follow-up or withdraws consent. Additional OS analysis may be conducted periodically until the end of the study. The study will end once survival follow-up has concluded.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: Informed Consent Document.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the

purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

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

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

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

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

10.1.3 FUTURE USE OF STORED SPECIMENS AND DATA

Clinical data collected for this study will be analyzed and stored at MCC. After the study is completed, the de-identified, archived clinical data will be stored within the Moffitt Cancer Center Sarcoma Department, for use by other researchers including those outside of the study (with appropriate data

sharing agreement in place). Permission to transmit de-identified data to other investigators will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored within the Mullinax Lab with the same goal as the sharing of data with other investigators involved in ACT research at Moffitt Cancer Center. These samples could be used to research the causes of cancer, its complications and other conditions for which individuals with cancer are at increased risk, and to improve treatment.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Principal Investigator after securing appropriate material or data transfer agreements as arranged by The Innovation Office.

10.1.4 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator
John Mullinax, MD
MCC
12902 Magnolia Drive, CSB-6, Sarcoma Department
(813) 745-8736
John.Mullinax@Moffitt.org

Moffitt Internal Protocol Data Safety Monitoring Committee (PDSMC) as described section 6.4.

10.1.5 SAFETY OVERSIGHT

Serious Adverse Events: Serious Adverse Events (SAEs) from this protocol will be reported concurrently to the IRB and the study sponsor. The Protocol Monitoring Committee (PMC) will review these SAEs in accordance with the protocol-specific DSMP. This trial will be continuously monitored according to the Moffitt Cancer Center's policy. A final safety and monitoring report will be submitted to the PMC within three months of the last patient treated. This protocol will be subject to periodic internal audits based on risk or as recommended by the PMC.

10.1.6 CLINICAL MONITORING

MCC's Internal Monitors will periodically monitor regulatory documents and case report forms according to the protocol specific clinical monitoring plan. Monitoring will include review of data for accuracy, completeness, and source verification, reporting of SAEs, and adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

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

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

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data will be captured in OnCore and/or MCC's electronic Clinical Trials Management System. For each subject enrolled, the electronic CRF must be completed by the assigned data manager or other authorized study staff. Any paper forms should be typed or filled out indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. This also applies to records for those subjects who fail to complete the study. If a subject stops dosing or terminates from the study, the dates and reasons must be noted on the CRF. If a subject terminates from the study because of a dose-limiting toxicity, thorough efforts should be made to clearly document the outcome.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Clinical data (including adverse events (AEs) and expected adverse reactions data) will be entered into Oncore, a data capture system provided by MCC. Clinical data will be entered directly from the source documents

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 10 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 10 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when there is no longer a need for these documents to be retained. Permission must be acquired from the State of Florida for document destruction after the 10-year minimum record-retention period described above has elapsed.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 3 working days of identification of the protocol deviation, or within 3 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported the Data Coordinating Center. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP. Deviations must be entered into the Clinical Trials Management System (CTMS).

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 10 years after the completion of the primary endpoint by contacting the Principal Investigator.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

ACT	Adoptive cell therapy
AE	Adverse Event
ANCOVA	Analysis of Covariance
BSA	Body surface area
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Science
CLIA	Clinical Laboratory Improvement Amendments
CLL	Chronic lymphocytic leukemia
CM	Complete medium
CMP	Complete Metabolic Panel
CMS	Centers for Medicare and Medicaid Services
CONSOR	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CTF	Cell therapy facility
CTLA-4	Cytotoxic T-lymphocyte–associated antigen-4
CTMS	Clinical Trial Management System
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DS	Double strength
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
EBV	Epstein-Barr virus
ECOG	Easter cooperative oncology group
EKG	Echocardiogram
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FEV	Forced expiratory volume
FTA	Fluorescent treponemal antibody
GCP	Good Clinical Practice
GCSF	Granulocyte-colony stimulating factor
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leukocyte antigen
HSA	Human serum albumin
HSV	Herpes simplex virus
HTLV	Human T-cell leukemia-lymphoma virus
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
ICMJE	International Committee of Medical Journal Editors

IDE	Investigational Device Exemption
IFN	Interferon
IL-2	Interleukin 2
IND	Investigational New Drug Application
IRB	Investigational Review Board
ISO	International Organization for Standardization
IV	Intravenous
MCC	MCC
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
MTD	Maximum tolerated dose
NIH	National Institutes of Health
NIH IC	NIH Institute & Center
NK	Natural killer
NS	Normal saline
OHRP	Office for Human Research Protections
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cell
PCP	*Pneumocystis carinii* Pneumonia
PI	Principal Investigator
PT	Prothrombin time
PTT	Partial thromboplastin time
QA	Quality Assurance
QC	Quality Control
RECIST	Response evaluation criteria in solid tumors
REP	Rapid expansion protocol
RPR	Rapid plasma regain
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SMX	Sulfamethoxazole
SOC	System Organ Class
SOP	Standard Operating Procedure
TIL	Tumor-infiltrating lymphocyte
TK	Thymidine kinase
TMP	Trimethoprim
UP	Unanticipated Problem
VZV	Varicella-zoster virus

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

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12 APPENDICES

12.1 APPENDIX A

Certificate of Analysis Cellular Therapy Product					
Documentation of review of release criteria for Tumor Infiltrating Lymphocytes (TIL)					
Principal Investigator				**Storage Temperature**	
Protocol		**Final Product Volume**		**Date/Time with Time zone manufactured**	
Total Number of Viable Cells for Infusion (≤ 2E11 viable cells)		**Final Product Additives**		**Expiration (24 hours from Harvest) with time zone**	

RELEASE CRITERIA

TEST	Method	SPECIFICATIONS	RESULTS	Acceptable (Circle One)	Tech
Gross contamination	Gram stain	No organisms seen (NOS)		Yes No N/A	
Sterility (From REP: Will not be final report)	Sterility culture	No growth to date		Yes No N/A	
Endotoxin (Final Product)	Endosafe	<5 EU/kg		Yes No N/A	
Mycoplasma contamination	qPCR	Negative		Yes No N/A	
Identity	Flow Cytometry	≥ 90% CD3 +/CD45+		Yes No N/A	
Viability	Dye Uptake	> 70% viable cells		Yes No N/A	

REVIEW OF FINAL PRODUCT FOR INFUSION SUITABILITY

Product meets all release criteria (check one): ☐ Yes ☐ No (If 'No' is checked: explain below and obtain approval from Laboratory Director and Medical Director)

_____

_____ _____

Quality Management or designee Date

Place Patient Label

If applicable: Comments:

_____

_____ _____

Laboratory Director or designee Date

12.2 APPENDIX B

Growth of TIL from Human Tumors

Purpose:

This technique is routinely used to grow tumor-infiltrating lymphocytes (TILs) and tumor cell lines from human solid tumors, including melanoma, sarcoma, non-small cell lung cancer, bladder cancer, renal cell carcinoma, colon adenocarcinoma, and penile squamous cell carcinoma. The method can also be used to grow lymphocytes from metastatic lymph nodes in these diseases. For reviews, see Yannelli, 1991 (J. Imm. Methods) and 1996 (Int. J. Cancer), and Bessner 2010 (Clin Cancer Res).

Procedure:

Complete cell culture medium (CM)

Human AB serum (Valley Biomedical) is heat inactivated, aliquoted into 10-mL tubes and then stored at -80°C for up to 6 months. One tube is randomly selected and tested for sterility. Complete medium (CM) for culturing TILs is prepared by supplementing RPMI-1640 (Invitrogen) with 10% human AB serum, HEPES (10 mM), Penicillin G (100 units/mL), Streptomycin (100 µg/mL), Gentamicin (50µg/mL), and β-mercaptoethanol (5.5 x 10-5 M). Complete media will be labeled with patient name, MCC number, date of preparation, and expiration date (7 days after preparation).

Deriving and Culturing TIL Cells from Tumor Explants

Tumors are resected or biopsied in the operating room or clinic and placed in a sterile container containing RPMI. The remaining tumor is delivered to the Cell Therapies Facility (see Appendix C) at the MCC.

For derivation of TILs, tumors are dissected with a scalpel or scissors into ~1 to 2 mm fragments in each of 3 dimensions. These fragments are then processed with enzyme media into a single-cell suspension, referred to as “tumor digest.” This digest is then cultured in a 24-well tissue culture plate containing 2 ml of CM supplemented with 6000 IU/mL recombinant human IL-2 (Prometheus Corp.). The plates are incubated in a humidified incubator at 37°C with 5% CO2 in air.

Once the TIL cells have begun to grow, half of the medium in the well is replaced with CM containing IL-2 (6000 IU/mL). This is accomplished by removing approximately 1 ml of media from the top half of the well and replacing with fresh CM. Once the cells have expanded to confluence, the culture is split from 1 well to 2 wells by diluting the cells with equal volumes of CM containing IL-2.

Cultures are continued until all tumor cells are eliminated and a cell number of ≥ 3×10^7 is obtained. This is typically accomplished in 21 to 28 days (5). If the TILs are not growing by 5 weeks and their concentration remains below 5×10^5/mL, the culture will not be used for the Rapid Expansion Protocol below for patient infusion. In this case, the cell culture will be discontinued.

All TILs from a given tumor will be tested individually against autologous tumor digest for tumor-specific activity. Briefly, $1x10^5$ TILs and $1x10^5$ tumor cells will be placed in a single well of a 96-well plate with 300uL of CM. After incubation for 24 hours, the supernatant of the co-culture will be collected and placed into an ELISA assay for IFN-gamma. Those TILs that produced IFN-gamma (> 250pg/mL) will be considered “reactive” for the next phase of cell product production

Regardless of reactivity, the TIL cultures will be pooled and expanded using the rapid expansion protocol (REP). Before the REP, some of the TIL will be cryopreserved and stored for functional analysis or for later use in the REP if required. For cryopreservation, selected cells are mixed with an equal volume of freeze medium containing DMSO (Edward's Life Science) as cryoprotectant at a final concentration of 7.5% and frozen in a controlled-rate freezer (refer to TIL cryopreservation SOP - SOP SC2.1).

During the initial TIL culture, the following in-process tests will be performed:

1. 14-Day Sterility (to be performed by MCC Pathology Laboratory or a designated licensed diagnostic laboratory):
   a. Within the first 24 to 72 hours after setup.
   b. As needed during the 5-week culture (if culture appears contaminated, etc).
   c. Final product at cryopreservation or when cells are brought to GMP for REP cultures.

2. Endotoxin levels on final product at cryopreservation or when cells are brought to GMP for REP cultures.

4. Viability on final product at cryopreservation or when cells are brought to GMP for REP cultures.

5 Cell count performed as needed during cell culture and on final product at cryopreservation or when cells are brought to GMP for REP cultures.

<u>Rapid Expansion Protocol</u>

TIL are washed in CM containing 50 μg/mL gentamicin as sole antibiotic (this washing as well as the washing and freezing above removes any penicillin remaining from the original cultures) and expanded to large numbers for patient treatment by using a REP, as approved by the FDA for use at the at the NCI in protocols 98-C-95, 99-C-158 and 03-C-0162. The expansion is based on T-cell stimulation with the monoclonal antibody OKT3 (anti-CD3) and irradiated allogeneic feeder cells, mixed together. Human recombinant IL-2 is added to the culture 2 days after the culture is started and is included in all fresh medium added later to the culture. All final expansions of TIL (REPs) are performed in G-REX flasks (Wilson-Wolff) in therapeutic-grade AIM V culture medium (Invitrogen) containing streptomycin (50 μg/mL) and gentamicin (10 μg/mL). There is no penicillin in this culture medium and thus no potential problem for penicillin-sensitive patients.

Feeder cells consist of a mixture of at least 3 allogeneic PBMCs. The source of these feeder cells in the REP are normal donors recruited specifically for this study; donors for these products have been screened for infectious diseases before collection of their product and determined to be negative before being used as feeders.

Feeders (collected from a minimum of 3 different donors) will be thawed, pooled, re-aliquoted and cryopreserved until use. The pooled feeders are completely anonymized at the time of thawing and pooling. The pools are relabeled to include a unique identifier number. This is the only identification for these feeder cells. A product data sheet containing the feeder source identity information is retained by the Director of the Cell Growth Laboratory.

On Day 14, the GREX-1000 flasks are harvested for infusion via centrifugation or membrane separation using a CytoMate machine (Nexell Therapeutics Inc.). During this concentration process, the TILs are washed with saline and resuspended in saline with 2.5% Human Serum Albumin (HSA) and 300 IU/mL of IL-2 for infusion.

During the rapid expansion period, the following in-process QC testing is performed:

1. Sterility at day 11 to 12 of REP (during the final 48 to 72 hours before harvesting and infusion.

In addition, a series of tests is performed for research purposes:

1. Cell-surface phenotype (for example, CD8, CD4, CD28, CD62L, and CD57) using flow cytometry analysis between days 7 and 12 of the REP.

Product is released for clinical use based on the results of day 11 to 12 sterility tests (needs to be negative to date) as well as results of lot release testing as follows:

1. Gram stain
2. Endotoxin levels on final product
3. Viability
4. Visual Inspection

Additional testing on final product (non-release criteria) includes 14-day sterility and mycoplasma testing.

12.3 APPENDIX C

Cell Therapy Facility at Moffitt Cancer Center

Leadership of Moffitt Cell Therapy Facility (CTF) is provided by Linda Kelley, PhD, Facility and Technical Director, James Mulé, PhD, Scientific Director, and Marco Davila, MD, PhD, Medical Director. CTF staffing consists of 40+ employees including 4 dedicated quality assurance specialists, 4 technical project managers, 31 technical staff, 1 dedicated training specialist, 1 dedicated IT specialist, and 1 dedicated administrative and grants assistant.

CTF is a newly constructed 10 000 square foot GMP-compliant state-of-the-art facility located in the M2GEN Building on the Moffitt McKinley Campus at 10902 N. McKinley Drive, Tampa, Florida, 33612. Moffitt McKinley Campus is approximately 0.5 mile from the main Moffitt Cancer Center (MCC) at 12902 Magnolia Drive, where inpatient and outpatient treatments are provided. CTF maintains a 1,000 square foot laboratory at MCC for the purpose of short-term storage of cryopreserved cell products and preparation of cell products for infusion. Receipt and shipment of fresh and cryopreserved cell products for off-site cell manufacturing occurs at MCC.

CTF laboratory facilities consist of 10 Class 10 000 clean rooms, each equipped with biological safety cabinets, CO_2 incubators, centrifuges and microscopes. A 1,000 square foot unclassified GTP laboratory is used for clinical procedures performed in closed systems. A separate quality control laboratory provides product testing including flow cytometry, quantitative PCR, ELISA, mycoplasma, endotoxin, gram stain, cell counts, and viability. Materials management and storage consists of separate quarantine receipt, kitting, and distribution locations. Liquid nitrogen storage of cell products occurs in new Chart MVE 1500 and 1800 series freezers in a secured 1 000 square foot facility. Source liquid nitrogen is supplied by an external 6 000 gallon bulk tank through vacuum-insulated piping under continuous electronic monitoring. All CTF equipment is monitored via the REES Centron Monitoring System. Quality Assurance compliance is facilitated with MasterControl quality management systems software. Laboratory data and information are documented with StemSoft Lab cell therapy management software.

CTF provides manufacturing of safe therapeutic cellular products in support of standard-of-care cellular therapies for the Moffitt Bone Marrow Transplant and Cellular Immunology Program as well as novel, investigator-initiated clinical trials. Strategic alliances with industry partners are in place to advance development of translational cell therapy protocols and to facilitate clinical cell product manufacturing for national and international clinical trials. Cell therapy products manufactured include chimeric antigen receptor (CAR) T cells, tumor-infiltrating lymphocytes (TIL), gene- and peptide-modified dendritic cell vaccines, tumor cell vaccines, and T regulatory cells (Treg). CTF has supported over 30 investigational new drug (IND) applications.

Funding for CTF services is provided by the Moffitt Cancer Center Support Grant (P30 CA076292), payer billing and via charge-back accounts. CTF serves as one of 5 national NIH/NHLBI Production Assistance for Cell Therapy (PACT) Centers from 2016-2022 (HHSN 268201600013I). Compliance

with standards set by the U.S. Food and Drug Administration (FDA), American Association of Blood Banks, and Foundation for Accreditation of Cellular Therapy (FACT) are consistently maintained.

12.4 APPENDIX D

Table: Toxicity of Treatment of 283 Patients With Melanoma or Renal Carcinoma With High-Dose Bolus Interleukin 2*

Toxic Effect	No. (%) of Courses by Diagnosis		
	Melanoma	Renal Cell	Total
Chills	42 (20%)	40 (17%)	82 (18%)
Pruritis	20 (10%)	20 (8%)	40 (9%)
Mucositis	0 (0%)	2 (1%)	2 (0%)
Nausea	78 (37%)	92 (39%)	170 (38%)
Diarrhea	68 (33%)	67 (28%)	135 (30%)
Malaise	37 (18%)	28 (12%)	65 (15%)
Peak bilirubin, μmol/L (mg/dL)			
1.7-34.2 (0.1-2.0)	30 (14%)	40 (17%)	70 (16%)
34.9-102.6 (2.1-6.0)	126 (60%)	153 (64%)	279 (62%)
104.3-171.0 (6.1-10.0)	41 (20%)	34 (14%)	75 (17%)
>171.0 (>10.0)	12 (6%)	11 (5%)	23 (5%)
Oliguria <80 mL/8 h	39 (19%)	60 (25%)	99 (22%)
Anuria <240 mL/24 h	3 (1%)	15 (6%)	18 (4%)
Weight gain, % of body weight			
0.0-5.0	53 (25%)	77 (32%)	130 (29%)
5.1-10.0	88 (42%)	99 (42%)	187 (42%)
10.1-15.0	44 (21%)	46 (19%)	90 (20%)
15.1-20.0	16 (8%)	13 (5%)	29 (6%)
>20.0	8 (4%)	3 (1%)	11 (2%)
Peak creatinine, μmol/L (mg/dL)			
8.8-176.8 (0.1-2.0)	98 (47%)	44 (18%)	142 (32%)
185.6-530.4 (2.1-6.0)	105 (50%)	172 (72%)	277 (62%)
539.2-884.0 (6.1-10.0)	6 (3%)	18 (8%)	24 (5%)
>884.0 (>10.0)	0 (0%)	4 (2%)	4 (1%)
Edema	3 (1%)	1 (0%)	4 (1%)
Respiratory distress	4 (2%)	14 (6%)	18 (4%)
Respiratory failure requiring intubation	4 (2%)	9 (4%)	13 (3%)
Pleural effusion	3 (1%)	2 (1%)	5 (1%)
Somnolence	3 (1%)	19 (8%)	22 (5%)
Coma	1 (0%)	9 (4%)	10 (2%)
Disorientation	25 (12%)	37 (16%)	62 (14%)
Hypotension	97 (46%)	136 (57%)	233 (52%)
Angina	2 (1%)	3 (1%)	5 (1%)
Myocardial infarction	0 (0%)	2 (1%)	2 (0%)
Arrythmias	17 (8%)	12 (5%)	29 (6%)
Anemia requiring transfusion, no. of units transfused			
0	171 (82%)	182 (76%)	353 (79%)
1-5	35 (17%)	47 (20%)	82 (18%)
5-10	3 (1%)	6 (3%)	9 (2%)
11-15	0 (0%)	3 (1%)	3 (1%)
Platelet nadir, cells x 109/L			
0-20	7 (3%)	9 (4%)	16 (4%)
20-60	72 (34%)	59 (25%)	131 (29%)

60-100	64 (31%)	73 (31%)	137 (31%)
>100	66 (32%)	97 (41%)	163 (36%)
Infection	9 (4%)	9 (4%)	18 (4%)
Line sepsis	3 (1%)	6 (3%)	9 (2%)
Death	0 (0%)	3 (1%)	3 (1%)
TOTAL	209 (100%)	238 (100%)	447 (100%)

*Reference 1; includes only grade 3 and 4 toxicity except where detailed.