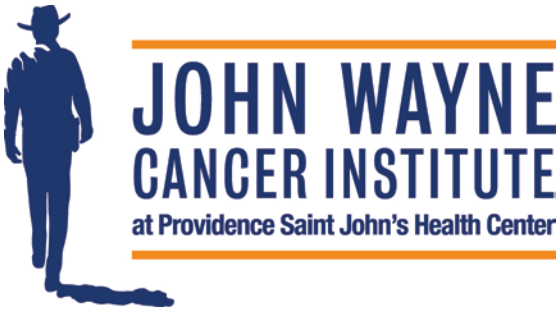

Title: A Phase II Study using Tumor-Reactive Autologous Tumor Infiltrating Lymphocytes (TIL) Plus IL-2 After Lymphocyte-Depleting Chemotherapy Regimen in Metastatic Melanomas

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Protocol #: TIL-TREATMENT-0614
Amendment 02 (Version Date: 11JUN2015)

IND Information

Drug Name:	NSC Number:	IND Number:
Cyclosphosphamide	NSC 26271	Commercial Sources
Fludarabine phosphate	NSC 312887	Commercial Sources
Melanoma Reactive TIL	NSC 733610	IND 16202
Proleukin	NSC 373364	Commercial Sources
Filgrastim	NSC 614629	Commercial Sources
Bactrim	NSC 618652	Commercial Sources
Fluconazole	NSC 754343	Commercial Sources
Acyclovir	NSC 335752	Commercial Sources
Mesna	NSC 113891	Commercial Sources

Protocol Summary

Background:

- Prior preclinical and clinical studies have shown that tumors from participants with advanced melanoma contain tumor-infiltrating lymphocytes (TIL) with anti-tumor reactivity targeting a variety of melanoma-associated antigens.
- Prior clinical trials have shown that these TIL (mentioned above)can be expanded in vitro using interleukin-2 (IL-2) with or without OKT-3 antibody stimulation and cause regression of melanoma when adoptively transferred back to the patient.
- Preclinical mouse models have shown that recipient immunosuppression prior to the adoptive transfer of tumor-reactive lymphocytes greatly enhances their anti-tumor effect.
- Using a preparative non-myeloablative chemotherapy regimen of cyclophosphamide and fludarabine, a single institution Phase II study of 35 participants showed a 51% objective response rate to TIL and IL-2, with some complete and durable responses in a patient population that had been heavily pre-treated (34 of 35 had had high-dose IL2)(1)
- Thus, in this Phase II trial, we will investigate whether similar results can be achieved and to determine the feasibility of broadly applying this approach to participants with advanced metastatic melanoma with few treatment options.

Objectives:

- To train participating staff of John Wayne Cancer Institute (JWCI) and Providence Saint John's Health Care (PSJHC) in the procurement, isolation and expansion of TIL.
- To determine whether TIL procurement, isolation and expansion is feasible and safe at the JWCI and PSJHC.
- To determine whether autologous TIL infused in conjunction with systemic high-dose IL-2 after non-myeloablative chemotherapy with cyclophosphamide and fludarabine can cause consistent and durable objective responses in participants who have metastatic melanoma.
- To evaluate and assess the health-related quality of life (QOL) of subjects undergoing high dose IL-2 by European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 tool.

Study Participation:

- Up to 22 participants who are ≥ 18 years of age, with metastatic melanoma, a successful harvest and expansion of TIL cells, and are physically able to tolerate high-dose IL-2 and non-myeloablative chemotherapy. Study participation is ~4 months.
- Study enrollment is estimated to be ~2 years, and study duration is estimated to be ~3 years.

Design:

- Participants will receive a non-myeloablative lymphocyte-depleting preparative chemotherapy regimen consisting of cyclophosphamide (60 mg/kg/day X 2 days IV) and fludarabine (25 mg/m^2/day IV X 5 days).
- Following the non-myeloablative chemotherapy, participants will receive an intravenous adoptive transfer of at least 10^9 TIL followed within 24 hours by high-dose intravenous IL-2 (600-720,000 IU/kg/dose every 8 hours for up to 12 doses).
- Supportive care is given until spontaneous hematopoietic recovery occurs.
- A tumor assessment will be conducted at 6 and 12 weeks after cell infusion. Response assessment per RECIST 1.1 criteria.
- A Quality of Life questionnaire (EORTC-QLQ-C30) will be administered at screening, pre-chemotherapy, day 30 post treatment, 6 weeks post treatment, and at 12 weeks post treatment.
- Up to 22 participants will be treated to determine if the combination of TIL infusion with high dose IL-2, after non-myeloablative lymphocyte depleting chemotherapy, is able to achieve an objective response rate that can rule out 15% in favor of a minimally desirable, targeted response rate of 40% with a one-tailed 0.05 alpha level and 85% power.

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Objectives

1.0 Study Objectives

- To train participating staff of John Wayne Cancer Institute (JWCI) and Providence Saint John's Health Care (PSJHC) in the procurement, isolation and expansion of TIL.
- To determine whether TIL procurement, isolation and expansion is feasible and safe at JWCI at PSJHC.
- To determine whether autologous TIL infused in conjunction with systemic high-dose IL-2 after non-myeloablative chemotherapy with cyclophosphamide and fludarabine can cause consistent and durable objective responses in participants who have metastatic melanoma.
- To evaluate the health-related Quality of Life in subjects who receive high dose IL-2 by EORTC-QLQ-C30

Background

2.0 Background and Rationale

Melanoma is the most lethal form of skin cancer. Prognosis for patients with metastatic melanoma is poor: median survival is 6 to 8 months and five-year survival rate is less than 6 % (3). Metastatic melanoma is notoriously resistant to chemotherapy and radiotherapy. There are several nonsurgical therapies resulting in long-term disease-free survival: biochemotherapy, vemurafenib, high-dose interleukin-2 (IL-2) and more recently ipilimumab, which is a monoclonal antibody that blocks cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (4-6). High-dose IL-2 can mediate durable complete responses in 3-5% of treated patients, but overall response rate is only about 13% to 16% and toxicity is significant (4, 7). Ipilimumab can enhance T-cell activation and proliferation, and increase median overall survival of treated participants from 6 months to 10 months; again, however, the response rate is low (11%) (7).

Clinical trials of adoptive cell therapy (ACT) with autologous tumor-infiltrating lymphocytes (TIL) are showing promising results in patients with metastatic melanoma; when TIL therapy was preceded by lymphocyte depletion, objective response rates were 49% to 72% and rate of complete remission was about 16% (8, 9). Patients who fail conventional Food and Drug Administration (FDA) approved treatments are eager to try ACT. However, because ACT requires infusion of a large number of tumor-reactive TIL from autologous tumor, there are significant logistic and technical difficulties; few cancer centers conduct ACT for melanoma. The John Wayne Cancer Institute (JWCI) is a world renowned cancer institute that treats a large population of patients with melanoma. These patients present at all stages of disease. Eligible patients with metastatic disease may be diagnosed during their follow up here or be referred after stage IV diagnosis at another center. Our intent is to demonstrate the feasibility of ACT at this center, outside of the NCI. We feel that our experience with melanoma immunotherapy will enable us to treat patients successfully.

2.1 Preclinical and Clinical Studies adoptive transfer of lymphocytes

As noted previously, when the lymphocytes which infiltrate human melanomas were found to frequently contain T-cells capable of recognizing autologous melanoma (and in many cases, MHC-matched allogeneic melanomas), Dr. Rosenberg's group at NCI explored the administration of these TIL to treat participants with metastatic melanoma. Laboratory studies had shown that the molecular targets of these TIL were frequently shared differentiation proteins associated with melanin production (e.g. MART-1, tyrosinase, gp100) or epitopes unique to the autologous tumor, presumably mutated proteins. Over 75 participants were treated with unselected autologous TIL, expanded with IL-2 *in vitro* and given with systemic high-dose IL-2 (some participants also received low doses of cyclophosphamide) (10). Although the objective response rates were 34% in participants who had had previous IL-2 and 32% in

IL-2-naïve participants, three quarters of all responding participants relapsed within a year. These results spurred efforts to identify lymphocyte populations with more potent tumor reactivity and possible effect of lymphodepletion of host to increase efficacy of ACT.

The use of immunosuppression prior to the adoptive transfer of lymphocytes from tumor bearing mice is based on a variety of murine models demonstrating improved therapeutic effectiveness of the adoptive transfer following immunosuppression of the host (11). Several mechanisms have been identified in mouse models for how lymphodepletion improves antitumor efficacy in ACT (12). These mechanisms include the elimination of cytokine "sinks" and accumulation of high levels of homeostatic cytokines like IL-7 and IL-15; elimination of CD4+CD25+ regulatory T cells (Tregs) that inhibit anti-tumor responses and activation of antigen presenting cells.

Several clinical trials of ACT with autologous TIL for metastatic melanoma in lymphodepleted hosts have been conducted by Dr. Rosenberg group at NCI. All clinical trials showed better objective responses compared to IL-2 or dacarbazine only which mediate objective response rates of approximately 15% (1, 13). ACT with nonmyeloablative chemotherapy alone showed an objective response rate of 49%, when 2or 12 Gy of total body irradiation (TBI) was added, the response rates were 52% to 72 %, respectively (14). There are 2 mechanisms that have been identified that are associated with improvement of antitumor efficacy of ACT with host lymphodepletion in humans; increased serum levels of the lymphocyte homeostatic cytokine IL-7 and IL-15 (14) and elimination of regulatory Tregs. The analysis of 4 clinical trials which were conducted by Dr. Rosenberg's group showed that the percentage and number of reconstructing Tregs observed in the peripheral blood was higher in non-responders than in responders who received non-myeloablative chemotherapy with or without total body irradiation before adoptive T cell transfer (15).

In fact, participants treated with the lymphocyte-depleting chemotherapy tolerated more consecutive doses of IL-2 compared to participants receiving only IL-2, suggesting that lymphodepletion might reduce IL-2 toxicities mediated through secondary cytokines produced by endogenous 'bystander' lymphocytes. The combination of cyclophosphamide and fludarabine was myelosuppressive, causing neutropenia, thrombocytopenia and anemia. The neutrophil nadir was on day 10 after the start of chemotherapy at a mean of 6/mm^3 and recovered to above 500/mm^3 on day 14 with filgrastim (G-CSF) support. Lymphocyte nadir was 6/mm^3 and recovered to above 200/mm^3. For participants receiving cyclophosphamide, fludarabine and IL-2, platelet nadir was 13/mm^3 and recovered to >20,000/mm^3 a mean of 18 days after the start of chemotherapy. Participants were usually discharged between 2-3 weeks after the initiation of the chemotherapy, with neutrophil counts above 500/mm^3 and platelet counts above 20,000/mm^3. No participants needed a stem cell transfusion to rescue marrow function. However, CD4 counts remained persistently low (at approximately day 200, the mean CD4 count was 156, range between 46 and 320), which is a known side effect of immunosuppression from fludarabine. Four participants developed transient herpes zoster but recovered completely. All participants recovered after treatments.

In a phase II portion of a clinical trial at the surgical branch of the NCI, 35 participants received the doses of cyclophosphamide (60 mg/kg/day twice) and fludarabine (25 mg/m^2/day for 5 days) and cloned lymphocytes, a heterogeneous population of autologous TIL was administered (1). With this regimen, major cancer regressions were seen in 51% of participants with metastatic melanoma (15 PR and 3 CR), some of who had not responded to the same non-myeloablative chemotherapy given with their cloned T-cells. In addition, 34 of the 35 participants were refractory to prior IL-2 and most had previously had chemotherapy. With this latest regimen, some participants achieved a clonal repopulation of anti-tumor lymphocytes that exceeded 80% of their circulating $CD8^+$ lymphocytes and this re-population could persist for months or even years (13). This was in sharp contrast to the rapid disappearance of TIL within a day of administration with no preparative chemotherapy regimen (16).

Non-hematologic and hematologic toxicities were similar to those described in the first study using cloned T lymphocytes referenced above. One difference was that autoimmunity was observed in the TIL therapy group. Four participants experienced vitiligo and one patient had an autoimmune uveitis that responded promptly to steroid eye drops. One patient had an RSV pneumonia requiring transient mechanical ventilatory support and recovered completely. One patient who showed a clonal repopulation from infused TIL cells and a dramatic regression of metastatic melanoma, developed Epstein-Barr virus (EBV)-associated B cell lymphoma several months after treatment was completed. This patient was EBV-naïve prior to treatment, had a depressed CD4 count after fludarabine and had received multiple blood product transfusions (17). Despite treatment, this patient died several months later of disseminated lymphoma. The risk of post-transplantation lymphoproliferative disorder (PTLD) arising from donor B cells infected with EBV can occur after allogeneic hematopoietic stem cell transplantation, but rarely occurs after autologous hematopoietic stem cell transplants. In a study of 18,014 participants who underwent allogeneic bone marrow transplantation at 235 centers world-wide, 78 recipients developed PTLD. Cumulative incidence was 1% at 10 years. In a study of 928 allogeneic renal transplants that were performed, 20 cases of EBV related PTLD were seen. There was a higher incidence of PTLD in EBV seronegative participants, although the overall incidence remained low (18).

In addition to the above toxicities, one patient developed polyneuropathy manifested by visual loss, motor and sensory defects, approximately 2 months after chemotherapy. The etiology for this complication is not known, but it may be neurotoxicity related to fludarabine. One patient who received the chemotherapy regimen plus high-dose IL-2 and bulk peripheral blood lymphocytes reactive to the melanoma, developed prolonged respiratory failure requiring mechanical ventilation and acute renal failure that required hemodialysis. Both toxicities have been reported with high-dose IL-2 therapy and the patient recovered from the toxicities.

Since the publication of the first 35 participants receiving this treatment (1), an additional 8 participants have been treated with TIL in the phase II portion of this protocol. Among these additional 8 participants, there have been two partial and one complete response. Response frequencies and durations for all 43 Phase II participants given the latest regimen are shown (Table 1)

The Surgery Branch, NCI experience with TIL and IL-2 following a preparative regimen of cyclophosphamide and fludarabine has reported a response frequency and durations not previously achieved for heavily pre-treated participants with metastatic melanoma (2). The fact that many participants had bulky disease and most had visceral involvement further substantiates that this is an extremely active regimen in this patient population. Because this approach involves patient-specific reagents, new technologies and a close collaboration between the clinic and a laboratory production team, there are unique obstacles to implementing its widespread evaluation and use. Therefore, the purpose of this protocol is to determine whether autologous TIL infused in conjunction with systemic high-dose IL-2 after non-myeloablative chemotherapy with cyclophosphamide and fludarabine can cause consistent and durable objective responses in participants who have metastatic melanoma at JWCI.

Table 1 Response rates after adoptive T cell therapy (2)

Treatment	*n* (%) of patients (duration in mo)			OR (%)
	Total	PR	CR	
No TBI	43	16 (37) 84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2	5 (12) 82+, 81+, 79+, 78+, 64+	21 (49)
200 TBI	25	8 (32) 14, 9, 6, 6, 5, 4, 3, 3	5 (20) 68+, 64+, 60+, 57+, 54+	13 (52)
1,200 TBI	25	8 (32) 21, 13, 7, 6, 6, 5, 3, 2	10 (40) 48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19	18 (72)
Total	93	32 (34)	20 (22)	52 (56)

NOTE: Data updated as of August 1, 2010.

Study Design

3.0 Study Design

This is a Phase II study and will include up to 22 treated participants with advanced metastatic melanoma. A "treated participant" is defined as one who has received the TIL infusion. Participants will be at least 18 years of age, able to provide informed consent, and be recruited from patient population in PSJHC outpatient Cancer Clinic and community referrals. It is predicted this study will take approximately 2 years to enroll and 3 years to complete. Study participation for each participant is ~4 months. Once successful TIL expansion is confirmed by the JWCI TIL Laboratory point of contact and date the adoptive transfer will be ready (Day 1), on day -7 participants will begin a non-myeloablative lymphocyte-depleting preparative chemotherapy regimen consisting of cyclophosphamide (60 mg/kg/day X 2 days IV) and fludarabine (25 mg/m^2/day IV X 5 days). On the day following the non-myeloablative chemotherapy, participants will receive an intravenous adoptive transfer of at least 10^9 TIL followed within 24 hours by high-dose IV IL-2 (600-720,000 IU/kg/dose every 8 hours for up to 12 doses). Supportive care will be given until spontaneous hematopoietic recovery occurs. A tumor assessment will be conducted at 4, 8, and 12 weeks after cell infusion (Day 1) per RECIST 1.1 criteria. A Quality of Life questionnaire (EORTC-QLQ-C30) will be administered at on Day -7, and 4, 8, and 12 weeks post treatment, and. Analysis of study data will determine if the combination of TIL infusion with high dose IL-2, after non-myeloablative lymphocyte depleting chemotherapy, is able to achieve an objective response rate that can rule out 15% in favor of a minimally desirable, targeted response rate of 40% with a one-tailed 0.05 alpha level and 85% power. In addition, analyses will determine the safety and feasibility of TIL procurement, isolation and expansion as well as assess the quality of life of treated participants.

4.0 Eligibility Criteria and Enrollment

4.1 Inclusion Criteria

- 4.1.1 Participants must have a suitable (sufficient size) by RECIST 1.1 metastatic lesion post successfully completing a TIL procurement through JWCI TIL Procurement-0614 Protocol.
- 4.1.2 Participants must be $\geq$ 18 years of age.
- 4.1.3 Participants of child bearing potential must agree to use an effective form of birth control during study and up to four months after receiving treatment.
- 4.1.4 Clinical performance status of ECOG 0-1.
- 4.1.5 Life expectancy of > three months.
- 4.1.6 Within 28 days prior to screening:
  - 4.1.6.1 Absolute neutrophil count greater than 1000/mm^3 without support of filgrastim.
  - 4.1.6.2 Platelet count greater than 100,000/mm^3 without the support of transfusions

4.1.6.3 Serum ALT/AST less than three times the upper limit of normal.
4.1.6.4 Serum creatinine less than or equal to 1.6 mg/dl.
4.1.6.5 Total bilirubin less than or equal to 2 mg/dl, except in participants with Gilbert's Syndrome who must have a total bilirubin less than 3 mg/dl.
4.1.7 Participants must be able to understand and sign the Informed Consent document.
4.1.8 Women who are pregnant or breastfeeding.
4.1.9 Life expectancy of less than three months.
4.1.10 Must have recovered immune competence after chemotherapy or radiation therapy as evidenced by normal ANC > 1000/mm^3 and absence of opportunistic infections. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Participants who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)

4.2 Exclusion Criteria

4.2.1 All systemic, cytotoxic therapy (including targeted therapies) must be stopped at least 5 weeks prior to Day 1-TIL infusion
4.2.2 Participants who have received prior treatment with anti-CTLA-4 antibody will be excluded unless a post anti-CTLA-4 antibody treatment colonoscopy was normal with normal colonic biopsies.
4.2.3 Participants who require immediate active treatment for symptomatic CNS lesions will not be eligible until after completing treatment of their symptomatic lesions. Less than 5 weeks has elapsed since any prior systemic therapy at the time the patient receives the non-myeloablative regimen Day -7. All participants' toxicities must have recovered to a grade 1 or less or as specified in the eligibility criteria. Participants may have undergone minor surgical procedures or focal palliative radiotherapy (to non-target lesions) within the past 5 weeks, as long as all toxicities have recovered to grade 1 or less or prior to Day -7 nonmyeloablative chemotherapy. Systemic steroid therapy more than the equivalent of 10mg/day of prednisone.
4.2.4 Hemoglobin less than 8gm/dl without support of transfusions.
4.2.5 Any active systemic infections, coagulation disorders or other active major medical illnesses of the cardiovascular, respiratory or immune system, as evidenced by a positive stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.
4.2.6 Any form of primary or secondary immunodeficiency. Seropositive for HIV antibody.
4.2.7 Participants with active hepatitis B or active hepatitis C.
4.2.8 a LVEF < 45% on a cardiac stress test (stress thallium, stress MUGA, dobutamine, echocardiogram or other stress test) with or without cardiac history. Cardiac history includes but not limited to: major EKG abnormalities, symptoms of cardiac ischemia or arrhythmias.
4.2.9 Symptoms of respiratory dysfunction and an abnormal pulmonary function test as evidenced by a FEV_1< 60% predicted.

Study Implementation

5.0 Study Visits

5.1.1 Screening Visit (SV): (PSJHC Outpatient Cancer Clinic)
5.1.2 Screening visits will be denoted as SV and numbered sequentially (SV1, SV2) if participants as more than one screening visit.
5.1.3 Review the study with the potential participant, answer any questions, and if potential participant is interested, complete consenting process per JWCI SOP Informed Consent.
5.1.4 Complete physical examination with ECOG performance status.
5.1.5 HR and BP, considered within normal range: HR >55, BP<140 systolic and 90 diastolic
5.1.6 Laboratory Tests (unless performed within past 28 days from screening visit and can provide study team with the official report)
5.1.7 Serum chemistries (CMP) including electrolytes, BUN, creatinine, amylase, liver function tests, and thyroid panel CBC, differential, PT/PTT,
5.1.8 Urinalysis with micro and culture, if signs or symptoms of a Urinary Tract infection
5.1.9 Anti HCV and anti CMV antibody titer; HSV serology, EBV panel
5.1.10 β-HCG pregnancy test on all women of child-bearing potential
5.1.11 Other Testing and Scan to be scheduled and performed unless performed in past 28 days and can provide official report (original or copy)
5.1.12 EKG
5.1.13 Baseline CT of the chest, abdomen and pelvis to evaluate the status of disease (All x-rays must be obtained within 4 weeks of patient entry). Additional scans and x-rays (e.g. brain MRI, bone scan, MRI) may be performed if clinically indicated based on participants' signs and symptoms.
5.1.14 Participants who have received prior treatment with anti-CTLA-4 antibody will undergo colonoscopy with biopsy (if not already done).
5.1.15 Baseline ophthalmological exam including slit lamp within a month of patient entry, because of the possibility of ocular inflammation in some participants after therapy. This will be repeated if the patient has any significant visual or ocular symptoms at any time after treatment begins.
5.1.16 Any other evaluations necessary to determine tolerability of IL-2 therapy per Investigator's discretion.
5.1.17 Once all screening tests are in, PI will determine if participant is eligible. Proceed to study implementation if deemed eligible by PI.
5.1.18 Subjects will be administered a QOL (EORTC-QLQ-C30 version 3)

5.2 Enrollment "visit" (PSJHC Outpatient Cancer Clinic)

Confirmation of eligibility does not require an outpatient visit. It may be determined and future appointments scheduled via communication devices with participants. Copies or notes of communications must be tracked with date/time and study staff identifier and maintained in participants study record.

Participants with metastatic melanoma are considered "enrolled"

1) Who fulfill the eligibility criteria,
2) Who have signed the informed consent, and
3) After EPIC registration updated to flag as a research patient

5.3 Preparation at JWCI, in TIL laboratory

Through the TIL Procure Study-0614, Participant's tumors will be minced into small fragments and 24 fragments cultured in media with recombinant IL-2. TIL will be grown in vitro and cells with anti-tumor activity (see Certificate of Analysis) will be considered for further expansion with OKT3 and feeder cells for patient administration as per FDA reviewed SOPs. Irradiated allogeneic PBMC (obtained from volunteers using standard blood banking procedures) will be used for this purpose (such irradiated allogeneic feeder cells were routinely used in earlier NCI Surgery Branch TIL protocols and JWCI pre-Clinical TIL lab successfully grow TIL with irradiated allogeneic feeder cells).

5.4 Non-Myeloablative Lymphocyte-Depleting Chemotherapy

5.4.1 Day -7 through Day -1 (Inpatient Unit, PSJHC

Once there is confirmation that cells are successfully expanding and day of infusion set (Day 1), participants will receive a non-myeloablative lymphocyte-depleting preparative regimen during the 7 days (Day -7 to -1)preceding the scheduled TIL Infusion Day 1.

5.4.1.1 Day -7
5.4.1.2 Participants will be registered and admitted to the inpatient oncology unit.
5.4.1.3 Oncologist to place all orders and follow patient to include ordering any lab assays and treating any symptoms as per standard of care through discharge
5.4.1.4 Venous access will be secured, preferable a Peripherally Inserted Central Catheter (PICC).
5.4.1.5 Start continuous infusion of Mesna
5.4.1.6 Targeted physical exam
5.4.1.7 Following Laboratory Tests: CMP, CBC w/diff, PT/PTT, U/A with microscopy, Urine Pregnancy test (if screening labs were within 14 days may use those results)
5.4.1.8 Pre-meds given prior to starting chemotherapy, (e.g. Odansetron)
5.4.1.9 Start first of two daily infusion of cyclophosphamide (60 mg/kg/day IVPB)
5.4.1.10 Document all concomitant medications
5.4.1.11 Record and report, as appropriate, all adverse events including start time, description, treatments, and time of resolution or change.
5.4.1.12 Subjects will be administered a QOL (EORTC-QLQ-C30 version 3)
5.4.1.13 Infection Prophylaxis Monitorin (Start on Day -7 per Institutional Standard of Practice)
5.4.1.14 Complete Day -7 Case Report Form (CRF)

5.4.1.15 Infectious Prophylaxis
5.4.1.16 Pneumocystis Carinii Pneumonia

All participants will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three days per week, beginning when engraftment occurs (neutrophil count > 500/ mm^3) and continuing for at least 6 months post chemotherapy and until the CD4 count is above 200 on two consecutive lab studies.

5.4.1.17 Herpes Virus Prophylaxis

Participants with positive HSV serology will be given valacyclovir starting the day after the last dose of fludarabine, orally at a dose of 500 mg daily, or Acyclovir 250 mg/m^2 IV every 12 hrs if the patient is unable to tolerate oral intake, and is continued until Day +100 AND patient is no longer neutropenic. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

5.4.1.18 Fungal Prophylaxis

Participants will start Fluconazole 400 mg P.O. starting the day after the last dose of fludarabine and continuing until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in participants unable to take it orally.

5.4.1.19 Empiric Antibiotics

Participants will start on broad spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone at single fever greater than or equal to 38.3 ^{0}C once or two temperatures of 38.0 ^{0}C or above at least one hour apart simultaneously with an ANC less than 500/mm^3. Aminoglycosides should be avoided unless clear evidence of sepsis. Infectious disease consultation will be obtained from all participants with unexplained fever or any infectious complications.

5.4.1.20 Cytomegalovirus surveillance

CMV PCR surveillance monitoring will be assessed if clinically indicated (e.g. unexplained fevers, pulmonary changes) and ganciclovir administered (5mg/kg I.V. q 12h x 7 days (induction) followed by daily 5 X week (maintenance) until two CMV PCR results are negative, one week apart. During treatment with ganciclovir, the valacyclovir/acyclovir prophylaxis will be temporarily held to avoid possible overlap.

5.4.2 Blood Product Support

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. Attempts will be made to keep Hgb>8gm/dl, and plts>20,000/mm^3. All allogeneic blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

5.4.5 Day -6

5.4.5.1 Administer 2nd of two daily doses of cyclophosphamide (60mg/kg/day IVPB)

5.4.5.2 Continue Mesna and hydration infusion

5.4.5.3 Document all concomitant meds

5.4.5.4 Record and report as appropriate per Reporting section 176.0 any new AEs, and any changes to previously recorded AEs, especially time of resolution.

5.4.5.5 Complete Day -6 CRF

5.4.6 Day -5

5.4.6.1 Continue Mesna and hydration infusion for 48 hours total from start on Day -7

5.4.6.2 Administer first dose fludarabine (25 mg/m^2/day x 5 days IVPB)

5.4.6.3 Document all concomitant meds

5.4.6.4 Record and report as appropriate per Reporting section 17.0 any new AEs, and any changes to previously recorded AEs, especially time of resolution.

5.4.6.5 After completion of IV medications and participant is assessed to be ready for discharge by covering Investigator, participant may go home with any instructions per Investigator per standard of care practices to return following day

5.4.6.6 Complete Day -5 CRF and Discharge CRF

5.4.7 Days -4 through -1 (per PSJHC standards of care)

5.4.7.1 Return daily to PSJHC to be registered

5.4.7.2 Obtain any lab assays ordered by Investigator prior to starting chemotherapy

5.4.7.3 Confirm venous access each day

5.4.7.4 Administer fludarabine (25 mg/m^2/day x 5 days IVPB)

5.4.7.5 Document any concomitant medications

5.4.7.6 Record and report as appropriate any AEs per Reporting section 17.0.

5.4.7.7 .Participants will return daily for next 5 days to PSJHC for administration of fludarabine.

5.4.7.8 Only new and unexpected Adverse Events will be recorded during chemotherapy, see Reporting Section 11.0

5.4.7.9 Complete the day's CRF

5.5 TIL Infusion Day 1 (PSJHC)

5.5.1 Participants will return and be admitted inpatient at PSJHC

5.5.2 Prior to infusion of TIL the following will be performed:

1) Targeted (symptom-driven) Physical Exam will be performed
2) Continued eligibility will be confirmed by PI
3) Baseline laboratories and research PBMC samples will be drawn

5.5.3 Secure IV access. A numbing agent may be used to place the catheter per PI discretion.

5.5.3.1 Record and report any new or changes to AEs per Reporting section 16.0

5.5.4 Document any concomitant meds

5.5.5 Participants will receive an IV adoptive transfer of at least 10^9 tumor-reactive lymphocytes.

5.5.6 Complete the Day 1 CRF TIL Infusion

5.5.7 The TIL will be released with a label identifying the product as "Caution- New Drug Limited by Federal Law to Investigational Use".

5.5.8 All Adverse Events will be collected through Day 7 post inpatient discharge; after which only new and unexpected SAEs will be reported, see section 17.0

5.5.9 The TIL must be administered over 20-30 minutes at room temperature using a standard infusion protocol or by hanging the infusion bag from a stand and allowing gravity to pull the cells down

5.5.10 If the patient experiences an infusion reaction such as those described in section 7.4.2, or any other adverse reaction, stop the infusion, assess the subject and treat as clinically indicated. Document and report this adverse event to the PI.

5.5.11 Once TIL administration is complete, discard the infusion bag and infusion set as per institutional policy for biohazardous materials.

5.6 High Dose Interleukin 2 (HD IL-2) Infusion

5.6.1 As per standard of care for Metastatic Melanoma patients, begin HD IL-2 600,000-IU/kg/dose every 8 hours (± 30 minutes) for up to 12 doses within 24 hours of receiving the TIL. If well tolerated and concurrence of the Investigator and PI dose may be increased to 720,000 IU/kg/dose.
5.6.2 High dose IL-2 will be administered per standard of care.
5.6.3 Participants will receive supportive therapy per a trained oncologist (Appendix X).
5.6.4 Once IL-2 infusions have been discontinued, participants will remain inpatient at PSJHC until ready for discharge to received appropriate supportive care by standards of care.
5.6.5 Participants will be discharged once Investigator assesses them ready for discharge.

5.7 Follow-up-Outpatient PSJHC Cancer Clinic-

At 4, 8, 12 weeks, post TIL infusion (Day 1)

5.7.1 Targeted Physical Exam
5.7.2 Vital Signs
5.7.3 Review of any open AEs and record and report any new SAEs per Reporting Section 17.0
5.7.4 Record any concomitant medications
5.7.5 Safety Laboratory Testing to include: CMP, CBC w/diff, and any deemed necessary by PI
5.7.6 Tumor Measurements and Evaluations by CT scans (abd and chest), CXR, Bone Scan
5.7.7 Subjects will be administered a QOL (EORTC-QLQ-C30 version 3) questionnaire (Appendix 7) per directions in Appendix 8.
5.7.8 Research PBMCs will be drawn

5.8 Post Study Evaluation

Complete evaluation of evaluable lesions with physical examination and CT of the chest, abdomen and pelvis will be performed within two weeks before starting chemotherapy and 4 and 12 weeks after the cell infusion. Toxicity assessment will be performed at the 4 week follow up visit. Other clinical evaluations will be used to determine tumor response. If a response or SD is present on the 4 week post-treatment CT scan, a confirmatory CT scan will be performed at least 4 weeks after the 8 week post-treatment scan to verify the response (Appendix 6). The participants will continue to undergo CT scanning as part of their standard of care after treatment. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan. If the patient has SD or tumor shrinkage, repeat complete evaluations will be performed every 1-3 months in accordance to standard of care.

5.9 Concomitant medications (Given to control side-effects of therapy will be given)

5.9.1 Any medications given during the participant's active participation, enrollment through 12 week follow-up, will be recorded on the Concomitant Medication CRF. Recorded elements include date started, date ended (if med is stopped), dose, reason for taking
5.9.2 Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg

q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg q8h). Participants who require transfusions will receive irradiated blood products.

5.9.3 Ondansetron hydrochloride will be obtained from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritus, constipation and urinary retention.

5.9.4 Furosemide will be obtained from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritus.

5.10 PBMC and Serum Collection

Peripheral blood mononuclear cells (PBMCs) from 30mL of peripheral blood will be obtained from participants before and after treatment and cryopreserved so that immunologic testing may be performed in an unknown future date. Subjects will have 30 ml of blood drawn at screening, prior to TIL infusion on Day 0 and at their 4, 8, and 12 week post treatment follow up visits. The PBMC and serum will be processed and stored frozen for future testing to study the effects of TIL plus high dose IL-2 on the immune system.

5.11 Off Study Criteria

Participants will be taken off study if the patient voluntarily withdraws, there is significant noncompliance, or if there is tumor progression without an option for retreatment. In addition, participants who meet criteria for PR or CR with confirmatory study at least 6 weeks post treatment CT scan will be followed using the standard of care. Participants who have evidence of dose limiting toxicity as defined in Section 5.2 (toxicity criteria) will be taken off treatment but continue in follow up until off study criteria are met. Participants who experience IL-2 related toxicity (Appendix 5) will be managed per the judgment of the Investigator and do not need to be removed from study treatment for well-known IL-2 related toxicity which responds to standard measures. If EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV seronegative subject occurs in any patient on this study, accrual of EBV-negative subjects will halt pending review of the event by the IRB and FDA.

Analysis

6.0 Data Collection and Evaluation

6.1 Response Criteria

6.1.1 Evaluation of target lesions

A maximum of 5 target lesions total (up to (2) per organ) per RECIST 1.1. Measurable Lesions must be ≥10 mm in longest diameter. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (SLD) for all target lesions will be calculated and reported as the baseline SLD.

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (SLD) of target lesions taking as reference the baseline

SLD.

Progression (PD):	At least a 20% increase in the SLD of target lesions taking as reference the smallest SLD recorded since the treatment started or the appearance of one or more new lesions.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest SLD.
Not Evaluable (NE):	If all lesions cannot be evaluated due to missing data or poor image quality the patient is not evaluable (NE) at that time point.

6.1.2 Evaluation of non-target lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level.
Non-Complete Response(NCR):	Persistence of one or more non-target lesions
Progression (PD):	Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

6.1.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (Table 3).

Table 3 Response Criteria

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	NE	No	PR
PR	Non-PD or NE	No	PR
SD	Non-PD or NE	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

6.2 Confirmatory Measurement/Duration of Response Confirmation

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

6.2.2 The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

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

6.3 Quality of Life Assessment

Quality of Life Evaluation assessments will be done prior to treatment and at specific time points post treatment (Appendix 6). The quality of life questionnaire module (EORTC-QLQ-C30 version 3, Appendix 7) and scoring procedures will be done per EORTC guidelines.

6.4 Statistical Plan

The primary objective is to evaluate the feasibility and safety of conducting this trial in a single-site setting. The another primary objective of the trial, on which the sample size will be based, is to determine if the combination of TIL infusion with high dose IL-2, after lymphocyte depleting chemotherapy, is able to achieve an objective response rate that can rule out 15% (15% response rate under the null hypothesis) in favor of a modest, targeted 40% response rate (40% response rate under the alternative hypothesis) in participants with metastatic melanoma refractory to standard treatments.

To achieve 85% power (15% probability of rejecting a good therapy) at 5% type I error (5% probability of accepting a poor therapy) and 85% power, 22 evaluable participants will be enrolled and treated on this trial. All 22 participants will be followed for minimum 12 weeks, or until develop new metastases or recurrence. At the end of 12 week follow up, the proportion of participants that showed clinical response (CR) determined by the disappearance of all target lesions, or partial response (PR) will be calculated. The patient is determined to have partial response as if 30% reduction in the sum of the longest diameter (SLD) of target lesions are shown from the baseline sum LD. If 9 participants our of 22 are showed CR or PR at the time of 12 weeks follow up, then further investigation of this treatment strategy is warranted.

Quality of Life will be assessed and scored per EORTC-QLQ-C30 version 3.0 requirements. The EORTC QLQ-C30 contains subscales for global health status, and physical, emotional, role, cognitive and social function with higher scores indicating better functioning (19). The change in QOL measured throughout the study period will be examined through mixed effect model adjusting for the baseline. Akaike information criteria (AIC) will be used to determine appropriate covariance structure.

6.5 Data Safety and Monitoring Plan

The Principal Investigator or their designee will monitor all final cell product functional data prior to administration, to insure delivery of TIL with adequate and consistent activity. The Medical Monitor will review adverse events within a month of each cell infusion to also evaluate trends and will require follow up plans from the principal investigator whenever a trend is identified. All SAE's reports will be will be expedited to the IRB

6.5.1 Toxicity Criteria

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of cell infusions will be performed. This study will utilize the CTC version 4.0 for toxicity and adverse event reporting. A copy of the CTC version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

6.5.2 Dose-Limiting toxicities

Participants experiencing the follow will have their IL-2 discontinued. Any grade 3 or greater hematologic or non-hematologic reaction excluding injection site reactions, skin rash, pruritus, and local adenopathy, and excluding those expected toxicities from the chemotherapy preparative regimen or support medication administration as listed below or in the package insert for commercial agents. Participants who experience known IL-2 related toxicities will be managed with standard supportive measures for IL-2 (Appendix 1) and do not need to be removed from study treatment unless indicated in Appendix 1.

7.0 Pharmaceutical Information

7.1 Cyclophosphamide

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration. It will be purchased from commercial sources.
Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8^{0}C.
Storage: Intact vials should be stored at room temperature.
Administration: 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 Appendix 4. Doses will be administered as an IV infusion in 250 mL D5W over 1 hour.
Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea, and vomiting occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of participants; 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 auroprotective agent in participants receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all participants. Participants who receive high-dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in participants with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, participants should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high-doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high-doses, cyclophophamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include

headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

7.2 Fludarabine

Description: Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite
How Supplied: It will be purchased from commercial sources.
Stability: Following reconstitution with sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8^{0}C; the single-use vial contains no antibacterial preservative; therefore, the reconstituted product should be discarded 8 hours after initial entry.
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, ribnucleotidereductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.
Storage: Intact vials should be stored refrigerated (2-8^{0}C).
Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. To prevent undue toxicity the dose will be based on 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, (See Appendix 4).
Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coombs 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 only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL participants treated with fludarabine. One patient developed EBV lymphoma after prolonged lymphopenia and two participants developed autoimmune uveitis which responded to steroid eye drops.

7.3 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

Description: Mesna will be obtained commercially and is supplied as a 100 mg/ml solution. It will be purchased from commercial sources.
Storage: Intact ampoules 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 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.
Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% Sodium Chloride Inj. and to be administered intravenously as a continuous infusion. Toxicities include

nausea, vomiting and diarrhea.

7.4 TIL cells

TIL is manufactured at JWCI using FDA reviewed SOPs. Certificate of Analysis of TIL will be completed before release. The agent contains T-lymphocytes derived from autologous tumor. The TIL is suspended in 0.9% sodium chloride containing 2.5% albumin and IL-2 (50 CU/mL).
The expected toxicities that may occur with this agent include fever, chills, headaches, malaise, shortness of breath and transient rash.

7.5 Interleukin-2 (Proleukin)

Description: Interleukin-2 (IL-2) is manufactured by the Prometheus, San Diego CA, and will be purchased commercially.
How Supplied: IL-2 is produced by Chiron Corporation in single-use vials containing 22 million IU (~1.3 mg) of IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8).
Formulation/Reconstitution: IL-2 (Chiron), NSC #373364, is provided as a lyophilized powder. The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.
Storage: Intact vials are stored in the refrigerator (2 - 8^0C) protected from light. Each vial bears an expiration date.
Dilution/Stability: Reconstituted IL-2 for high-dose intravenous administration should be further diluted with 50 mL of 5% Human Serum Albumin (HSA) or sterile water for injection, USP. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. IL-2 is chemically stable for 48 hours at refrigerated and room temperatures, 2 - 30C.
Administration of high-dose IL-2: The final dilution of IL-2 will be infused over 15 minutes. IL-2 will be administered as an inpatient.
Toxicities: Grade III toxicities common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Interleukin-2 (Proleukin)
Reporting: Only severe or unexpected (grade 4 and all grade 5) and other unexpected toxicities will require expedited reporting to JWCI. Reporting of any grade 3 or 4 expected laboratory abnormalities that are easily reversible without organ dysfunction are not required. (see Appendix 1)

7.6 Filgrastim

Filgrastim (granulocyte colony-stimulating factor, G-CSF, filgrastim, Neupogen) will be obtained commercially and is supplied in 300 µg/ml and 480 mcg/1.6 ml vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

7.7 Bactrim DS

Trimethoprim and Sulfamethoxazole double strength (TMP / SMX DS, Bactrim) will be obtained from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO bid twice a week beginning on day -7 and continuing for at least 6 months and until CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, Bactrim can cause allergies, fever, nausea, and vomiting and photosensitivity. 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.

7.8 Fluconazole

Fluconazole will be obtained from commercial sources. It will be used to guard against fungal infections. It is available in 200 mg tablets. Participants will start Fluconazole 400 mg p.o. on the same day as the cell infusion (day 0). It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in participants who cannot tolerate the oral preparation, Fluconazole comes in 2 MG/ML solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in participants unable to take it orally.
Hepatic injury: fluconazole has been associated with rare cases of serious hepatic toxicity, including fatalities primarily in participants with serious underlying medical conditions. In cases of Fluconazole DIFLUCAN-associated hepatotoxicity, no obvious relationship to total daily dose, duration of therapy, and sex or age of the patient has been observed.
Fluconazole hepatotoxicity has usually, but not always, been reversible on discontinuation of therapy. Participants who develop abnormal liver function tests during Fluconazole therapy should be monitored for the development of more severe hepatic injury. Fluconazole should be discontinued if clinical signs and symptoms consistent with liver disease develop that may be attributable to fluconazole.

7.9 Herpes Virus Prophylaxis

7.9.1 Valacyclovir

Valacyclovir (Valtrex) will be obtained from commercial sources in 500mg tablets. It will be used orally to prevent the occurrence of herpes virus infections. Valcyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in participants with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

6.9.2 Acyclovir

Acyclovir will be obtained from commercial sources. It will be used intravenously 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 to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Acyclovir will be started the day after the last dose of fludarabine, at a dose of 250 mg/m^2 IV q 12 hrs if the patient is unable to tolerate oral intake.

Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; diaphoresis; hematuria; hypotension; and thrombocytosis have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling. Common side effects include anorexia, nausea, bronchospasm, cough, dyspnea (inhalation only), elevated liver function tests, rash, and nephrotoxicity.

Regulatory

8.0 Adverse Event Reporting

FDA Serious Adverse Event Reporting Requirements (21 CFR 312.64 (b)

8.1 Sponsor Reporting

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

8.2 Expedited Reporting

Expedited reporting requirements for serious adverse events that occur on this study within 30 Days of the Last Administration of the Investigational Agent/Intervention, Day 1 TIL Infusion.

8.3 Serious Adverse Event

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
   Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention.

8.4 Expedited AE reporting timelines

1) “24-Hour; 5 Calendar Days” - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
2) “10 Calendar Days” - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.
3) Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

4) A complete report is to follow with 10 working days.

8.5 Adverse Event Reporting in the Continuing Review Report

The IRB requires a summary report of adverse events that have occurred on the protocol since the previous continuing review. The method of presentation should provide the IRB with the information necessary to clearly identify risks to participants and to make a risk: benefit determination. The summary report is based on the following guidance:

1) Any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk: benefit of study participants in the narrative.
2) Grade 1 events are not required.
3) Grade 2 unexpected related to the research events is required.
4) Grade 3 and 4 expected and unexpected events related to the research are required except for reversible toxicities known to be associated with the administration of high-dose IL-2 (see Appendix 5).
5) Grade 5 (all) events are included regardless of attribution.

   Based on protocol-associated risks to participants, the local IRB retains the authority to establish more frequent continuing review periods than the customary annual review period.

8.6 Serious Adverse Event (SAE)

Definition of Serious Adverse Event: An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention.

ALL SERIOUS adverse events (SAEs) that meet the above criteria MUST be immediately reported to the JWCI within the timeframes detailed in Table 4.

Table 4 Reporting Guidelines for SAEs

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days

8.6.1 Expedited 24-hour notification followed by complete report within 5 calendar days for All Grade 4/5 AEs

8.6.2 Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

8.7 Halting Rules

1) If one of the first four participants (or 2 of the first 6 participants, or 3 of the first 9 participants, or 4 of the first 12 participants) develops a grade 3 or greater non-hematologic toxicity due to the prior conditioning regimen, IL-2 or the cell product that does not resolve in 72 hours, or experiences a toxicity requiring intubation, vasopressors or continuous veno-venous hemofiltration (CVVH).
2) If the results of post lot-released TIL sterility testing show contamination. In this case, the PI must notify JWCI Compliance, accrual will be temporarily suspended pending the results of an internal investigation. These results will be reported to the IRB and the FDA.
3) If one of the first ten treated participants dies of treatment-related complications before Day 100.
4) If EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject occurs, accrual of EBV-negative subjects will halt pending review of the event by the IRB and FDA.
5) In the case of exceeding grade 4 toxicity rates as per the package insert for IL-2 for the following: cardiovascular system, hyperbilirubinemia, creatinine increase, nervous system, and respiratory system. IRB Serious Adverse Event Reporting Requirements the PI or designees will report:

8.8 Unexpected and Related SAE

- All serious events as defined by the FDA that are unexpected and related to the research;
- All deaths (grade 5: CTCAE) expected and unexpected, related and unrelated to the research. All Grade 3 and 4 (CTCAE), unexpected, and related to the research with attributions of possibly, probably, or definitely within 24 hours of the event or notification of the event. The PI or designees must report all serious and unexpected adverse events to the IRB using the IRB 24 hour Serious,
- Unexpected Adverse Event Report Form. A follow up form must be submitted within 10 working days of the event or notification of the event. Information may be sent via SECURE e-mail to:

Western Institutional Review Board® (WIRB®)
1019 39th Avenue SE Suite 120
Puyallup, Washington 98374-2115
Telephone: 1-800-562-4789 or 360-252-2500
Fax: 1-310-252-2498
E-mail: Help@wirb.com

9.0 Human Subjects Protections

9.1 Rationale for Patient Selection

The participants to be entered in this protocol have metastatic cancer and limited life expectancies and

have measurable metastatic melanoma that is refractory to standard treatment. Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is not information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

9.2 Evaluation of Benefits and Risks

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in section 13.0 Toxicity Criteria and Section 18 Pharmaceutical Information, and in Appendix 5. Because all participants in this protocol have metastatic melanoma and limited life expectancies the potential benefit is thought to outweigh the potential risks.

9.3 Consent Document

All participants are thoroughly screened for eligibility prior to admission onto this study. During this time, the patient, along with family members, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent form is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. Informed consent will be obtained by the principal investigator, a sub-investigator, research nurse or a medical staff fellow.

9.4 Participation of Children

Children under the age of 18 are excluded because melanoma is not common in children and they may not be representative of adults affected by this disease. Although there is an equally prognosis associated in minors, this age group will be excluded until this treatment approach shows benefit in adults.

10.0 Regulatory Obligations

10.1 Independent Review Board/Independent Ethics Committee (IRB/IEC)

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material or materials to be distributed to study subjects must be submitted to the IRB/IEC for written approval. A copy of the written approval of the protocol and informed consent form must be received and on file prior to recruiting subjects into the study. The investigator must submit and, where necessary, obtain approval from the IRB/IEC for all subsequent protocol amendments and changes to the informed consent document. The investigator is to notify the IRB/IEC of deviations from the protocol or serious adverse events occurring at the site and other adverse event reports, in accordance with local procedures. The investigator is responsible for obtaining annual IRB/IEC approval/renewal throughout the duration of the study.

10.2 ICH/GCP Conformance

In compliance with federal regulations and International Conference on Harmonisation (ICH) GCP Guidelines, it is required that the investigator and institution permit authorized representatives of regulatory agencies, JWCI and PHS compliance, and the IRB/IEC direct access to review the subject's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform and obtain the consent of the subject to permit such individuals to have access to his/her study-related records, including personal information.

10.3 Protocol Amendments and Study Termination

The IRB/IEC must be informed of all amendments and give approval. The investigator must retain a copy of the approval letter from the IRB/IEC to the investigator. The investigator reserves the right to terminate the study at any time. The investigator is to notify the IRB/IEC in writing of the study's completion or early termination and retain a copy of the notification with the essential regulatory documentation.

10.4 Study Documentation and Archive

The investigator is to maintain a list of appropriately qualified persons to whom he/she has delegated study duties. All persons authorized to make entries and/or corrections on CRFs will be included on the Delegation of Authority Log. Source documents are original documents, data, and records from which the subject's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. The investigator and study staff are responsible for maintaining a comprehensive filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives of regulatory agencies, JWCI and PHS compliance, and the IRB/IEC. Elements to include:

- Subject files containing completed CRFs, informed consent forms, and subject identification list
    - Investigational product-related correspondence including certificates of analysis, manufacture records, product accountability record(s), as applicable.

In addition, all original source documents supporting entries in the CRFs must be maintained and readily available. Retention of study documents will be governed per federal, state and institutional policies.

11.0 References

1. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol. 2005;23(10):2346-57. PMCID:15800326
2. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, Morton KE, Laurencot CM, Steinberg SM, White DE, Dudley ME. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res.17(13):4550-7. PMCID:21498393
3. Barth A, Wanek LA, Morton DL. Prognostic factors in 1,521 melanoma patients with distant metastases. J Am Coll Surg. 1995;181(3):193-201. PMCID:7670677
4. Smith FO, Downey SG, Klapper JA, Yang JC, Sherry RM, Royal RE, Kammula US, Hughes MS, Restifo NP, Levy CL, White DE, Steinberg SM, Rosenberg SA. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res. 2008;14(17):5610-8. PMCID:18765555
5. Bernatchez C, Radvanyi LG, Hwu P. Advances in the treatment of metastatic melanoma: adoptive T-cell therapy. Semin Oncol. 2012;39(2):215-26. PMCID:22484193
6. Sapoznik S, Hammer O, Ortenberg R, Besser MJ, Ben-Moshe T, Schachter J, Markel G. Novel anti-melanoma immunotherapies: disarming tumor escape mechanisms. Clin Dev Immunol. 2012;2012:818214. PMCID:22778766
7. Davar D, Tarhini A, Kirkwood JM. Adjuvant therapy: melanoma. J Skin Cancer. 2011;2011:274382. PMCID:22220281
8. Dudley ME, Gross CA, Langhan MM, Garcia MR, Sherry RM, Yang JC, Phan GQ, Kammula US, Hughes MS, Citrin DE, Restifo NP, Wunderlich JR, Prieto PA, Hong JJ, Langan RC, Zlott DA, Morton KE, White DE, Laurencot CM, Rosenberg SA. CD8+ enriched "young" tumor infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin Cancer Res. 2010;16(24):6122-31. PMCID:20668005
9. Radvanyi LG, Bernatchez C, Zhang M, Fox P, Miller P, Chacon J, Wu RC, Lizee G, Mahoney S, Alvarado G, Glass M, Johnson V, McMannis JD, Shpall EJ, Prieto VG, Papadopoulos NE, Kim KB, Homsi J, Bedikian AY, Hwu WJ, Patel S, Ross MI, Lee JE, Gershenwald JE, Lucci A, Royal R, Cormier JN, Davies MA, Mansaray R, Fulbright OJ, Toth C, Ramachandran R, Wardell S, Gonzalez A, Hwu P. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2012. PMCID:23032743
10. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst. 1994;86(15):1159-66. PMCID:8028037
11. Mills CD, North RJ. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient. Inhibition by suppressor T cells. J Exp Med. 1983;157(5):1448-60. PMCID:6189937
12. Wrzesinski C, Paulos CM, Kaiser A, Muranski P, Palmer DC, Gattinoni L, Yu Z, Rosenberg SA, Restifo NP. Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. J Immunother.33(1):1-7. PMCID:19952961
13. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298(5594):850-4. PMCID:12242449

14. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, Robbins PF, Huang J, Citrin DE, Leitman SF, Wunderlich J, Restifo NP, Thomasian A, Downey SG, Smith FO, Klapper J, Morton K, Laurencot C, White DE, Rosenberg SA. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008;26(32):5233-9. PMCID:18809613
15. Yao X, Ahmadzadeh M, Lu YC, Liewehr DJ, Dudley ME, Liu F, Schrump DS, Steinberg SM, Rosenberg SA, Robbins PF. Levels of peripheral CD4(+)FoxP3(+) regulatory T cells are negatively associated with clinical response to adoptive immunotherapy of human cancer. Blood.119(24):5688-96. PMCID:22555974
16. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Karson EM, Lotze MT, Yang JC, Topalian SL, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990;323(9):570-8. PMCID:2381442
17. Shahinian VB, Muirhead N, Jevnikar AM, Leckie SH, Khakhar AK, Luke PP, Rizkalla KS, Hollomby DJ, House AA. Epstein-Barr virus seronegativity is a risk factor for late-onset posttransplant lymphoroliferative disorder in adult renal allograft recipients. Transplantation. 2003;75(6):851-6. PMCID:12660514
18. Curtis RE, Travis LB, Rowlings PA, Socie G, Kingma DW, Banks PM, Jaffe ES, Sale GE, Horowitz MM, Witherspoon RP, Shriner DA, Weisdorf DJ, Kolb HJ, Sullivan KM, Sobocinski KA, Gale RP, Hoover RN, Fraumeni JF, Jr., Deeg HJ. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood. 1999;94(7):2208-16. PMCID:10498590
19. Revicki DA, van den Eertwegh AJ, Lorigan P, Lebbe C, Linette G, Ottensmeier CH, Safikhani S, Messina M, Hoos A, Wagner S, Kotapati S. Health related quality of life outcomes for unresectable stage III or IV melanoma patients receiving ipilimumab treatment. Health and quality of life outcomes. 2012;10:66. PMCID:22694829
20. Proleukin® (aldesleukin) package insert, Prometheus, San Diego, CA
21. O'Day, Gammon G, BoasbergPD, Martin MA, Kristedja TS, Guo M, Stern S, Edward S, Fournier P, Weisberg M, Cannon M, Fawzy NW, Johnson TD, Essner R, Foshag LJ, Morton DL. Advantages of concurrent biochemotherapy modified by decrescendo interleukin-2, granulocyte colony-stimulating factor, and tamoxifen for patients with metastatic melanoma. J Clin Oncol. 1999;17(9):2752-61. PubMed PMID: 10561350

Appendices

Appendix 1: Expected IL-2 Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritus	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg q4h p.r., prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; DiphenoxylateHCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bed rest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No

*Unless the toxicity is not reversed within 12 hour

Appendix 1 continued

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hour

Appendix 2: Schedule of Events					
Tests/Exams	**Screening w/in 28 days**	**Cycloph. & Mesna Day -7**	**Cycloph. & Mesna Day -6**	**Fludarabine Days -5**	**Fludarabine Days -4 to -1**
Inpt/outpt	OUTPT-CLINIC	INPT-ONCOLOGY UNIT			OUTPT-SHORT STAY
Registration	**X**	**X**			**X**
I/E Criteria	**X**				
Surg/MedHX	**X**				
Targeted PE	**X**			**X**	
Vital Signs [e]	**X**	**X**	**X**	**X**	**X**
ECOG	**X**				
QOL		**X**			
AEs		**X**	**X**	**X**	**X**
Con Meds	**X**	**X**	**X**	**X**	**X**
Chem [a] and CBC w/diff	**X**	**X**	**X**	**X**	**X**
PT/PTT	**X**				
UA w/micro [b]	**X**				
HIV, HTLV I/II, NAT	**X**				
HCV, HbsAG,CGS Ab	**X**				
EBV,CMV,HSV	**X**				
B-HCG	**X**				
ECG	**X**				
CXR	**X**				
Tumor Measurements	**X**				
CT Scan-abd, chest	**X**				
Bone Scan	**X**				
Colonoscopy w/biopsy [c]	**X**				
Opthalmology Exam [d]	**X**				
Echo/Muga	**X**				
PFT's	**X**				

Appendix 2: Schedule of Events continued										
Tests/Exams	**TIL Infusion Day 1**	**IL-2 Day 2**	**IL-2 Day 3**	**IL-2 Day 4**	**IL-2 Day 5**	**Recovery on Med Onc unit**	**4wk f/u +/- 3 days**	**8 wk f/u +/- 1wk**	**12 wk f/u +/- 1wk**	**Off Tx f/u**
Inpt/outpt	INPT-ICU					INPT 1-3 DAYS	OUTPT-CLINIC	OUTPT-CLINIC	OUTPT-CLINIC	OUTPT-CLINIC
Targeted PE	**X**					**X**	**X**	**X**		
Vital Signs [e]	**X-f**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**Xp**
QOL							**X**	**X**	**X**	
AEs	**X-f**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	
Con Meds	**X-f**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	
Chem [a]	**X-f**	**X**	**X**	**X**	**X**					
CBC w/diff	**X-f**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**
PT/PTT	**X-f**									
UA w/micro [b]	**X-f**									
B-HCG	**X-f**									
ECG	**X-f**							**X**	**X**	**X**
CXR								**X**	**X**	
Tumor Measurements							**X**	**X**	**X**	
CT Scan-abd, chest,[g]								**X**	**X**	
Bone Scan [g]										
PBMC & Serum	**X-f**						**X**	**X**		
G-CSF 10mcg/kg/day		**X**	**X**	**X**	**X**					
TMP/SMX	**qd X3/week when ANC>500 until 6mos post chemo AND CD4>200**									
Fluconazole	**until the absolute neutrophil count is greater than 1000/mm3**									
Acyclovir/Valacyclovir	**If Serology is + until Day +100 AND patient is no longer neutropenic**									
a. Chemistries to include CMP, amylase, LFT's and TSH										
b. U/A w micro (within 28 days of registration and 14 days of start of chemotherapy); cx will be done if indicated by initial U/A results										
c. For those who have received prior treatment with anti-CTLA-4 antibody, if not already done										
d. Baseline ophthalmology exam to include slit lamp										
e. Vital signs include blood pressure, heart rate, temperature, respiration rate (weight is at screening and daily when inpatient; height is only at screening)										
f. Prior to TIL infusion										
g Post treatment scans will be driven by standard of care and Clinical Provider; what scans are available will be reviewed at follow-up visits										

Appendix 3: Life-Threatening Adverse Events
(Grade 4; n=525) (Cited from reference (20), Table 4)

BODY SYSTEM	# (%) PATIENTS	BODY SYSTEM	# (%) PATIENTS
Body as a whole		**Metabolic & Nutritional Disorders**	
Fever	5 (1%)	Bilirubinemia	13 (2%)
Infection	7 (1%)	Creatinine increase	5 (1%)
Sepsis	6 (1%)	SGOT increase	3 (1%)
Cardiovascular		Acidosis	4 (1%)
Hypotension	15 (3%)	**Nervous**	
Supraventricular tachycardia	3 (1%)	Confusion	5 (1%)
Cardiovascular disorder	7 (1%)	Stupor	3 (1%)
Myocardial infarct	7 (1%)	Coma	8 (2%)
Ventricular tachycardia	5 (1%)	Psychosis	7 (1%)
Cardiac arrest	4 (1%)	**Respiratory**	
Digestive		Dyspnea	5 (1%)
Diarrhea	10 (2%)	Respiratory disorder	14 (3%)
Vomiting	7 (1%)	Apnea	5 (1%)
Hemic & Lymphatic		**Urogenital**	
Thrombocytopenia	5 (1%)	Oliguria	33 (6%)
Coagulation disorder	4 (1%)	Anuria	25 (5%)
		Acute kidney failure	3 (1%)

Appendix 4: Toxicities of Concurrent Biochemotherapy
(Cited from reference (21), Table 6)

Toxicity	Patients (n = 45)		Treatment Cycles (n = 217)	
	No.	%	No.	%
Capillary leak syndrome				
Systolic hypotension < 90 mm Hg	20	44	43	20
Body weight gain				
0-5%	25	56	171	79
6-10%	18	40	44	20
> 10%	2	4	2	1
Infection	19	42	28	13
Catheter-related	15	33	18	8
Staphylococcus epidermidis	11	24	13	6
Staphylococcus aureus	4	9	5	2
Clostridium difficile	4	9	4	2
Others	5	11	6	3
Gastrointestinal toxicity				
Grade 3	13	29	21	10
Grade 4	2	4	4	2
Renal				
Grade 3	1	2	2	1
Grade 4	1	2	1	0.5
Catheter-related deep vein thrombosis	1	2	1	0.5
Intracranial hemorrhage/death	1	2	1	0.5