PHASE II STUDY OF CELLULAR ADOPTIVE IMMUNOTHERAPY USING AUTOLOGOUS CD8+ ANTIGEN-SPECIFIC T CELLS AND ANTI-CTLA4 FOR PATIENTS WITH METASTATIC MELANOMA

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2. INTRODUCTION

Adoptive T cell therapy represents a promising strategy for the treatment of patients with cancer. Phase I and II studies using adoptively transferred antigen-specific T cells have led to the conclusion that its effectiveness may be enhanced by extending the *in vivo* persistence of transferred T cells and broadening the repertoire of immune responses to limit the outgrowth of antigen-loss tumor variants.

CTLA4 is a molecule that is up-regulated on activated T cells and functions as an inhibitory receptor. When engaged to its B7-ligand, CTLA4 delivers inhibitory signals that impede TCR signaling, IL-2 transcription, and T cell proliferation (1). The use of anti-CTLA4 antibody to counteract this effect can lead to an enhanced T cell response and the induction of a broadened T cell response to antigens released by killed tumor cells.

We propose a combined biologic strategy involving the use of adoptively transferred antigen-specific CD8+ CTL and the concomitant administration of anti-CTLA4 as a means of enhancing the transferred T cell response and promoting the generation of endogenous T cell responses against a broader panel of tumor-associated antigens that are released in a pro-inflammatory environment following lysis of melanoma tumors by transferred CTL.

We will evaluate, in a Phase II study of adoptive cellular therapy, up to 20 patients with metastatic melanoma to evaluate the influence of anti-CTLA4 administration on the duration of *in vivo* persistence of transferred antigen-specific CTL and the induction of a multivalent T cell response against non-targeted epitopes. Results will be correlated to clinical responses and/or the outgrowth of antigen-loss tumor variants if this last event occurs. Patients will receive an infusion of antigen-specific CTL (10^{10} cells/m^2) preceded by low-dose cytoxan (300 mg/m^2). They will receive in addition to low-dose IL-2, one dose of anti-CTLA4 3mg/kg the day after the CTL infusion followed by anti-CTLA4 for a total of 3 successive doses at 3mg/kg every 3 weeks thereafter.

2.1 Immunotherapy of Malignant Melanoma

The incidence of malignant melanoma has steadily doubled since 1975 to reach 20 new cases per 100'000 inhabitants per year in 2004. Unlike the trend for other common cancers, the death rate for invasive forms at time of diagnosis has not decreased and has remained steady since 1991, suggesting very little improvement in the 5-year survival rate for the past 15 years (2). The median age of patients with melanoma is 45 years and thus, the loss of productive years is one of the most significant among cancers (2). Chemotherapy and radiation therapy represent the standard treatment for patients with extensive disease at presentation or with recurrent disease after surgical resection, but response rates are low and the responses are not usually durable. As a result many patients are treated in a palliative way or are enrolled in investigational studies (3).

In recent years, the identification of immunogenic targets in melanoma and other malignancies and the development of novel technologies to elicit effector cells *in vivo* and *ex vivo* have led to clinical trials of adoptive cellular therapy and vaccination studies. Evidence

of tumor regression and measurable responses in patients with progressive metastatic disease who were otherwise refractory to conventional therapy has heralded a renewed interest in antigen-specific cellular immunotherapy.

2.2 Adoptive T Cell Therapy

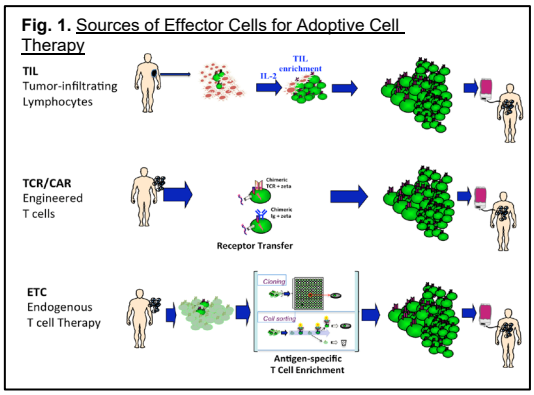


Fig. 1. Sources of Effector Cells for Adoptive Cell Therapy

Significant advances in understanding and manipulating the tumor immune response over the last decade have shown that tumor-associated antigens can be identified that can elicit a robust T cell response in patients and, thus, strategies to isolate and expand such T cells ex vivo would allow the adoptive transfer of anti-tumor immunity to the treated patient. This concept is the basis for adoptive cellular therapy (ACT), which is a form of immunotherapy that involves the ex vivo isolation and expansion of antigen-specific T cells for infusion, with the expectation that this bolus of tumor-reactive, effector cells can traffic to tumor sites, mediate regression, and maintain a durable response. In general, there are three sources of T cells for ACT (**Fig. 1**): (1) **tumor infiltrating lymphocytes (TIL),** comprised of an IL-2-expanded population of tumor-derived T cells; (2) **chimeric antigen receptor (CAR) or T cell receptor (TCR)** engineered lymphocytes, genetically modified to redirect specificity to tumor-associated target antigens; and (3) **endogenous T cells (ETC)** isolated from peripheral blood, which, by various enabling technologies, can be selected on the basis of antigen-specificity and expanded ex vivo for adoptive transfer. Since circulating tumor-reactive, antigen-specific T cells are typically present in patients at very low frequencies, ETC represents a technically challenging strategy, but allows for greatest flexibility for targeting tumors and rapid deployment into the clinical arena.[1]

Endogenous T Cell (ETC) Therapy

In the last decade, the identification of an increasing number of tumor-associated antigens that are capable of eliciting an antigen-specific immune response (i.e., an immunogenic target) has heralded the development of antigen-specific immunotherapy, and led to strategies to either isolate such rare antigen-specific T cells from the peripheral blood (ETC) or to present a means to engineer T cells to express the cognate TCR of interest. Engineered TCR and CAR T cell therapies have recently demonstrated remarkable success, in particular for studies using CAR T cells targeting CD19 for the treatment of refractory B cell leukemias and lymphomas. This approach represents an attractive 'off-the-shelf' strategy; however, it is encumbered with its unique set of regulatory constraints and both scientific and technical hurdles.[2, 3] Over the last 18 years, we have taken another approach to the generation of antigen-specific T cells and pioneered ETC as a novel form of adoptive cellular therapy.[1, 4, 5] Unlike TIL therapy, which is constrained by accessibility to tumor of sufficient size and a tumor type known to harbor infiltrating lymphocytes, ETC therapy requires only access to peripheral blood and thus provides a broader, unskewed T cell repertoire. Based on the premise that tumor-reactive T cells can be found in this extant circulating T cell repertoire,[6] we have developed enabling technology to isolate rare antigen-specific T cells present at very low frequency (< 1: 100,000) in the peripheral blood, aided by tetramer-guided cell sorting,[7, 8] and to expand these T cells to a cell dose of > 10^{10}/ m^2 for adoptive transfer. Furthermore, we demonstrated for the first time that exposure of peripheral blood T cells to

IL-21 during in vitro antigen priming leads to the generation of a unique population of central memory type CD8 T cells (T_{CM}) characterized by high surface expression of CD28 and CD127 (IL-7 receptor).[9, 10] CD28 signaling is directly linked to the antigen-driven autocrine production of IL-2, and gives rise to the helper-independent properties of these long-lasting central memory type CD8 T cells. Further, the intrinsic properties of T cells grown in this manner allow for a gentler pre- and post-infusion regimen; thus, ETC therapy is not encumbered by the intensive hospital monitoring associated with high–dose lymphodepletion, and post-infusion high dose IL-2. [11]

Using this approach, we have successfully generated antigen-specific T cells targeting virus-associated antigens (polyoma T antigen)[12] differentiation antigens (MART-1, gp100),[4, 13-15] self-antigens (WT1)[16] and cancer testis antigens (NY-ESO1)[17] for use in several first-in-human clinical trials for the treatment of patients with leukemia, sarcoma, melanoma, breast cancer, pancreatic cancer and Merkel cell carcinoma.

2.3 Target antigens

2.3.1 MART-1/ MelanA antigen

MART-1 also known as melanoma antigen recognized by T cells, and MelanA was identified as a tumor-associated antigen by Kawakami et al and Coulie et al, respectively in 1994. This antigen is broadly expressed in cutaneous melanoma (upwards of 90%) but following treatiment with immune checkpoint therapy, has been found less frequently. For this reason, we are also proposing to target a CT antigen, PRAME; the CT antigens are believed to be upregulated following epithelial mesenchymal transformation and immune resistance that may occur after immune checkpoint therapy failure.

2.3.2 PRAME antigen

PRAME, also known as preferentially expressed antigen in melanoma, is a germinal tissue-specific gene expressed in placenta and endometrium, and both hematological and solid tumor malignancies. Several HLA-A*0201 restricted epitopes have been identified, one of which, PRAME-004, has been shown to be presented in melanoma with high immunogenicity and prevalence33. This epitope has been found only on tumor samples, and not normal tissues. In the TCGA database, it is expressed at very high levels in melanoma, ovarian and uterine/endometrial cancers (> 40 RPKM) with no expression in normal tissues except at very low levels in adrenal gland. Its prevalence of expression in melanoma is > 80%.

2.4 Antigen-loss variants and antigen-spreading

Following *in vivo* targeting of a single antigen with a T cell clone, we and others have shown that antigen-loss tumor variants can appear. In these cases, effective induction of an endogenous multivalent response through antigen-spreading (i.e. the induction and/or expansion of pre-existing host T cell responses to non-targeted antigens) clearly did not occur. There is accumulating evidence that such antigen-spreading can occur and be of therapeutic benefit. Both murine and human studies have demonstrated tumor regression following immunologic targeting of only a single antigen or epitope in settings in which the antigen is known to be heterogeneously expressed (6, 12-16). In a vaccine study of melanoma patients,

anti-tumor CTL recognizing non-targeted antigens MAGE-C2 and gp100 expanded several-fold *in vivo* after MAGE-3 vaccination, exceeded the frequency of anti-MAGE-3 CTL, and were associated with complete tumor regression. In our own studies using $CD4^+$ T cell clones to the single tumor-associated antigen, NY-ESO-1, we demonstrated the induction of a broader multivalent response appearing in a patient who developed a complete regression of his tumor (17).

2.4 Anti-CTLA4 Therapy for Cancer

CD28 and CTLA4 represent counter-receptors to the T cell co-stimulator ligands B7-1 and B7-2 that regulate T cell activation. Whereas CD28 is constitutively expressed and provides positive co-stimulatory signals during the early phase of T cell activation, CTLA4 is upregulated following activation and provides an inhibitory effect on T cell activation, attenuating the magnitude of the T cell response. CTLA4 localizes to the immunologic synapse in direct proportion to the TCR signal strength, leading to inhibition of IL-2 production and cell cycle progression (18-20); hence strategies that abrogate CTLA4 engagement may lead to enhanced T cell responses. Murine studies demonstrate that CTLA4 blockade alone can promote rejection of immunogenic tumors, and, if combined with vaccination or CD25 depletion, can also promote eradication of established poorly immunogenic tumors such as B16 melanoma (21). Since CTLA4 blockade can promote expansion of not only effector, but also of regulatory T cell populations (with the ratio of effector to regulator cells being a major determinant of anti-tumor efficacy) (22), anti-CTLA4 therapy would likely be most effective in a setting in which the regulatory T cell population has been reduced with methods such as CD25 depletion or low-dose cyclophosphamide administration (21).

The treatment of melanoma and ovarian cancer patients with a combination of GM-CSF-transduced autologous tumor cell vaccine and anti-CTLA4 led to extensive tumor necrosis and $CD8^+$ T cell infiltrates in 3 of 9 patients, and reduction or stabilization of disease in 2 others (23). At this time, a number of studies have used the anti-CTLA-4 antibody (ipilimumab) in monotherapy for melanoma and patterns of tumor response observed vary from a classic tumor response in baseline lesions, a slow and steady decline in tumor mass, response after initial increase in tumor burden and a response in tumor burden in the presence of new lesion. These patterns of response are thought to derive from the fact that immune activation precedes tumor shrinkage from weeks to and have been defined as immune-related responses (24).

In this study, the doses of ipilimumab will consist of the standard approved dosage of 3mg/kg, cyclophosphamide and T cell infusions.

2.5 Cyclophosphamide as an Immunopotentiator

The effectiveness of immunotherapy may be enhanced when combined with conventional cytotoxic agents, cyclophosphamide being the most extensively studied drug (26). Since the original observation almost 40 years ago that CY could augment immune responses (27), this effect has been evaluated in experimental animals and clinical studies at doses ranging from 40 to >6000 mg/m^2 (28-32). In human studies, CY potentiated delayed type hypersensitivity (DTH) responses to a vaccine in patients with metastatic cancer at doses as low as 300 mg/m^2

(33-36). Studies have suggested that low doses of CY (200-400 mg/m^2) can selectively deplete suppressor activity whereas higher doses (1000-4000 mg/m^2) induced a more global lympho-depletion but may have had a beneficial 'bystander' effect promoting preferential homeostatic expansion of responding T cells in lymphodepleted hosts (31, 35).

2.6 Regulatory T Cells

$CD4^+$ regulatory T cells (Treg) comprise 5-10% of $CD4^+$ cells in human peripheral blood and have been phenotypically characterized as $CD4^+$ cells constitutively expressing surface CD25. Treg $CD4^+$ cells, in contrast to recently activated $CD4^+$ T cells that are $CD25^{med}$ or resting $CD4^+$ T cells that are $CD25^{lo}$, can be isolated based on being phenotypically $CD25^{hi}$, and further distinguished by expression of the forkhead transcription factor, Foxp3. Cyclophosphamide has been shown to deplete Treg cells, and in murine studies cyclophosphamide-mediated Treg depletion enhanced anti-tumor immunity (21, 37-39), suggesting this may in part be the basis for the immuno-potentiating effect of cyclophosphamide in tumor therapy models. In patients, circumstantial evidence suggests that the levels of Treg cells may correlate with disease stage and prognosis (40), and selective depletion of $CD25^+$ cells using an IL-2-diphtheria-toxin conjugate is the subject of several ongoing clinical trials (25, 41).

2.7 Interleukin-2

The Use of IL-2 in Patients with Metastatic Melanoma

IL-2 promotes the proliferation of immune effector cells such as T cells and NK cells by binding to high and intermediate affinity surface receptors. Initial studies using high dose IL-2 for the treatment of metastatic melanoma and renal cell carcinoma have demonstrated responses in 3 - 24% of patients with rare durable remissions. These doses, in the range of 18-28 x 10^6 U/m^2 every 8 hours resulted in significant morbidity as a result of capillary leak syndromes and occasional mortality (43). In contrast, patients receiving low-dose recombinant IL-2 at doses up to 3 x 10^6 U/m^2 for periods of 1 month or more do not experience significant toxicity (WHO classification, Grade III or IV) (44-46). While such doses have seldom resulted in clinical response, doses as low as 100,000 U have been demonstrated to induce cellular immunologic effects *in vivo* while doses greater than 10^6 U/m^2 were associated with systemic symptoms of malaise and myalgias. Following subcutaneous (s.c.) administration, IL-2 exhibits a half-life of between 3 to 12 hours, sustained serum levels of 10- 25 U/ml, and receptor saturating serum concentrations of 22 pM after an injection of 250,000 U/m^2 (47-50). Administration of 3 x 10^6 U/m^2 of IL-2 for up to 21 days for the treatment of melanoma results in response rates of 0 to 9% (51). Therefore, patients receiving low-dose IL-2 in this trial, (250,000 U s.c twice daily), are unlikely to receive significant benefit from the IL-2 alone, rather, the IL-2 is administered in a manner designed to promote persistence of infused T cells *in vivo*.

High-dose IL-2 (600,000 U/kg three times daily) is the U.S. FDA-approved dose and schedule for the treatment of patients with metastatic melanoma or renal cell cancer and has a demonstrated response rate in patients with melanoma of 15% and a complete remission (CR) rate of 6-8%; however most patients experiencing a CR go on to enjoy a long term durable remission. The toxicities associated with high-dose IL-2, in particular the vascular leak

syndrome secondary to cytokine-mediated permeability of capillary vessels leads to interstitial edema, hypotension, fever and oliguria. IL-2 associated toxicities are reversed in most cases upon cessation of IL-2 therapy and go on to complete an average of 10-12 doses of IL-2 with the first course.

2.7.1 The Use of Interleukin-2 in Adoptive T Cell Therapy

In our clinical studies, adoptively transferred CTL clones had a median survival of 6.6 $^{+}$/- 0.8 days in the absence of IL-2. The addition of a 14-day course of low-dose IL-2 (250,000 U/m^2 twice daily) following T cell infusion significantly prolonged median CTL survival to 16.8 $^{+}$/- 1.6 days without associated toxicity (6). The frequency of transferred CTL *in vivo* began to fall before the end of the 14-day course, suggesting limited benefit might be expected with continued administration of IL-2 at this dose beyond 14 days.

High-dose IL-2 (720,000 U/kg three times daily) has been administered following adoptive transfer of CD8$^+$ T cells (7, 8). In one study, clonal T cells were promulgated to very high numbers and clinical responses were observed; however, due to the design of the study, no conclusions could be drawn as to whether high-dose IL-2 was required for the *in vivo* survival or expansion of transferred T cells. In addition to a direct effect on T cell expansion, it could be speculated that increase vascular permeability induced by high-dose IL-2 provides improved tissue access to tumor sites. Additionally, our own studies and that of others (42) have shown that exposure to high-dose IL-2 (1000 U/ml) leads to rapid, antigen-independent expansion of CTL clones *in vitro* (in a manner similar to IL-15) while exposure to low-dose IL-2 had little effect. Of note, responses to high-dose IL-2 were only observed with antigen-specific CTL clones generated by cyclical re-stimulation using PBMCs of vaccine-naïve donors. CTL clones generated from G209M-vaccinated donors and exposed to high doses of IL-2 *in vitro* failed to respond to either high-dose IL-2 or IL-15, thus concurring with the hypothesis that the manner and source from which T cells are generated can influence their *in vivo* behavior.

2.8 Potential toxicities associated with CD8$^+$ CTLs

The infusion of large numbers of antigen-specific *ex vivo* derived CD8$^+$ T cells may potentially lead to recognition by the same T cells of antigen expressed in normal tissue. Furthermore, tissue-specific toxicities associated with T cells targeting cancer-testis antigens have not been observed with vaccine (52) or adoptive therapy studies (17). However, if antigen spreading occurs and the patient develops a *de novo* response to his own melanoma cells, recognition of normal tissue may occur. Melanoma antigen is expressed in cells derived from the neural crest such as melanocytes of the skin and choroid and retinal cells. The pigmented tissues of the eye may be protected from potential toxicity since the sub-retinal space and vitreous cavity are considered immunologically privileged sites, like the testis, by virtue of physical barriers and immunosuppressive factors such as TGF-beta, alpha-MSH and other low molecular weight proteins present in these sites (53-55).

In humans, there have been reports of patients treated with immunomodulators such as gamma-interferon or IL-2 in whom the appearance of vitiligo parallels their response to therapy (56). Patients with metastatic melanoma have also been treated with infusions of large numbers of (up to 10^{11}) CD8$^+$ TIL cells that were subsequently shown to be reactive to

antigens (gp100, Melan-A and antigen) shared by melanomas and normal melanocytes. Although vitiligo was observed in a small proportion of these patients, there was no evidence of visual or neurologic toxicity (57). More recently, we reported on the development of a targetoid rash surrounding normal pigmented skin cells on a patient who received MART-1 specific $CD8^+$ T cells. The infused T cell clone was retrieved from these sites of inflammation (9). The rash resolved without sequelae, and the patient did not develop evidence of ocular or neurologic toxicity. There have been no toxicities associated with the infusion of PRAME-specific CTL.

2.9 Rationale and Proposed Study of Adoptive T Cell Therapy for the Treatment of Patients with Metastatic Melanoma

In this study we propose to examine the benefits of a combined biologic approach: adoptive transfer of antigen-specific CTLs together with anti-CTLA4. CTLA4 functions as an inhibitory receptor for B7 ligands and is upregulated in T cells proportionally to the strength of the TCR signal from antigen recognition. The higher affinity for binding to B7 of CTLA4 compared to CD28 permits disruption of co-stimulatory signals from CD28 as well as delivery of inhibitory signals that impede TCR signaling, IL-2 transcription, and T cell proliferation(1). Although CTLA4 was initially shown to play a role during primary induction of antigen-specific T cells, it is also believed to contribute to secondary T cell responses (58). We postulate that administration of anti-CTLA4 may not only enhance the adoptively transferred T cell response, but also promote the generation of additional T cell responses against a broader panel of tumor-associated antigens that are released in a pro-inflammatory environment following lysis of tumors by antigen-specific CTL.

Antigen Specificity and Patient Treatment

Disease Diagnosis & Staining Outcome	Antigen-Specific CTL
Pt. tests for HLA-A*0201, MART1 +	MART 1 cells (10^{10} cells/m^2)
Pt. tests for HLA-A*0201, PRAME +	PRAME cells (10^{10} cells/m^2)
Pt. tests for HLA-A*0201, MART1 + and PRAME+	MART1 cells and PRAME cells (10^{10} cells/m^2 total, with no fewer than 2^9 cells/m^2 of each)

3. OBJECTIVES

3.1 Primary Objectives

1. Evaluate the safety and efficacy of adoptively transferred CTL targeting melanoma tumors combined with anti-CTLA4

3.2 Secondary Objectives

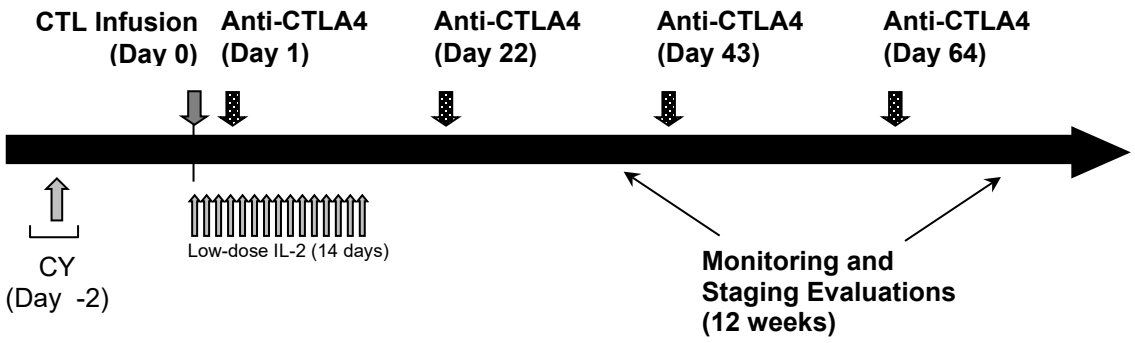
1. Evaluate the influence of anti-CTLA4 on the duration of in vivo persistence and anti-

tumor efficacy achieved following adoptive transfer of antigen-specific CTL.

2. Evaluate the influence of anti-CTLA4 on the induction of T cells to non-targeted tumor-associated antigens (antigen-spreading) following adoptive transfer antigen-specific CTL, and the correlation of these responses with clinical outcome.

4. STUDY DESIGN

The proposed study is a Phase II trial aimed at treating up to 20 HLA-A*0201^{+} individuals with metastatic melanoma. Patients will first receive a lympho-depleting conditioning regimen comprised of a single dose of cytoxan at 300mg/m^2 on day -2. On day 0, patients will receive an infusion of 10^{10} T cells/m^2, followed by a 14-day course of low-dose s.c. IL-2. Anti-CTLA4 will be administered at a dose of 3 mg/kg intravenously over ~90 minutes 24 hours after the T cell infusion. Thereafter anti-CTLA4 will be given every 3 weeks at a dose of 3mg/kg intravenously for a combined total of 4 doses.



For the purposes of registration, the CORe system will be employed. All patients will be registered in CORe before any study specific tests are performed.

This protocol is designed to treat metastatic melanoma patients with measurable residual disease and no CNS metastasis.
Based on the entry criteria requiring a performance status of >70% (85% of total patients, n=119) and HLA-A2 expression (40% of these patients), an average of 45 patients/year will be eligible for this study.

5. PATIENT ELIGIBILITY

5.1 Eligibility for Enrollment

a Histopathologic documentation of melanoma concurrent with the current or prior diagnosis of metastatic disease or in-transit disease
b Male or female subjects ≥18 years of age.
c Expression of HLA-A2.

d ECOG/ Zubrod performance status of '0-1' at screening visit

e Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study in such a manner that the risk of pregnancy is minimized. Suggested precautions should be used to minimize the risk or pregnancy for at least 1 month before start of therapy, and while women are on study for up to 3 months after T cell infusion, and at least 8 weeks after the study drug is stopped. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal

f Men must be willing and able to use an acceptable method of birth control, during and for at least 3 months after completion of the study, if their sexual partners are WOCBP.

g Willing and able to give informed consent.

h Adequate venous access- consider PICC or central line.

i Evaluation of BRAFV600 mutation status.

j Measurable tumor (by RECIST criteria).

k MART 1 or PRAME (+) assay results. (If patients have not had staining test in the past, the test will be run after patient consent is obtained, but before enrollment).

5.2 Exclusion for Enrollment

a Any other malignancy from which the patient has been disease-free for less than 5 years, with the exception of adequately treated and cured basal or squamous cell skin cancer, superficial bladder cancer, carcinoma in situ of the cervix.

b Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception. Women of childbearing potential with a positive pregnancy test within 3 days prior to entry.

c Active and untreated central nervous system (CNS) metastasis (including metastasis identified during screening MRI or contrast CT):

- No signs or symptoms of CNS mets within the last 30 days (from enrollment evaluation).
- No single lesion larger than 1cm
- No more than 5 lesions

d Autoimmune disease: Patients with a history of Inflammatory Bowel Disease are excluded from this study, as are patients with a history of autoimmune disease (e.g. Systemic Lupus Erythematosus, vasculitis, infiltrating lung disease) whose possible progression during treatment would be considered by the Investigator to be unacceptable.

e Any underlying medical or psychiatric condition, which in the opinion of the Investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events, such as a condition associated with frequent diarrhea.

f Positive screening tests for HIV, Hep B, and Hep C (referencing blood draw at leukapheresis screening). If positive results are not indicative of true active or chronic infection, the patient can be treated.

5.3 Eligibility for Treatment (Includes Cyclophosphamide, T cell, anti-CTLA4 infusions and s.c. IL-2)

a ECOG/Zubrod performance status of '0-1'

b At least 4 Weeks must have elapsed since the last chemotherapy, radiotherapy or major surgery. At least 6 Weeks for nitrosoureas, mitomycin C and liposomal doxorubicin. **If started before T-cell administration, Ipilimumab infusions must be least 21 days apart.**

c Toxicity related to prior therapy must either have returned to ≤ grade 1, baseline, or been deemed irreversible.

d Persons of reproductive potential must agree to use and utilize an adequate method of contraception throughout treatment and for at least 8 weeks after study drug is stopped.

e Willing and able to give informed consent.

5.4 Exclusion Criteria for Treatment

a CBC and Chemistry profile prior to cyclophosphamide and T cell infusions:

- WBC ≤ 1000/uL
- Hct ≤ 24% or Hemoglobin ≤ 8 g/dL
- ANC ≤ 500
- Platelets ≤ 50,000
- Creatinine ≥ 3.0 x ULN
- AST/ALT ≥ 2.5 x ULN
- Bilirubin ≥ 3 x ULN

b Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception. Women of childbearing potential with a positive pregnancy test within 3 days prior to entry.

c Steroids are not permitted 3 days prior to T cell infusion and concurrently during therapy.

d Any non-oncology vaccine therapy used for the prevention of infectious disease within 1 month before or after any ipilimimab dose.

e Patients may not be on any other treatments for their cancer aside from those included in the protocol. Patients may not undergo another form of treatment concurrently with this study.

f Active and untreated central nervous system (CNS) metastasis (including metastasis identified during screening MRI or contrast CT):

- No signs or symptoms of CNS mets within the last 30 days (from enrollment evaluation).
- No single lesion larger than 1cm
- No more than 5 lesions

6. STUDY AGENTS

6.1 Antigen-specific CD8$^+$ T cells

All T cells administered will be T cells derived from the peripheral blood lymphocytes of metastatic melanoma patients. Briefly, T cells demonstrating antigen-specific cytolytic function will be tested for:

- CD3$^+$, 8$^+$ surface phenotype
- Class I MHC restricted lysis of antigen-expressing target cells
- Mycoplasma, fungal and bacterial sterility

T cells will be infused intravenously at a rate of not more than 500cc/hour through a peripheral vein or central catheter. The infusion bag will be gently mixed every 5 minutes during the infusion.

6.2 Cyclophosphamide

Cyclophosphamide at 300 mg/m^2 will be administered intravenously. Standard Practice Policy guidelines will be followed and its administration will be completed at least 48 hours prior to the T cell infusion.

6.3 Anti-CTLA4 / Ipilimumab

Ipilimumab (Yervoy™) is a human cytotoxic T-lymphocyte antigen 4 (CTLA-4) blocking antibody indicated for the treatment of unresectable or metatstatic melanoma. Ipilimumab will be purchased by M.D. Anderson Pharmacy from commercial sources. Ipilimumab administration will be initiated approximately 24 hours after the T cell infusion at a dose of 3mg/kg over approximately 90 minutes intravenously.

6.4 Interleukin-2

Low-dose s.c IL-2 will be initiated within 6 hours of T cell infusion.
Low-dose IL-2 will be administered at 250,000 U/m^2 s.c. twice daily x 14 days. The patient or their caregiver will be instructed on s.c. self-administration.

7. PLAN OF TREATMENT/STUDY SCHEDULE (Appendix A)

The timing of visits and procedures after T cell infusion are expected to occur +/- 3 days of the designated date.

7.1 Screening visit/Consent visit

- Patients will be screened for their eligibility on the study based on eligibility for enrollment inclusion/exclusion criteria.
- Patients will be screened for Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, and HIV 1/HIV 2 Antibody within 7 days of leukapheresis.
- EKG performed prior to enrollment (within the last 6 months).
- Patients may be consented.
- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, ALT, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets (see Appendix A, Schedule of treatment and evaluation).
- Leukapheresis visit may be scheduled.

7.2 Tumor Biopsy (Optional)

- Patients undergoing surgery to remove or biopsy tumor as part of the usual management of their disease will be asked to consent to the use of a non-diagnostic portion of their tumor for research purposes (including measurement of antigen and MHC expression).
- Patients may be asked to consent to an optional tumor biopsy should the tumor be measureable and accessible (e.g. skin or subcutaneous site) and risks, in the opinion of the surgeon, are minimal.

7.3 Leukapheresis Visit

- Leukapheresis is scheduled at a time prior to anticipated interval systemic therapy or, if the patient is receiving treatment, at a time at least two weeks from last treatment cycle and when blood counts satisfy criteria for leukapheresis (see section 5.4). Peripheral blood mononuclear cells (PBMC's) will be used for the generation of antigen-specific T cells in the lab. Patients ineligible for leukapheresis because of poor venous access or patients who elect not to undergo leukapheresis but still wish to remain on the study may undergo a maximum of four weekly phlebotomies to obtain PBMC's necessary to establish T cell cultures. On each of these blood draws, 50 – 100 cc of blood will be obtained.

- Leukapheresis may be deferred for patients with active infections or oral temperature > 38.2 C within 72 hours prior to planned Leukapheresis.

- Leukapheresis may be deferred for patients with Hct < 30%, WBC <2500/uL and platelets <50,000 immediately prior to Leukapheresis.

Typically, a leukapheresis procedure will collect at least 1 billion white blood cells from which we generate the T cells for the clinical trial. If more than 1 billion cells are collected, then we may use some portion of the unused white blood cell collection to perform laboratory research studies that will improve the ability to grow these cells and improve

their function for future clinical trials. The excess cells will be used for laboratory research only at the discretion of the principal investigator.

7.4 Pre-Infusion Visit (Week -4 to -2)

- Patients for whom antigen-specific CTL have been generated will be evaluated prior to the anticipated treatment date.
- At this visit, eligibility criteria for treatment will be assessed (refer to section 5.3 and 5.4).
- If patient has not had in the last seven (7) days, perform perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation)
- Patients will be excluded from treatment if CBC and Chemistry profile prior to cyclophosphamide and T cell infusion is:
    - WBC $\leq$ 2000/uL
    - Hct $\leq$ 24% or Hemoglobin $\leq$ 8 g/dL
    - ANC $\leq$ 1000
    - Platelets $\leq$ 50,000
    - Creatinine $\geq$ 3.0 x ULN
    - AST/ALT $\geq$ 2.5 x ULN
    - Bilirubin $\geq$ 3 x ULN
- Staging for measurable disease: Patients must have bi-dimensional measureable disease by palpation on clinical exam or radiographic imaging (CT scan).
- At least 4 Weeks must have elapsed since the last chemotherapy, radiotherapy or major surgery. At least 6 Weeks for nitrosoureas, mitomycin C and liposomal doxorubicin. **If started before T-cell administration, Ipilimumab infusions must be least 21 days apart.**
- Steroids are not permitted 3 days prior to T cell infusion and concurrently during therapy.
- Patients may not be on any other treatments for their cancer aside from those included in the protocol. Patients may not undergo another form of treatment concurrently with this study.
- Bi-dimensionally measurable disease by palpation on clinical exam, or radiographic imaging (CT scan).
- If patients agree to proceed with T cell therapy and all criteria are met, then a cyclophosphamide infusion visit (outpatient) and T cell infusion visit (inpatient) appointment will be scheduled

7.5 Cyclophosphamide Infusion visit (Day -2)

- If patient has not had in the last seven (7) days, perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets (see Appendix A, Schedule of treatment and evaluation).

- Cyclophosphamide will be administered at 300 mg/m^2 intravenously 48 to 72 hours prior to T cell infusion as an outpatient procedure. For women of child-bearing potential, the confirmation of the absence of pregnancy will be required prior to infusion.

7.6 T Cell Infusion Visit (Day 0)

- Prior to infusion perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).
- Administer T cells at a dose of 10^{10} cells/m^2. T cells from *in vitro* cultures will be washed three times with RPMI-Hepes solution and re-suspended in 250-500 ml of 0.9% NaCl in preparation for infusion.
- Patients will have vital signs and oxygen saturation measured by pulse oximetry pre-infusion, approximately every 15 minutes for the first hour, then approximately every 30 minutes for the next two hours followed by approximately every 6 hours overnight.

7.7 Interleukin-2 Administration (Day 0 to Day +14)

- Low-dose IL-2 (250,000 U/m^2 s.c. q12h) will begin within 6 hours of T cell infusion and continue for a total of 14 days (28 doses).

7.8 Anti-CTLA4 (Ipilimumab) Administration (Day +1 to Day +64)

- Ipilimumab will be administered ~24 hours after the T cell infusion at a dose of 3mg/kg over ~90 minutes intravenously. Subsequent infusions will be administered on days +22, +43 and +64 (+/- 1 day) at a dose of 3mg/kg.

7.9 Follow Up: (Day +3):

- Perform: comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.10 Follow Up: (Day +7):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation)

7.11 Follow Up: (Day +14):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation.
- End Low dose IL-2 (Day 13).

7.12 Follow Up: (Day +22):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation.
- 2nd dose of Ipilimumab will be administered at a dose of 3mg/kg over ~90 minutes intravenously.

7.13 Follow Up: (Day +28):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation.

7.14 Follow Up: (Day +35):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).
- Perform staging for measurable disease (Day +35 to +42)

7.15 Follow Up: (Day +43/6 weeks):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation.
- 3nd dose of Ipilimumab will be administered at a dose of 3mg/kg over ~90 minutes intravenously.

7.16 Follow Up: (Day +49):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation.

7.17 Follow Up: (Day +56):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.18 Follow Up: (Day +64):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).
- 4nd dose of Ipilimumab will be administered at a dose of 3mg/kg over 90 minutes intravenously.

7.19 Follow Up: (Day +70):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.20 Follow Up: (Day +77):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).
- Perform staging for measurable disease (Day +77 to +84)

7.21 Follow Up: (Day +84/12 weeks):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.22 Follow Up: (Day +112):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.23 Follow Up: (Day +140):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests and LDH; Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

7.24 Follow Up/End of Study: (Day +168):

- Perform: physical exam; vital signs; comprehensive chemistry panel to include electrolytes, BUN, Cr, baseline Thyroid Function Testing, Liver function tests and LDH (including TSH, free T4, T3, AST, alkaline phosphatase, total bilirubin, and direct bilirubin); Complete blood count and differential, platelets; collect research blood samples (see Appendix A, Schedule of treatment and evaluation).

Follow up visits that do not require the patient to return to MD Anderson, may be performed by the patients' local oncologist/doctor. The collection of data and blood samples will be coordinated by the assigned MD Anderson Study Coordinator. All outside labs will need to be reviewed, signed and dated by the Principal Investigator or treating physician prior to submission to the electronic medical record. The PI or treating physician should appraise abnormal lab results and determine/document clinical significance.

Retreatment may be considered if no serious unexpected adverse toxicities were experienced with the initial treatment and any adverse toxicities from the initial treatment (including those related to ipilimumab administration) have resolved to Grade I or lower prior to re-treatment.

7.25 End of Patient Monitoring

The end of patient monitoring is planned for 6 months after the T cell infusion. Patients who have detectable transferred cells >0.5% of $CD8^+$ T cells at the end of monitoring, show tumor response (refer to section 8.2 for definitions) or show antigen spreading, will continue to be monitored on study with visits every 4-6 weeks or as often as clinically indicated. MD Anderson Immune Monitoring Core Facility will perform the analysis using tetramer staining and/or ELISPOT analysis.

7.26 Outside Physician Participation During Treatment

- MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician. (This will be documented in the patient record).
- A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (Appendix M).
- Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it.
- Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.
- A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.
- Documentation to be provided by the local physician will include drug administration records, progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.
- The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
- Patients will return to MDACC at time-points (D+35 to D+42) and (D+77 to D+84) months for evaluation (staging for measurable disease).
- Collection of data and blood samples will be coordinated by the assigned MD Anderson Study Coordinator.

8. SCHEDULE OF EVALUATIONS

8.1 General Toxicity Assessment

8.1.1 Vital signs (temperature, pulse, breathing, blood pressure), Physical exams, oxygen saturation, Comprehensive chemistry panel, Complete blood counts, differentials and platelet counts will be completed according to the schedule as outlined in Appendix A.
Of note, the dates listed on the study calendar are approximate as many patients reside out of the area and cannot always follow the time points as dictated by the protocol.
8.1.2 All adverse events will be recorded and graded according to the NCI CTCAE v4.1 and the table in section 10.10. The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

8.2 Efficacy Assessment

8.2.1 Clinical Response

Radiographic imaging and clinical assessment of residual disease will be compared with pre-infusion assessment. A complete response (CR) will be defined as total regression of all tumor, a partial response (PR) as 30% or greater decrease in the sum of the longest diameter of target lesions and progressive disease (PD) as 20% increase in the sum of the longest diameter of target lesions (RECIST criteria) This assessment will be performed 12 weeks following T cell infusion and then every 3 months until disease progression or intervening therapy. The overall response rate (OR) will be after 1 cycle (12) weeks.

8.2.2 Delayed Response Endpoints

Ipilimumab is expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months. Some patients with advanced melanoma may have objective volume increase of tumor lesions within 12 weeks after inclusion in the study. Such patients may not have had sufficient time to develop the required immune activation or, in some patients, tumor volume increases may represent infiltration of lymphocytes into the original tumor. In conventional studies, such tumor volume increases during the first 12 weeks of the study would constitute PD and lead to discontinuation of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response. Therefore, in this study, patients with tumor volume increase detected prior to Week 12, but without rapid clinical deterioration, will continue to be treated with ipilimumab and clinically observed with a stringent imaging schedule to allow detection of a subsequent tumor response. Such assessments will be using identical criteria considering index and non-index lesions. Best overall response assessments will therefore include responses occurring at any time before disease progression and after early progression, within the first twelve weeks of the study. This will improve the overall assessment of the clinical activity of ipilimumab and more likely capture its true potential to induce clinical responses.

8.2.3 Best Overall Response Rate (BORR)

BORR is defined as the total number of patients whose BOR is CR or PR, divided by the total number of patients.

8.2.4 Duration of Response

A patient's Duration of Response is defined as the time between the date measurement criteria are first met for subsequently-confirmed OR of PR or CR (whichever status is recorded first) and the date of disease progression or death. Duration of Response will be calculated for all patients with CR or PR using the Kaplan-Meier product limit method. For patients who undergo tumor resection on study, Duration of Response will be censored on the date of last tumor assessment prior to resection. For those patients who remain alive and have not progressed, Duration of Response will be censored on the date of last tumor assessment. The subset of patients whose Duration of Response (CR or PR) is $\geq$ 24 weeks will also be captured. This is referred to as Major Durable Response (MDR). The Major Durable Response Rate (MDRR) is defined as the total number of patients with MDR divided by the total number of patients.

8.2.5 Time to Response

A patient's Time to Response is defined as the time from the first dose of study therapy until measurement criteria are first met for subsequently confirmed OR of PR or CR (whichever status is recorded first). Every confirmed CR or PR, whether occurring before or after progression of disease, is included in this assessment. Descriptive statistics on Time to Response will be computed, as treated, for all treated patients whose BOR is PR or CR. Time to Response will also be analyzed for all treated patients, as treated, using the Kaplan-Meier product limit method. In this case, for patients who do not have BOR of CR or PR, Time to Response will be censored on the date of last tumor assessment. For patients undergoing tumor resection before a confirmed PR or CR is observed, Time to Response will be censored on the date of the last tumor assessment prior to resection.

8.2.6 Progression Free Survival (PFS)

PFS is defined as the time between infusion date and the date of progression or death. A patient who dies without reported prior progression will be considered to have progressed on the date of death. For a patient who undergoes tumor resection on study, PFS will be censored on the date of the last tumor assessment prior to resection. For those who remain alive and have not progressed, PFS will be censored on the date of last tumor assessment. For patients who have PD prior to Week 12 and a subsequent assessment of SD, PR or CR, the date of PD following response (where available) will be used in the analysis of PFS; otherwise these patients will be censored on the date of their last tumor assessment. PFS is estimated using the Kaplan-Meier product limit method.

8.2.7 Progression Free Survival (PFS) Rate at Week 12

PFS Rate at Week 12 is defined as the probability that a patient survives and is progression free at Week 12 following infusion date. It is estimated using the Kaplan-Meier product limit method. PFS Rate at Week 12 will also be evaluated as a proportion, calculated as the total number of patients who have an OR assessment of SD, PR or CR at the Week 12 assessment, divided by the total number of patients. Patients who have PD prior to the Week 12 assessment and a subsequent assessment of SD, PR or CR at Week 12 will be analyzed as having SD, PR or CR, respectively, at the Week 12 assessment.

8.3 Immune Monitoring

Patients will have 60 mls of heparinized blood (green top tube) drawn at the pre-infusion visit, immediately prior to the $CD8^+$ T cell infusion, and on days +1, +3 and +7 and then weekly thereafter for 12 weeks, followed by every 4 weeks until 6 months post-infusion, corresponding to the discontinuation of patient monitoring.

Patients will also have 5ml gray top tube drawn at the pre-infusion visit, immediately prior to the $CD8^+$ T cell infusion, and on day +1, +7, +14, +28, +43, +56, +84, +112, +140 and +168 after the T cell infusion.

Samples will be used to evaluate the duration of *in vivo* persistence and function of infused T cells as well as antigen spreading.

Immune monitoring will be carried out in the PI research laboratory, with the support of the Core Immune Monitoring Lab.

Primary endpoint:
The numeric and functional persistence of transferred CTL will be performed on peripheral blood obtained from patients prior to T cell infusion (baseline sample), on Days $^+1$, $^+3$, $^+7$, and weekly thereafter. To prevent inter-test bias, all samples will be cryo-preserved, and then thawed and assayed simultaneously. The numeric frequency of transferred T cells will be determined using peptide MHC-tetramer analysis or specific CDR3 TCR quantitative PCR of transferred CTL if necessary. The function of transferred CTL will be determined by intracellular cytokine staining of $tetramer^+$ $CD8^+$ cells following *in vitro* stimulation as previously described.

Secondary endpoint:
Responses to non-targeted antigens will be evaluated for evidence of "antigen-spreading". Blood samples taken pre-infusion and at 1 month, 3 months, and every 3 months up to one year following T cell infusion and/or at the time of observed partial or complete clinical response, will be analyzed for the induction of non-targeted T cell responses. Two approaches will be used – tetramer analysis and ELISPOT assay:

a Tetramer analysis. Since all patients on study will be HLA-A2+, the use of tetramers to track several well-characterized tumor-associated HLA-A2-restricted responses will be feasible. These include responses to several melanosomal antigens: (tyrosinase, MART-1/MelanA, gp100, SLC45A2) and CT antigens: (PRAME, MAGE-A1, MAGE-A10). Flow-based tetramer staining permits multiparametric analysis such that surface markers of differentiation (CD62L, CCR7, CD27, CD28) can be used to further characterize the effector, effector memory or central memory phenotype of the induced population of antigen-specific T cells.

b ELISPOT analysis. The use of RNA-transfected autologous APCs as stimulator cells will provide a more comprehensive analysis of induced responses by detecting responses regardless of the HLA-restricting element, as well as identifying CD4+ T

cell responses to tumor-associated antigens (we have demonstrated the feasibility of this assay in ongoing adoptive therapy trials).

8.4 Tumor Immunophenotyping

In the absence of complete regression, immunophenotype analysis of the tumor pre-infusion will be compared with persistent tumor nodules or new tumor nodules post-infusion to evaluate for the outgrowth of antigen-loss tumor variants. New nodules will be sampled when they arise. Persistent disease will be sampled 2 weeks following the T cell infusion. Immunophenotyping will evaluate the expression of targeted antigen as well as other melanoma antigens. The level of expression will be scored by the pathologist in 'blinded' samples, as 0-4^+ with a score of 0 being no expression, 1^+, 25% of cells positive; 2^+, 50% of cells, 3^+, 75% of cells, 4^+ 100% of cells. Antigen loss will be defined as the absence of expression of targeted antigens or a significant decrease in expression (defined as a ≥ 2 level decrease in score) in post-infusion sample as compared with the pre-infusion sample.. Persistent expression of non-targeted antigen, e.g. gp100, in the post-infusion tumor sample in the presence of antigen-loss as defined above will be considered evidence of outgrowth of antigen-loss variants as a result of immune targeting. Where possible, single cell suspensions of tumor samples will be stained with anti-Class-I and anti-HLA-A2 antibodies in order to evaluate HLA allele loss as a possible alternative mechanism of immune evasion.

8.5 T cell Localization to Disease Sites

Localization of transferred antigen-specific CTL to tumor sites will be evaluated in selected patients with surgically accessible disease (disease that is accessible by needle or core biopsy, or that can be excised). Single cell suspensions will be prepared and stained to identify antigen-specific CTL by tetramer analysis. Tetramer positive cells will be sorted and analyzed for identity to the original infused CTL by PCR of a cell-specific CDR3 region of the T cell receptor. Where possible, these results will be compared with a pre-infusion tumor sample and the peripheral blood. A T cell frequency at least five-fold higher than that found in the peripheral blood or pre-infusion sample will be considered evidence of T cell localization/accumulation in disease sites.

9 MANAGEMENT OF TOXICITIES AND COMPLICATIONS

9.1 Dose Limiting Toxicity (DLT)

- Any $\geq$ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to $\leq$ Grade 1 severity within 2 weeks of starting therapy, OR, requires systemic treatment;
- Any $\geq$ Grade 3 bronchospasm or other hypersensitivity reaction;
- Any adverse event, laboratory abnormality or inter current illness which, in the judgment of the Investigator, presents a substantial clinical risk to the patient with continued dosing
- Any other $\geq$ Grade 3 non-skin related adverse event that does not return to baseline levels within 7 days, with the exception of events listed below:

9.2 Exceptions

- Cytokine Release Syndrome (CRS) that, in the opinion of the investigators, is attributable to T cell infusions: Including but not limited to asthemia, flu-like symptoms, myalgia, lymphopenia, neutropenia that returns to a Grade 2 toxicity on 7 days from day of onset.
- Skin rash/Erythroderma (grade 2 toxicity) lasting for ≤7 days
- Reversible inflammation (< Grade 4), potentially attributable to a local anti-tumor reaction and therapeutic response. This includes inflammatory reactions at sites of tumor resections or draining lymph nodes, at sites suspicious for, but not diagnostic of metastasis.
- Hospitalization for ≤ Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the Investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy.
  - Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy. Note: ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.
- Fever > 40C for less than 24 hours.
- WBC < 1000 for less than 7 days.
- Platelets < 50,000 for less than 7 days.
- ANC < 500 for less than 7 days.

9.3 Criteria for Discontinuation of Therapy

- Permanent discontinuation of ipilimumab, T cell infusion and IL-2 will be required for any patient experiencing a dose-limiting toxicity (9.1) with exceptions as noted in 9.2.
- Withdrawal of informed consent (subject's decision to withdraw from the study for any reason).
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the Investigator, indicates that continued treatment with study therapy is not in the best interest of the subject.
- Pregnancy: all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation and therapy will be discontinued.
- Imprisonment or the compulsory detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

9.4 Management of Symptoms during T Cell Infusion

Mild transient symptoms have been observed with tumor infiltrating lymphocytes (TIL), lymphokine activated killer (LAK) therapy and antigen-specific T cell infusions. The management of these symptoms is outlined below:

- Fever, chills and temperature elevations > 101°F will be managed with acetaminophen 650 mg p.o. q 4-6 hrs. All subjects that develop fever or chills will have a blood culture drawn. Naproxen 250 mg po q 12 hours prior to and during high-dose IL-2 will be administered prophylactically per standard practice guidelines for IL-2 administration.
- Headaches may be managed with acetaminophen following a neurologic examination.
- Nausea, vomiting will be treated with a non-steroidal anti-emetic of choice.
- Hypotension will initially be managed by intravenous fluid administration and further measures as dictated by standard medical practice.
- Hypoxemia will initially be managed with supplemental oxygen and further measures as dictated by standard medical practice.

9.5 Toxicities Warranting Ablation of Adoptively Transferred T Cells

Severe toxicity occurring after $CD8^+$ T cell infusions and, in the best judgment of the investigator, are related to $CD8^+$ T cells toxicity as opposed to a transient albeit severe cytokine release syndrome, may warrant ablation. Patients that receive ablation must be discontinued from the study.

Examples of such scenarios include:

- Hypotension (systolic BP <90 mmHg and > 20 mmHg below baseline), tachycardia (HR > 130), tachypnea (RR > 32) and/or hypoxemia (arterial O2 saturation < 90%), not responsive to supportive care.
- Grade 2 or greater allergic reaction, consisting of bronchospasm or generalized urticaria.
- Grade 3 or greater toxicity occurring in any other organ system following administration of antigen-specific cytotoxic T cells, and not attributable to a different cause. Exceptions to this are:
    - Fever of >40˚C which lasts less than 48 hours following infusions
    - Lymphopenia of Grade 3 that does not revert to Grade 2 within 96 hours

Patients will receive corticosteroids if treatment-related toxicity warranting ablation of T cells is observed and the following dose schedule is suggested:

Day 1 Intravenous Solu-Medrol at 2 mg/kg
Day 2 Intravenous Solu-Medrol at 2 mg/kg
Day 3-4 Prednisone at 30 mg po b.i.d.
Day 5-6 Prednisone at 15 mg po b.i.d.
Day 7-8 Prednisone at 10 mg po b.i.d.
Day 9-10 Prednisone at 10 mg po q.d
Day11-12 Prednisone at 5 mg po q.d.

9.6 Management of Symptoms During Ipilimumab Administration

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypo- or hypertension, bronchospasm or other symptoms. No prophylactic pre-medication will be given unless indicated by previous experience in an individual patient.

Reactions will be treated based upon the following recommendations:

For mild symptoms (e.g., localized cutaneous reactions such as mild pruritus, flushing, rash):

- Decrease the rate of infusion until recovery from symptoms, remain at bedside and monitor patient;
- Complete the ipilimumab infusion at the initial planned rate ;
- Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring;
- Premedication with diphenhydramine may be given at the discretion of the Investigator for subsequent doses of ipilimumab.

For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mmHg):

- Interrupt ipilimumab;
- Administer diphenhydramine 50 mg IV;
- Monitor patient closely until resolution of symptoms;
- Corticosteroids may abrogate any beneficial immunologic effect, but may be administered at the discretion of the treating physician;
- Resume ipilimumab infusion after recovery of symptoms;
- At the discretion of the treating physician, ipilimumab infusion may be resumed at *one half the initial infusion rate, then increased incrementally to the initial infusion rate.*
- If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab should be administered that day;
- The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above;
- At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.

For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure <80 mm Hg, or angioedema):

- Immediately discontinue infusion of ipilimumab, and disconnect infusion tubing from the subject;
- Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.

- Patients should be monitored until the Investigator is comfortable that the symptoms will not recur;
- No further ipilimumab will be administered;

In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

Treatment of Ipilimumab Related Isolated Drug Fever

- In the event of isolated drug fever, the Investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology.
- If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (Investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses.
- If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the Investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

9.7 Concomitant Therapy

- Active infections occurring after initiating the study should be treated according to the standard of care.
- Patients may not be on any other treatments for their cancer aside from those included in the protocol. Patients may not undergo another form of treatment concurrently with this study.
- The following agents are not allowed while on study:
  - Systemic corticosteroids (except as outlined for management of transferred T cell toxicity in section 9.6),
  - Immunotherapy (for example, interleukins, interferons, melanoma vaccines, intravenous immunoglobulin, expanded polyclonal TIL or LAK therapy)
  - Pentoxifylline
  - Other investigational agents.

9.8 Premature Discontinuation

Subjects who do not complete the study medication will be considered to have prematurely discontinued the study. The reasons for premature discontinuation (for example, voluntary withdrawal, toxicity, death) must be recorded on the case report form. If possible, final study evaluations should be completed at the time of discontinuation.

Potential reasons for premature discontinuation include:

- Patients who are enrolled but do not meet treatment eligibility criteria.
- Development of a life-threatening infection.
- Judgment by the principal investigator that the patient is too ill to continue.
- Patient noncompliance with study therapy and/or clinic appointments.
- Pregnancy.
- Voluntary withdrawal; a patient may remove himself/herself from the study at any time without prejudice.
- Significant and rapid progression of melanoma requiring alternative medical or surgical intervention including, but not limited to, the development of CNS metastasis.
- Grade III or IV toxicity judged to be possibly or probably related to study therapy according to criteria and exceptions as described above.
- Initiation of any additional non-study cancer treatment regardless of the reason
- Termination of the study by the principal investigator, the DSMB, Institutional Review Office or the Food and Drug Administration.

10. REPORTING ADVERSE EVENTS

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

10.1.1 Death

10.1.2 A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.

10.1.3 Inpatient hospitalization or prolongation of existing hospitalization.

10.1.4 A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

10.1.5 A congenital anomaly/birth defect.

10.2 Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. 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 (21 CFR 312.32).

10.3 Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

10.4 All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in

"The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

10.5 **All life-threatening or fatal events**, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

10.6 Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

10.7 Serious adverse events will be captured from the time of the first protocol-specific intervention until 30 days after the last study treatment/intervention, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event. Adverse events occurring one week after pheresis prior to cytoxan infusion (Day-2) will not be recorded in the CRF.

10.8 Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include development of a secondary malignancy.

10.9 **Reporting to the FDA**

10.9.1 Serious adverse events will be forwarded to the FDA by the IND sponsor (Safety Project Manager IND Office) according to 21 CFR 321.32

10.10 **It is the responsibility of the PI and the research team to ensure 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.**

10.11 All Adverse Events will be recorded according to the table below. Adverse events will be captured from the time of T-cell infusion until 30 days after the last dose of study drug.

Recommended Adverse Event Recording Guidelines					
Attribution	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Unrelated	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible					

	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III

11. DATA ENTRY AND PROTOCOL MANAGEMENT

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

12. STATISTICALCONSIDERATIONS

This is a single-arm, open-label, phase II clinical trial to evaluate the efficacy and safety of autologous CD8+ antigen-specific T cells and anti-CTLA4 treating patients with metastatic melanoma. The regimen will be considered successful if it elicits a higher overall response rate and no higher toxicity rate in this patient population than Ipilimumab (standard of care). The primary endpoint for evaluating efficacy will be the overall response rate (OR = complete or partial response) at 12 weeks after T cell infusion and subsequently every 3 months until disease progression. Toxicities are defined as serious adverse events as noted in Section 9.1. Expected accrual is 1 to 2 patients per month. A maximum of 30 patients will be enrolled in this study on an intent-to-treat basis, and MDACC will enroll 20 patients. Data from an additional patients is available through data sharing on patients enrolled at Fred Hutch Institute.

12.1 SAMPLE SIZE AND ENDPOINT MONITORING

Overall response and toxicity will be monitored simultaneously using the Bayesian approach of Thall, Simon, Estey (1995, 1996) and the extension by Thall and Sung (1998). The OR rate of Ipilimumab is less than 20%. A serious toxicity rate of 30% or less is considered acceptable in this patient population. Using this information, we constructed the Dirichlet parameters for the joint prior distribution of efficacy and toxicity for the standard of care:

Table 1. Dirichlet parameters for the joint prior distribution of overall response and serious toxicity assuming information on 100 patients and independence among response and adverse events.

	Objective Response	Others
Toxicity	6	24
No Toxicity	14	56

Patients will be considered non-responders after 1 cycle (12 weeks).

A flat Dirichlet prior distribution for the study regimen was chosen reflective of information from the equivalent of 2 patients with the same marginal distributions in table 1 (i.e., OR rate of 20% and serious adverse event rate of 30%).

This new regimen will be considered worthy of further investigation if it elicits a 10% increase in overall response to 30% with acceptable toxicity. Thus, interim monitoring rules, assuming the prior distributions above, were constructed that meet the following two conditions,

1) $Pr(\theta_{E,\,Tox} > \theta_{S,Tox} | \text{ data}) > 0.90$ and,
2) $Pr(\theta_{E,\,Resp} > \theta_{S,Resp} + 0.1 | \text{ data}) < 0.01$

$\theta_{E,Resp}$ and $\theta_{S,Resp}$ are the OR rates for the study treatment and Ipilimumab, respectively. Similarly, $\theta_{E,Tox}$ and $\theta_{S,Tox}$ are the serious adverse event rates for study treatment and Ipilimumab, respectively. The first rule provides for stopping the study if the serious adverse event rate with new treatment exceeds that of Ipilimumab with high probability (i.e., probability > 90%). The second condition will stop the study early for futility, i.e., if the data suggest that it is unlikely the OR response rate with this new treatment is better than Ipilimumab (probability < 1%).

The continuous monitoring rule for serious adverse events, based on these assumptions and monitoring conditions is found in table 2. Both toxicity and futility monitoring will start after 10 patients have been enrolled. For example, accrual will cease if 6 patients experience serious AE among the first 10-11 patients treated.

Table 2. Stop accrual if the number of serious adverse events is equal to or greater than indicated (i.e., # Serious AE's) among the number of patients accrued (i.e., # Patients).

# Serious AE's	6	7	8	9	10	11	12	13
# Patients	10-11	12-13	14-16	17-19	20-21	22-24	25-26	27-29

Overall response will be monitored according to the rule listed in Table 3. Again, monitoring rule will be implemented after 10 patients enrolled. For example, accrual will cease if no patient experience OR in the first 10-16 patients treated.

Table 3. Stop accrual if the number patients with an overall response is less than or equal to that indicated (i.e., # OR's) in the number of patients accrued (i.e., # Patients).

# OR's	0	1	2	3
# Patients	10-16	17-22	23-27	28-29

Simulation (10,000 replicates) was used to evaluate the performance of these stopping rules on the conduct of the study (Table 4). The probability of stopping the study early if the objective response rate of the new regimen was not better than Ipilimumab was

37% given low toxicity and 90% given high toxicity. This study design was carried out using Multc99 version 2.1.

Table 4. Operating characteristics, based on 10,000 simulations per scenario, of simultaneous monitoring of overall response and serious adverse event rates for patients treated with study treatment.

Response/Serious AE Relationship	True Response and Seriou AE Rates	Probability of Stopping Early	Achieved Sample Size Quartiles		
			25th	50th	75th
Low / Low	20%/30%	0.37	17	30	30
Low / High	20%/50%	0.90	9	11	19
High / Low	30%/30%	0.17	30	30	30
High / High	30%/50%	0.86	9	11	21

ANALYSIS PLAN

Continuous variables (e.g., age, etc.) will be summarized using the mean (SD) or median (range). Frequency tables will be used to summarize categorical variables. Logistic regression will be used to assess the impact of patient characteristics on the overall response rate. The distribution of time-to-event endpoints (e.g., CR duration, overall survival) will be estimated using the method of Kaplan and Meier. Comparison of time-to-event endpoints by important subgroups of patients will be made using the log-rank test. Cox (proportional hazards) regression will be used to evaluate multivariable predictive models of time-to-event outcomes.

12.2 Projected targeted accrual

Neither gender nor ethnicity is criteria for enrollment on this study. Since melanoma afflicts primarily (> 95%) a Caucasian population, we do not expect to see significant minority representation. It is anticipated that CTL may not be derived for some enrolled patients and some patients may opt not to receive the study treatment. A maximum of 30 patients will be enrolled in this study on an intent-to-treat basis, and MDACC will enroll 20 patients. Data from additional 10 patients is available through data sharing on patients enrolled at Fred Hutch.

Toxicities are defined as serious adverse events as any Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to >/= Grade 1 severity within 2 weeks of starting therapy, OR, requires systemic treatment; any >/= Grade 3 bronchospasm or other hypersensitivity reaction; any adverse event, laboratory abnormality or inter current illness which, in the judgment of the Investigator, presents a substantial clinical risk to the patient with continued dosing; any other >/= Grade 3 non-skin related adverse event that does not return to baseline levels within 7 days, with the exception of events listed below:

Exceptions

· Cytokine Release Syndrome (CRS) that, in the opinion of the investigators, is attributable to T cell infusions: Including but not limited to asthemia, flu-like symptoms, myalgia, lymphopenia, neutropenia that returns to a Grade 2 toxicity on 7 days from day of onset.
· Skin rash/Erythroderma (grade 2 toxicity) lasting for ≤7 days
· Reversible inflammation (< Grade 4), potentially attributable to a local anti-tumor reaction and therapeutic response. This includes inflammatory reactions at sites of tumor resections or draining lymph nodes, at sites suspicious for, but not diagnostic of metastasis.
· Hospitalization for ≤ Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.

Patients with the following conditions where in the Investigator's opinion continuing study drug administration is justified:

Ocular toxicity that has responded to topical therapy.
· Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy. Note: ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

· Fever > 40C for less than 24 hours.
· WBC < 1000 for less than 7 days.
· Platelets < 50,000 for less than 7 days.
· ANC < 500 for less than 7 days

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
ETHNIC CATEGORY	***Sex/Gender***		
	Females	Males	Total
Hispanic or Latino	0	0	0
Not Hispanic or Latino	5	5	10
Ethnic Category Total of All Subjects*	5	5	10
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	5	5	10
Racial Categories: Total of All Subjects *	5	5	10

13. ADMINISTRATIVE CONSIDERATIONS

13.1 PROTOCOL MONITORING

The study will be monitored by the M.D. Anderson IND office and a protocol specific monitoring plan will be followed.

The principal investigator will monitor the data and toxicities to identify trends. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The MD Anderson IRB will review serious adverse events as they are submitted. Serious adverse events will be submitted to the FDA by the IND Sponsor (Safety Project Manager, IND Office). The principal investigator will also review serious adverse events and evaluate trends. Whenever a trend is identified, the principal investigator will determine an appropriate follow up plan.

Changes to the Protocol:
Any change or addition to this protocol requires a written protocol amendment that must be approved by the IND Office and the IRB. A copy of the written approval of the IRB must be received by the IND Office and the principal investigator before implementation of any changes. The IRB must review and approve all amendments to the protocol.

Ethics and Good Clinical Practice:
This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.

US 21 Code of Federal Regulations dealing with clinical studies
(including parts 50 and 56 concerning informed consent and IRB regulations).

Declaration of Helsinki, concerning medical research in humans
(Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice

13.2 Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

13.3 Consent

The Principal Investigator or designee must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with federal regulations (21 CFR 312), all patients must sign the IRB-approved consent form in the presence of a witness.