TITLE: Phase II study of MK-3475 in conjunction with lymphodepletion, TIL, and high or low dose IL-2 in patients with metastatic melanoma

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1.0 TRIAL SUMMARY

Abbreviated Title	TIL with MK-3475
Trial Phase	II
Clinical Indication	Stage IIIc unresectable and stage IV melanoma
Trial Type	Treatment
Type of control	n/a
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	Arm 1: high dose IL-2 Arm 2: low dose IL-2
Number of trial subjects	36
Estimated duration of trial	3 years
Duration of Participation	3-5 years

2.0 TRIAL DESIGN

2.1 Trial Design

This is a randomized phase II study of treating patients with lymphodepletion, *ex-vivo* expanded melanoma specific tumor infiltrating lymphocytes (TIL) followed by high dose (Arm 1) or low dose (Arm 2) IL-2. All patients will have had prior surgical excision of a melanoma tumor deposit to isolate the TIL and amplify to over a billion cells. Once patients have completed and passed extensive pre-treatment screening evaluations, they will be randomized to one of two treatment groups. Arm 1 will consist of 18 patients who receive standard high dose IL-2 after TIL infusion. Arm 2 will consist of 18 patients who will receive low dose IL-2. Patients will be randomized based on tumor stage (stage IIIc/M1a vs. M1b/M1c and LDH (elevated or normal). All patients will receive standard lymphodepletion consisting of fludarabine and cyclophosphamide chemotherapy. This will be followed by melanoma specific tumor infiltrating lymphocyte infusion and IL-2 will be administered starting approximately 12-16 hours post completion of the T cell infusion. Approximately 21 days after T cell infusion, all patients will initiate on the anti PD-1 antibody MK-3475 200mg IV flat dosing every three weeks for up to 2 years.

In addition to clinical endpoints of overall response rate, progression free survival and overall survival, this trial emphasizes correlative research with built-in research blood collection and biopsies to facilitate identification of biomarkers of response or failure to therapy (see section 4.2.2.2).

2.2 Trial Diagram

Figure 1a: Treatment interventions for Cohort 1 (HD-IL2)

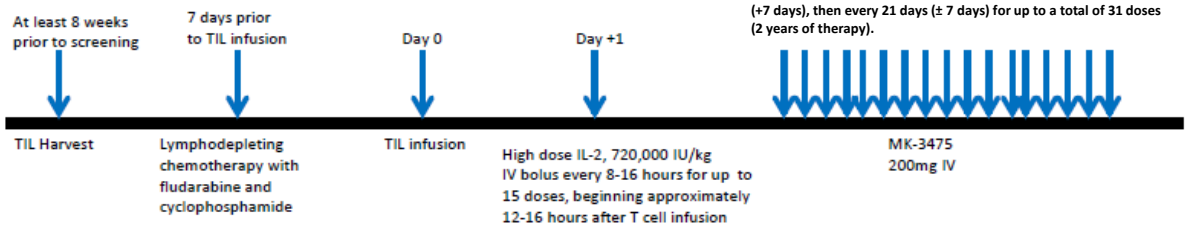
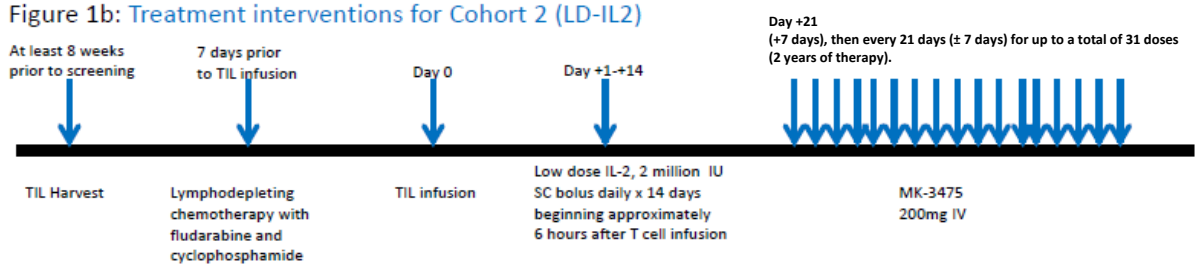


Figure 1b: Treatment interventions for Cohort 2 (LD-IL2)



3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objectives

Objectives: Evaluate the overall response rates of MK-3475 combined with lymphodepletion, TIL and high or low dose interleukin-2 therapy in patients with metastatic melanoma.

Secondary Objectives

Objectives:

(1) Comparison of progression free survival between the treatment arms

(2) Comparison of overall survival between the treatment arms

(3) Comparison of deep tumor responses (defined as over 60% reduction in tumor burden) between the treatment arms as per RECIST criteria

(4) Number of complete responses in both treatment arms

(5) Safety evaluations by CTCAE v 4.0

3.2 Exploratory Objective

Objective: Identification of biomarkers predictive of treatment response or failure through immunohistochemistry, flow cytometry, gene expression changes as assessed by NanoString codeset, neo-antigen identification and CDR3 sequencing from blood and tumor samples acquired from baseline and on-treatment samples.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Adoptive Cell Therapy (ACT) for Melanoma

ACT using expanded TIL has shown great promise for the treatment of Stage IV melanoma patients failing all other types of therapy [1-3]. ACT clinical trials reported by the NCI (Bethesda) using a lymphodepleting pre-conditioning regimen before TIL and IL-2 infusion have had clinical response (CR) rates of up to 51% [1-3]. A number of immune-related mechanisms account for this improved persistence and better clinical response [2, 4, 5]. The current methodology for ACT requires a two-phase process. The first phase involves the expansion of TIL, isolated from surgically-resected tumor fragments, *ex vivo* with IL-2 in small scale over a period of 4-5 weeks [4, 6]. TIL cultures from patients exhibiting a minimal rate of expansion are then expanded in large-scale using a Rapid Expansion Protocol (REP) in a GMP facility [6, 7]. A typical post-REP TIL product for infusion consists of 25 to 150 billion T-cells, with increased numbers of infused $CD8^+$ TIL correlating to better clinical response (Fig. 2 and Fig. 3A). We have established a comprehensive and successful immunotherapy program at the MDACC aimed at further enhancing the efficacy of ACT by improving TIL expansion protocols and developing synergistic therapies. MDACC is the largest center for ACT of melanoma outside of the NCI. At present, we are conducting a Phase II clinical trial assessing the efficacy of ACT using expanded TIL *ex vivo* using the classical REP described above together with lymphodepletion before TIL infusion [2, 3]. Since starting our program in 2006, we have cultured melanoma tumor fragments from over 600 patients with Stage IV melanoma and have successfully expanded minimal numbers of TIL (at least 4×10^7) needed for the large-scale REP from approximately 60% of patients. So far we have treated over 70 patients with their expanded TIL with the pace of treatment accelerating to 2-3 patients per month.

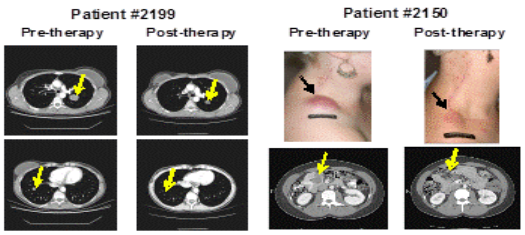


Fig. 2. Pre- and post- treatment photos and CT scans of responding metastatic lesions in two ACT patients. Post-treatment analysis was done 12 weeks after TIL infusion. Patient #2199 received an autologous DC co-vaccine with multiple lung lesions responding partially or completely. Patient #2150 (no DC co-vaccine) had a complete response of subcutaneous and lymph node lesions. Arrows point to the respective lesions.

Fig. 2 shows some examples of the significant

tumor regressions that have occurred in patients responding to TIL in our ACT program; these patients were refractory to all other therapies. The clinical response rate in our ACT program so far has been very encouraging (Table 1) with 45% of the 71 patients evaluated post-infusion so far (32/71) exhibiting a clinical response (PR/CR) according to RECIST criteria after CT scans of target lesions starting at 6 weeks after treatment. Duration of responses has varied with some patients achieving years of clinical benefit (Table 1). Immunocorrelative studies have revealed that $CD8^+$ T-cells are critical in mediating anti-tumor responses and that higher numbers of infused $CD8^+$ TIL correlates with greater objective tumor regression (Fig. 3A, and data not shown).

Progression-free and Overall Survival (as of September 2013)

	Best Overall Response (n=71)			
	irRC Responders (45%)		irRC Non-Responders (55%)	
	CR	PR	SD	PD
Number of patients	3	29	22	11
Progression-free survival (months)	42+, 34+ 8+	58+, 43, 58+, 15, 55+, 55+, 53+, 48+, 48+, 48+, 45+, 39+, 37+, 39+, 55+, 33+, 17, 25+, 30+, 28+, 23+, 7, 21+, 18+, 10+, 7, 11, 7+, 3	3, 3, 4, 4, 4, 7+, 7+, 8, 3, 5+, 8+, 3, 3+, 3+, 5, 3, 10+, 7+, 8+, 5+, 4+, 4+	
Overall survival (months)	42+, 34+ 8+	58+, 43+, 58+, 15, 55+, 55+, 53+, 48+, 48+, 48+, 45+, 39+, 37+, 39+, 55+, 33+, 17+, 25+, 30+, 28+, 17, 23+, 7, 21+, 18+, 10+,10+, 7, 11	4, 12, 25, 8, 8, 8, 17+, 35+, 14, 5, 25+, 23+, 4, 17+, 21+, 7, 4, 10+, 8+, 5+, 4+, 4+	3, 18, 4, 5, 8, 21, 2, 14, 24+, 18+, 3

Updated stats → original data was published in 2012
Radvanyi,...Hwu, Clin Cancer Res, 2012

Table 1: updated progression free and overall survival for 71 TIL treated patients as of September 2013.

During REP anti-tumor $CD8^+$ TIL not only greatly increase in number, but also further differentiate and acquire more potent anti-tumor killing function [7]. This is needed for anti-tumor CTL activity, but is also associated with an acquisition of a terminally differentiated CTL phenotype and a loss of effector-memory (EM) $CD8^+$ T-cells ($CD27^+CD28^+$) capable of long-term persistence and expansion in the host after adoptive transfer [5, 7, 8]. However, maintenance of CD28 expression in particular has been shown to be critical for TIL to respond to further antigenic stimulation, long-term cell division and persistence *in vivo*, as found by studies at the NCI as well as data from our group [5, 7, 9]. We have tracked the persistence of specific TIL clonotypes after ACT using TCR Vβ sequencing of isolated PBMC post-infusion, and found that the persistence for over 1 month of one or more major

TCR clonotypes in the TIL correlates with clinical response (Fig. 3B).

Our laboratory has been actively engaged in identifying biomarkers which correlate with clinical response in TIL treated patients. We have found that patients who achieve responses to TIL tend to have higher absolute numbers of TIL infused. Additionally, patients with higher proportions of CD8+ T cells in their infusion product also tend to have better responses. An unexpected finding was that the negative co-stimulatory markers PD-1, BTLA and TIM3 on infused TIL correlated with improved patient responses. Indeed, patient TIL with higher frequencies of BTLA + T cells seemed to have improved clinical responses [10]. Much work is required to validate the significance of these potential biomarkers.

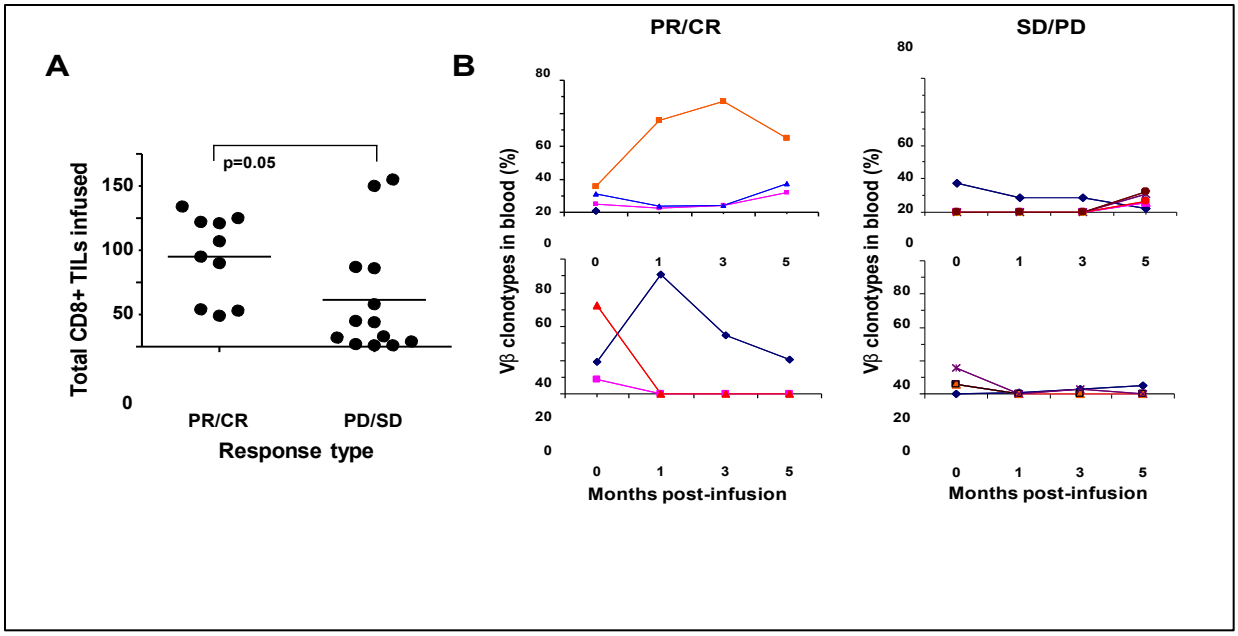


Fig. 3. Persistence of major TIL TCR clonotypes and total CD8$^+$ TIL infused is associated with clinical response to ACT. A. Higher number of infused CD8$^+$ TIL correlates with clinical response. **B.** Vβ TCR clonotype analysis of post-infusion PBMC following infusion in lymphodepleted patients showing persistence of major TIL Vβ clonotypes in two representative patients with a PR/CR. In contrast, the major TCR clonotypes rapidly disappear by 1 month in non-responding patients (SD/PD).

4.1.2 Impact on IL-2 Dosing in Adoptive Cell Therapy

Use of high dose intravenous IL-2 beginning approximately 12 hours after TIL infusion has been utilized as a mechanism to promote survival and enhanced efficacy of transferred T cells [10-13]. However, HD-IL2 is associated with significant toxicities including grade 3 capillary leak syndrome, hypotension, diarrhea, nausea, vomiting, skin changes, anorexia, mucositis, dysphagia, constitutional symptoms, and laboratory changes. Rarely, grade 4 toxicities and death have been seen with the HD-IL2 regimen. Due to expected high level adverse effects associated with HD-IL2, we do not offer this regimen to patients over the age of 65 or to patients with significant cardiovascular or pulmonary compromise at baseline which thus limits our ability to offer ACT to a significant portion of melanoma patients.

However, we do not yet know how much addition of HD-IL2 to the TIL treatment protocol really adds to the induction of clinical responses associated with this regimen and it is possible that similar efficacy could be appreciated by using lower doses of IL-2. Multiple prior studies have investigated use of ACT with low doses of IL-2 [1, 14, 15] and while it seems that better response rates are seen with the high dose regimen, low dose IL-2 administered IV or SC has also produced durable responses with much better tolerance to therapy. A groundbreaking study in 2002 treated patients with four infusions of autologous CD8+ T cell clones against MART1, Melan -A and gp100 tumor associated antigens to 10 patients with metastatic melanoma. Very low doses of IL-2 were used (0.25, 0.50 and 1.0 x 10^6 units/m2 twice daily for the second, third and fourth infusions, respectively). These autologous T cells were found to persist *in vivo*, localized to the tumor sites and led to clinical responses in eight of ten treated patients with responses seen for up to 21 months. This regimen was very well tolerated and there were no AEs attributed to the administered SC LD-IL2 [14].

In an early study phase I study of adoptive cell therapy, patients with metastatic melanoma were treated with non-myeloablative lymphodepleting chemotherapy consisting of cyclophosphamide and fludarabine and were then infused with *in vitro* expanded, tumor reactive autologous T cell clones selected for high avidity recognition of melanoma antigens. There were three cohorts of patients who then went on to receive either no interleukin 2, low dose IL-2 (72,000 IU/kg IV three times a day to a maximum of 15 doses) or high dose IL-2 (720,000 IU/kg intravenously three times a day for a maximum of 12 doses). Although there were no durable responses in this clinical trial, this was thought to be more due to need for additional manipulations of the transferred T cell population as transient responses were seen in both the low dose and high dose IL-2 treated patients [1].

In a more recent pilot study using low dose IL-2, six patients with metastatic melanoma

refractory to prior therapies were treated with standard non-myeloablative lymphodepletion and TIL infusion followed by low dose IL-2 administered as 2 million IU daily for 14 days. Two of the six patients had ongoing complete responses at the time of publication (+10 and +30 months), 2 patients had stable disease of 4 and 5 month duration and two patients progressed shortly after treatment. The median time to progression was 8.2 months and overall survival was 12 months [15].

4.1.3 PD1 blockade with ACT is synergistic in preclinical models

An important pre-clinical tool utilized by our laboratory has been the development of an adoptive T cell therapy model in mice. In these studies, B16 tumors were inoculated into mice and allowed to grow for 7 days prior to treatment with adoptively transferred pmel T cells expressing a T cell receptor recognizing a specific B16 tumor antigen restricted epitope of gp100. This was then followed by a gp100 peptide-pulsed vaccine and interleukin-2. Sacrifice of mice approximately 6 days post infusion showed accumulation of PD-1 positive endogenous and transferred T cells in the tumor site compared to T cells localized in the spleen. These T cells were not fully functional when interferon-gamma production in response to re-stimulation with gp100 pulsed cells was assessed, thus indicating potential limited therapeutic efficacy of these cells [16].

For the next set of experiments, MC38/gp100 tumor-inoculated mice were treated with anti PD-1 antibody on day of and two and four days post adoptive transfer of luciferase- expressing T cells. Six days after adoptive transfer of the T cells, luciferase activity scoring was performed showing approximately 3 fold more transferred T cells at the tumor site compared with mice receiving the control antibody (Figure 4A, B, C) [16].

Additionally, use of anti PD-1 antibody in both MC38/gp100 and B16 tumor inoculated mice after adoptive transfer of T cells lead to statistically significant shrinkage of tumor compared to mice treated with T cells or anti PD-1 antibody alone (Figure 4 D and E) with little evident toxicity. Further work was done to characterize the mechanism of this apparent synergy and

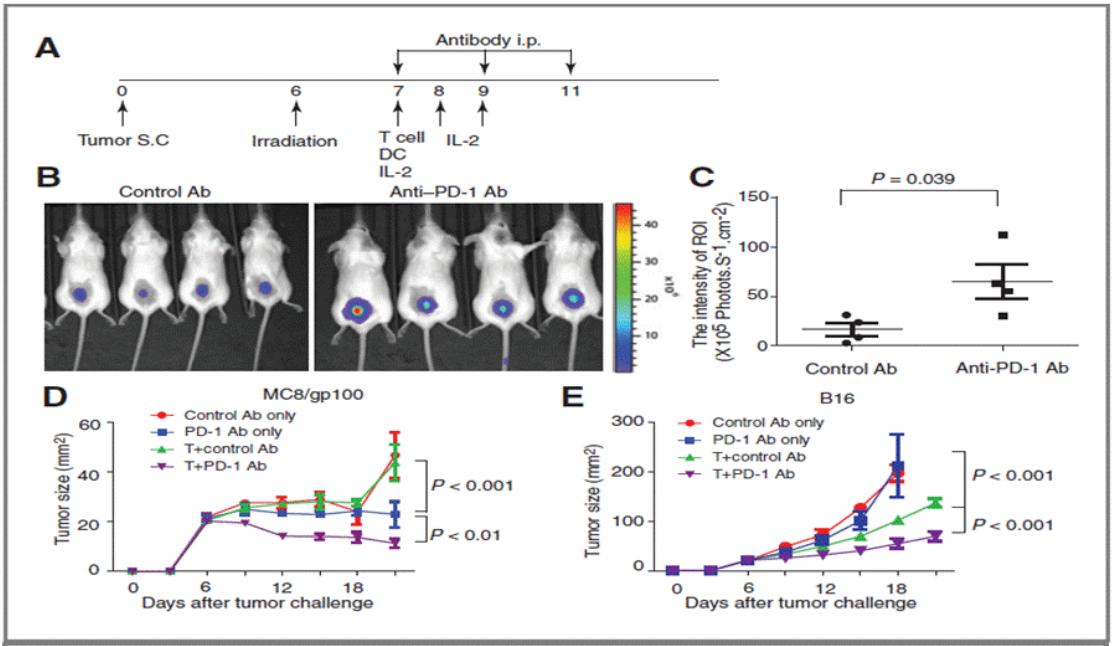


Fig 4. Increased accumulation of pmel-1 T cells to tumor sites and enhanced antitumor immune response in mice receiving ACT combined with anti-PD-1 antibody treatment. A, schematic representation of treatment schedule. B, in vivo trafficking of transferred pmel-1 T cells. Luciferase-expressing pmel-1 T cells (1 106) were transferred into mice bearing established 7-day MC38/gp100 tumors. DC vaccine and IL-2 treatment were conducted as previously described. Mice were intraperitoneally injected with 250 mg either control antibody or anti-PD-1 Ab on days 0, 2, and 4 after T-cell transfer. Imaging was conducted on day 6 after T-cell transfer. Data shown were from representative mice. C, quantitative imaging analysis of transferred T cells in tumor-bearing mice. Intensities of the luciferase signal at tumor sites in all tumor-bearing mice are depicted (N ¼ 4 per group). D, tumor growth curve of MC38/gp100 tumor-bearing mice receiving anti–PD-1 Ab with or without adoptive T-cell transfer (N ¼ 5 per group). E, tumor growth curve of B16 tumor-bearing mice receiving anti–PD-1 Ab with or without adoptive T-cell transfer (N ¼ 5 per group).

it was found that ACT combined with PD-1 blockade did not reduce the number of immunosuppressive regulatory T cells or myeloid derived suppressor cells. However, there was evidence of increased expression of the interferon gamma inducible (INF-γ) chemokine CXCL10 within the tumor micro-environment. This was verified by finding increased INF-γ levels within the tumors of mice undergoing combination ACT with anti PD-1 antibody compared with mice treated with ACT alone. There was no significant difference in the expression of other cytokines such as IL-10, transforming growth factor-beta and IL-17 [16].

4.1.4 Anti PD-1 Antibody: Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [17]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [18-29]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated

T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [30, 31]. The structure of murine PD-1 has been resolved [32]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T cell signaling cascade [31, 33-35]. The mechanism by which PD-1 down modulates T cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [36]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B cells, T regs and Natural Killer cells [37, 38].Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [39]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [40-43]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T cell activation triggered through the T cell receptor. PD-L1 is expressed at low levels on various nonhematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T cell function in peripheral tissues [43]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

A recent study of TIL from fresh melanoma tumors showed that expression of inhibitory receptors including PD-1 on CD8+ TIL identified and selected for a diverse repertoire of autologous tumor-reactive cells. Additionally TCR deep sequencing showed that the most highly expanded clonotypes were the ones which had the ability to recognize autologous tumor. These data suggest that PD-1 identifies clonally expanded CD8+ tumor-reactive populations and suggests that expression of PD-1 on CD8+ TIL could function as a potential predictive biomarker of response to checkpoint inhibition [44].

MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

4.1.5 Preclinical and Clinical Trial Data on MK-3475

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Trial Rationale

ACT using TIL has consistently shown response rates over 40% with a substantial proportion of patients achieving durable responses over 12 months in over 60% of responding patients [10]. The most widely adopted TIL protocol utilizes high dose IL-2 after TIL infusion to produce an environment conducive to the survival and proliferation of transferred cells. However, HD-IL2 is associated with significant grade 3 toxicities that contribute to lack of widespread use of this regimen. Recent advancements in checkpoint blockade in melanoma have shown response rates of approximately 40% with the anti PD-1 antibody MK-3475 and early data indicates durable responses although data need to continue to mature [45]. It has been shown that PD-1 + T cells increase in the tumor microenvironment after adoptive cell therapy in patients as well as in mouse models. Preclinical data from MDACC demonstrates solid pre-clinical proof that addition of anti PD-1 antibody improves the therapeutic efficacy of adoptive cell therapy and also demonstrates a potential mechanism of these improved responses in mice. We hypothesize that addition of MK-3475 to lymphodepletion, TIL infusion and IL-2 will be synergistic leading to improved response rates, improved progression free and overall survival. Additionally, we believe that addition of MK-3475 to this regimen will produce enough synergy with the adoptively transferred cells that the use of high dose IL-2 would not be required and treatment with LD-IL2 may be non-inferior to results seen with historical controls. This protocol provides us with the ability to combine two of the most effective therapies for melanoma and thus gives us the chance to potentially cure more patients and positively impact on the lives of our patients and enhance our understanding of the immune system.

4.2.1 Rationale for Dose Selection/Regimen

4.2.1.1 Interleukin-2 (Aldesleukin, Proleukin)

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

In this study, there will be two arms of patients that differ based on the dosage of IL-2 that is administered after adoptive cell therapy. Arm 1 will receive the classical high dose IL-2 regimen which is administered as an inpatient treatment. 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 durable remissions in approximately 6% of treated

patients [46]. Grade III toxicities common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, constitutional symptoms, and laboratory changes. Additional Grade IV and V toxicities have been seen with IL-2 (See Appendix H).

Arm 2 will receive low dose IL2 after completion of adoptive cell therapy. 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 [46-48]. 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% [49]. Therefore, patients receiving low-dose IL-2 in this trial, (2 million IU SC daily x 14 days), 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*. Patients treated with low dose IL-2 do not experience significant toxicity [50-52] and it is rare to have CTCAE grade 3-4 events which is directly in contrast to what is expected with patients treated with high dose IL-2.

4.2.1.2 Fludarabine

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

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

Fludarabine is administered as an I.V. infusion in 100 ml 0.9% sodium chloride, USP over approximately 15-30 minutes. At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression. However, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Hemolytic anemia has been reported after one or

more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fatigue, anorexia, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is rare at the currently administered doses. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, and allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of Chronic Lymphocytic Leukemia (CLL).

4.2.1.3 Cyclophosphamide (Cytoxan)

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

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

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

Cyclophosphamide will be obtained from commercially available sources by the M. D. Anderson pharmacy. It will be diluted in 250 ml NS and infused over approximately two hours. The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Tables (Appendix—K). Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea, and vomiting may occur, especially after high-doses. Diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported in patients. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than

that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an uroprotective agent Mesna may not be effective in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity due to allopurinol induction of hepatic microsomal enzymes. At high doses, cyclophosphamide can also result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. Cardiotoxicity has been observed at high doses of cyclophosphamide. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis.

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

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

Mesna was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis. Analogous to the physiological cysteine-cystine, mesna is rapidly oxidized to its major metabolite, mesna disulfide (dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic metabolites, resulting in their detoxification.

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

4.2.1.5 G-CSF (Granulocyte Colony-Stimulating Factor)

G-CSF may be given in the form of Filgrastim or PEG-Filgrastim at the appropriate doses. 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.

4.2.1.6 Antimicrobials

Antimicrobials will be instituted as per standard of care to prevent bacterial, fungal and viral infections typically encountered during the period of immunosuppression post lymphodepletion.

4.2.1.7 T-cell preparation

The procedures and reagents for expanding the human TIL cells and the Certificates of Analysis are contained in the CMC located in the IND office.

4.2.1.8 MK-3475

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings and the dose of 200mg IV every 3 weeks is the dose chosen by Merck in future clinical trials.

4.2.2 Rationale for Endpoints

4.2.2.1 Efficacy Endpoints

The primary endpoint is RR to demonstrate the anti-tumor activity of MK-3475 in both study groups. The primary measure for assessment of tumor response is based on RECIST 1.1 by investigator review. Secondary endpoints will include assessment of PFS, OS, number of deep responses, number of complete responses and safety analyses.

4.2.2.2 Biomarker Research

Biomarker research is heavily emphasized in this clinical trial. We will have access to stored tumor and blood for the time of TIL harvest. Research blood and tumor biopsy (specifications detailed in Section 7.2.8) will also be collected prior to hospital admission for lymphodepletion. Blood will be collected at day +7 (+/-2 days). Blood and tumor (tumor biopsy if feasible) will be repeated on day +21 (+/-7 days) which is prior to the first dose of MK-3475 to assess changes by the two different IL-2 dosing schemas utilized in this protocol. Blood collection and tumor biopsy is also mandatory (tumor biopsy if feasible) on day +42 (+/-7 days) just prior to the second dose of MK-3475 to assess changes in circulating T cells and tumor microenvironment from exposure to MK-3475. Research blood and biopsy of residual disease if feasible will be performed at week 9 with the first restaging scan and then at every subsequent restaging scan every 12 weeks.

Planned biomarker research includes:

1. Phenotypic analysis of TIL infusion product by flow cytometry: Panels of antibodies will be designed to answer specific questions about phenotype and function of the T cells. State of differentiation can be determined (CD3, CD4, CD8, CD45RA, CD45RO, CCR7, CD27, CD28) in combination with functional type by intracellular staining for various cytokines and mediators of CTL activity (IL-2, IL-4, IL-10, IL-8, IFN-γ, IL-9, IL-17, Granzyme B, perforin) or transcription factors associated with commitments to different functional paths (Eomes, Tbet).

2. Immune analysis of tumors will be performed using our customized immunohistochemistry panel to evaluate immune cell phenotype (CD3, CD4, CD8, FoxP3, CD68, CD56, CD20, CD45RO) and markers of functionality (granzyme B, perforin) as well as determinants of immune modulation (PD-1, LAG3, TIM3, , BTLA, and proliferative status (e.g. TUNEL, Ki-67). Using this panel, we will be able to perform correlative studies between response to therapy and immune cell subtype numbers and function or the presence of immunodulatory molecules in the tumor microenvironment. We expect to see high expression of PD-L1 within the tumor due to IFN-γ expression triggered by the homing of activated T cells into the tumor post treatment. We also expect to see increased proliferation and markers of functionality on the T cells in the tumor post treatment as compared to the baseline tumor sample. Samples will be shared with Merck specifically for PD-L1 testing using their proprietary assay.

3. CDR3 sequencing: We will track the fate of bulk CD8+, CD4+ or sorted tumor specific TIL clones after infusion and monitor if tumor specific clones migrate preferentially to the tumor or not. We will also determine which sorted subpopulations persist longer post-infusion and determine if persistence of the infused TIL correlate with response to TIL + MK-3475 therapy.

4. Assessment of gene expression changes from baseline to on-treatment samples and correlation with clinical response is planed through use of our validated NanoString code-set of 788 pertinent signaling and immune-related genes.

5. Neoantigen discovery will be performed on patients with excellent clinical response to allow us to ascertain novel antigens involved in development of immune responses to this therapy. At MDACC, we have devised an in-house neo-antigen discovery platform that combines whole exome sequencing of the tumor tissue to define mutations existing in the patient's tumor, algorithm-based prediction of epitope binding affinity of the predicted peptides to the different HLA molecules, and TIL screening of the identified peptides to identify reactive T cells. Our group has optimized a system whereby the MHC class I bound peptides are eluted off of the patient's own tumor tissue and analyzed using tandem mass spectrometry (MS/MS). In addition, part of the tumor tissue is sent for whole exome sequencing and immunogenic peptides are predicted based on the patients' own unique HLA type.

5.0 METHODOLOGY

5.1 Entry Criteria

Patient evaluation for eligibility and registration will occur utilizing a two-turnstile design.

5.1.1 Turnstile I – Screening – Initiation of TIL Expansion/TIL Harvests Entry Criteria

1. Patients must have metastatic melanoma or stage III in-transit, subcutaneous, or regional nodal disease.

2. Patients must have a lesion amenable to resection for the generation of TIL on MD Anderson protocol 2004-0069.

3. Patients must receive an MRI/CT/PET of the brain within 6 months of signing informed consent. If new CNS lesions are present, patient must have definitive treatment (including surgery or radiation). PI or his designee should make final determination regarding enrollment.

4. Age greater than or equal to 18 years.

5. Clinical performance status of ECOG 0 – 1 within 30 days of signing informed consent.

6. Patients previously treated with immunotherapy, targeted therapy, or no therapy (treatment naïve) will be eligible.

7. Patients receiving cytotoxic agents will be evaluated by the PI or his designee for eligibility suitability.

8. Patients with a negative pregnancy test (urine or serum) must be documented within 14 days of screening for women of childbearing potential (WOCBP). A WOCBP has not undergone a hysterectomy or who has not been naturally postmenopausal for at least 12 consecutive months (i.e. who has not had menses at any time in the preceding 12 consecutive months).

5.1.2 Turnstile II-- Treatment – Chemotherapy/Cell Infusion Entry Criteria

1. Patients must sign the treatment consent document before Turnstile II screening procedures. Patients must fulfill all of the following criteria to be eligible for Turnstile II of the study.

2. Patients must have adequate TIL that were previously harvested and then cryopreserved on MDACC protocol 2004-0069.

3. Patients who have had prior therapy (BRAF inhibitors, ipilimumab, anti PD-1 antibody or anti PD-L1 antibody) or treatment naïve patients are eligible as long as toxicity from therapy is ≤ grade 1 or at baseline.
4. Patients must have at least one biopsiable measurable metastatic melanoma, lesion ≥ 1cm and must be amenable to undergoing serial biopsies through the course of therapy (see Section 7.2.7). This lesion must not be documented as one of the target lesions

5. Patients may have CNS metastases which have been treated and are radiographically stable for at least 4 weeks

6. Patients of both genders must practice birth control for four months after receiving the preparative regimen (lymphodepletion) and continue to practice birth control throughout the study. Patients must have a documented negative pregnancy test (urine or serum) for women who have menstruated in the past 12 months and without sterilization surgery.

7. Unless surgically sterile by bilateral tubal ligation or vasectomy of partner(s) or if the patient is post-menopausal, the patient agrees to use 2 methods of contraception throughout the study such as: condom, diaphragm, hormonal, IUD, or sponge plus spermicide. Abstinence is an acceptable form of birth control.

8. Pregnancy testing will be performed within 14 days of screening for women of childbearing potential (WOCBP). A WOCBP has not undergone a hysterectomy or who has not been naturally postmenopausal for at least 12 consecutive months (i.e. who

has not had menses at any time in the preceding 12 consecutive months). .

9. Clinical performance status of ECOG 0-1 within 30 days of signing informed consent. .

10. A stress cardiac test (stress thallium, stress MUGA, dobutamine echocardiogram or other stress test that will rule out cardiac ischemia) within 1 month of lymphodepletion.

11. 12-lead EKG showing no active ischemia and QTc interval less than 480 msec

12. Pulmonary function tests (FEV1>65% or FVC>65% of predicted) within 1 month of lymphodepletion.

13. Have measurable disease based on RECIST 1.1 and irRC criteria (Appendix D).

14. Demonstrate adequate organ function as defined in Table 2, all screening labs should be performed within 30 days of admission to the hospital for initiation of lymphodepletion.

Table 2 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
(GFR can also be used in place of creatinine or CrCl)	
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

5.2 Subject Exclusion Criteria

5.2.1 Turnstile I – Screening – Initiation of TIL Expansion/TIL Harvests Exclusion Criteria

1. Active systemic infections requiring intravenous antibiotics, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system. PI or his designee shall make the final determination regarding appropriateness of enrollment

2. Primary immunodeficiency and need for chronic steroid therapy, Exception: Patients on chronic physiologic dose of steroid equivalent to prednisone < 10 mg/day is allowed.

3. Patients who are pregnant or nursing.

4. Presence of a significant psychiatric disease, which in the opinion of the principal investigator or his designee, would prevent adequate informed consent.

5. Presence of an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded. Subjects with hypophysitis stable on physiologic dose steroid will not be excluded from the study.

5.2.2 Turnstile II-- Treatment – Chemotherapy/Cell Infusion Exclusion Criteria

1. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of lymphodepletion (with the exception of MD Anderson protocol 2004-0069).
2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to initiation of lymphodepletion. Exception: Patients on chronic physiologic dose of steroid equivalent to prednisone < 10 mg/day is allowed.

3. Has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to investigational or standard agents administered more than 4 weeks earlier.

4. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to lymphodepletion or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

- Note: Subjects with ≤ Grade 2 neuropathy, alopecia, hypophysitis stable on physiologic dose of steroid equivalent to prednisone < 10 mg/day, hypothyroidism stable on hormone replacement are an exception to this criterion and may qualify for the study.

- Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

5. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

6. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to initiation of lymphodepletion.

7. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study. Subjects with hypophysitis stable on physiologic dose of steroid will not be excluded from the study.

8. Has evidence of interstitial lung disease or has a history of non-infectious pneumonitis that required steroids or current pneumonitis.

9. Has an active infection requiring systemic therapy.

10. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

11. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

12. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

13. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

14. Has received a live vaccine within 30 days prior to the initiation of lymphodepletion.

15. Any active systemic infections requiring intravenous antibiotics, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, such as abnormal stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease. PI or his designee shall make the final determination regarding appropriateness of enrollment.

5.3 Trial Treatments

5.3.1 Treatment Plan

36 patients will be treated in two separate arms. Both arms will receive standard lymphodepleting chemotherapy and TIL infusion. Arm 1 will receive high dose IL2 after lymphodepletion and TIL infusion. Arm 2 will receive low dose IL2 after lymphodepleion and TIL infusion. If patients develop stable or partially responding disease, up to 31 doses (2 years) of MK-3475 will be administered, with the first dose beginning approximately 21 (+7 days) after TIL infusion. Patients who have a CR to therapy can discontinue MK-3475 if CR is appreciated for six months or longer.

5.3.1.1 T Cell Growth

T-cells will be expanded in a state-of-the-art GMP facility that will allow compliance with all FDA regulations regarding investigational cell transfer products. We have demonstrated that we can successfully expand TIL on the majority of patients and have treated patients with up to 150 billion TIL. The procedures and reagents for expanding the human TIL cells and the Certificates of Analysis are contained in the CMC located in the IND office.

5.3.1.2 Lymphodepletion

All patients will receive lymphodepleting chemotherapy with cytoxan and fludarabine to enhance T cell persistence and effectiveness *in vivo*. Cytoxan will be administered at 60 mg/kg/day I.V. in 250 ml NS over approximately 2 hours on Days –7 and –6. Mesna 60 mg/kg with D5W or NS at 125 ml/hr infused intravenously over 24 hours on Days -7 and -6.

The dose will be based on the patient's body weight, but to prevent undue toxicity, it will not exceed a dose greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Tables (Appendix K). Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 15-30 minutes on Days –5 to –1. To prevent undue toxicity with fludarabine, the dose will be based on body surface area (BSA), but will not exceed a dose calculated on surface areas based on body weights greater than 140% of the maximum ideal body weight per Metropolitan Life Insurance Company, Height and Weight Tables (Appendix K).

Variations from the lymphodepletion (e.g. infusion times; schedule of treatments, etc) prior to day 0 will be documented in the medical record but will not be considered protocol violations/deviations.

5.3.1.3 TIL Infusion

On day 0, TIL will be infused as an inpatient by I.V. over approximately 15-60 minutes depending on the volume of cells to be infused. Up to 150 billion cells will be infused. Tylenol (acetaminophen) will be given by mouth before the T-cell infusion to decrease the risk of these side effects.

5.3.1.4 IL-2 Administration

After completing the T cell infusion, patients will begin treatment with either high or low dose interleukin-2 (IL-2) depending on their treatment assignment.

Patients randomized to receive high dose IL-2 in arm 1 will receive therapy on an inpatient basis at the standard dose of 720,000 IU/kg as an intravenous bolus over an approximate 15 minute period every 8-16 hours for up to 15 doses starting 12-16 hours after T cell infusion. Doses will be skipped if patients reach Grade III or IV toxicity due to high dose IL-2, except for the reversible Grade III toxicities common to high dose IL-2 such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes (i.e. platelets, creatinine, total bilirubin) as detailed in Appendix H. If the toxicity is easily reversed by supportive measures, then additional doses may be continued.

Patients randomized to receive low dose IL-2 in arm 2 will initiate this therapy approximately 6 hours after completion of T cell infusion. Therapy will be administered as a subcutaneous injection for 14 days. Patients will be allowed to leave the hospital prior to 14 days as long as their blood counts have recovered adequately (ANC >500, hemoglobin >8, platelets >30,000) and they have received educating on injecting the IL-2 while out of the hospital.

5.3.1.5 MK-3475 Administration

On day +21 (+7 days), patients will receive their first dose of MK-3475 200mg IV over 30 minutes (- 5 minutes/ + 10 minutes). MK-3475 will be administered every 21 (+/-7) days. Patients will receive up to 31 doses (2 years of therapy).

5.3.2 Dose Selection/Modification

5.3.2.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.2.1

5.3.2.2 Dose Modification

MK-3475 will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life- threatening AEs as per Table 3 below.

Table 3: Dose modification guidelines for drug-related adverse events.

Toxicity	Grade	Hold Treatment (Y/N)	Timing for Restarting Treatment	Dose/Schedule for Restarting Treatment	Discontinue Subject
Hematological Toxicity	1, 2	No	N/A	N/A	N/A
	3* *Excluding Grade 3 neutropenia, anemia, and thrombocytopenia	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for*
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	
Non-hematological Toxicity <u>Note:</u> Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference Section 5.6.1.1.	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to Grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule (reference Section 5.7.2.2 for recommendations regarding pneumonitis) *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	3, 4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued after consultation with the principle investigator. With investigator agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of adverse events, see Section 5.7.2

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of MK-3475 should be discontinued from trial treatment.

5.3.3 Timing of Dose Administration

Administration of lymphodepletion, TIL and IL-2 should be as specified in section 5.3.1.1-5.3.1.4.

Administration of MK-3475 will be as a 30 minute (- 5 minutes/ + 10 minutes) IV infusion (treatment cycle intervals may be increased due to toxicity as described in Table 3).

5.3.4 Trial Blinding/Masking

This is an open-label trial; therefore, the investigator and subject will know the treatment administered.

5.4 Randomization or Treatment Allocation

Subjects will be approved for treatment after fulfilling the screening evaluations as specified by Turnstile 1 and Turnstile 2 (sections 5.1.1 and 5.1.2). Subjects will then be assigned a subject number and will be eligible for randomization. Subject randomization will be implemented by MD Anderson Cancer Center's Clinical Oncology Research System (CORe) . Once a subject number has been assigned, it cannot be reassigned to any other patient. If the subject is prematurely discontinued from the study without having received the prescribed treatment, an additional subject may be enrolled as a replacement subject.

5.5 Stratificatio n

Patients will be stratified based on tumor stage (stage IIIc/M1a vs. M1b/M1c) and LDH (elevated or normal).

5.6 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required.

5.6.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be documented in the medical record but will not be captured in the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, IV medications, supporting prophylactic medication, and fluids.

Concomitant medications administered during the trial and 30 days after the last dose of trial

treatment should be recorded for SAEs and ECIs as defined in Section 7.4.8.1.

5.6.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than MK-3475
- Radiation therapy
    - Note: Radiation therapy to asymptomatic solitary lesion or to the brain may be allowed
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed..
- Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. Exception: Patients on chronic dose of steroid equivalent to Prednisone < 10 mg/day is allowed. Patients requiring glucocorticoids prior to CT scans are allowed for patients who are sensitive to CT scans contrast.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.7 Rescue Medications & Supportive Care

5.7.1 Prophylaxis

Patients treated with lymphodepletion are subject to opportunistic infections and appropriate infectious agent prophylaxis is required.

5.7.1.1 Infection Prevention and Pneumocystis carinii Pneumonia (PCP) Prophylaxis

Patients will receive levofloxacin at 500 mg daily (or an equivalent antibiotic) until ANC recovers to greater than 500/mm^3 and the fixed combination of trimethoprim (TMP) and sulfamethoxazole (SMX) as double strength (DS) tablet [DS tabs = TMP 160 mg/tab and SMX 800 mg/tab] p.o. b.i.d. twice a week. TMP/SMX-DS will be taken by patients beginning on Day -7 and continuing for a minimum of 6 months post chemotherapy. Patients with sulfa allergies, Pentamidine 300 mg IV will be given starting Day -1 and will be administered every 21 days for six months after lymphodepletion. If IV Pentamidine is not feasible after discharge, Pneumocystis Jirovecii Pneumonia (PCP) prophylaxis can be substituted with oral antimicrobials such as Atovaquone as per standard of care for 6 months after lymphodepletion.Patients will be given prophylactic antibiotics intravenously during high dose IL-2 therapy.

5.7.1.2 Herpes Virus Prophylaxis

At the time of the T cell infusion, patients will be administered valtrex 500 mg p.o. daily for 6 months after lymphodepletion, if the patient is able to take oral medications. If patient needs intravenous medication give acyclovir 5 mg/kg IVPB every 8 hours, which is continued until absolute neutrophil count is greater than 1000/ml. Reversible renal insufficiency has been reported with IV administered acyclovir but not with oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. If symptoms occur, a dosage adjustment will be made or the drug be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs (e.g. ganciclovir), which interfere with DNA synthesis. In patients with renal disease, the dose is adjusted as per product labeling.

5.7.1.3 Fungal Prophylaxis

Patients will begin Fluconazole 200 mg p.o. daily with the T cell infusion (Day 0) and Continue for 6 months after lymphodepletion..

5.7.1.4 Ondansetron hydrochloride (Zofran)

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. Consult the package insert for a complete list of side effects and specific dose instructions.

5.7.1.5 Furosemide (Lasix)

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. Consult the package insert for a complete list of side effects and specific dose instructions.

5.7.1.6 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fevers ≥38.5 C 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 patients with unexplained fever or any infectious complications.

5.7.1.7 Blood Product Support

In order to reduce neutropenia following chemotherapy and T cell infusion, G-CSF will be given at 5 µg/kg/day daily subcutaneously until neutrophil counts reach >500/mm^3 Using daily CBC's as a guide, the patient will also receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and platelets >20,000/ml. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection. Irradiated blood and blood products should be used.

5.7.2 Supportive Care Guidelines for MK-3475

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator including but not limited to the items outlined below. More detailed instructions on management of events of clinical interest and immune-related adverse events can be found in Appendix G

- Diarrhea: Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic subjects, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered.

    - In subjects with severe enterocolitis (Grade 3), MK-3475 will be permanently discontinued and treatment with systemic corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over at least 1 month.

    - In subjects with moderate enterocolitis (Grade 2), MK-3475 should be withheld and anti-diarrheal treatment should be started. If symptoms are persistent for more than one week, systemic corticosteroids should be initiated (e.g., 0.5 mg/kg/day of prednisone or equivalent). When symptoms improve to Grade 1 or less, corticosteroid taper should be started and continued over atleast 1 month. Regarding guidelines for continuing treatment with MK-3475, see Section 5.3.2.2 Table 3.

    - All subjects who experience diarrhea should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

- Nausea/vomiting: Nausea and vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Subjects should be strongly encouraged to maintain liberal oral fluid intake.

- Anti-infectives: Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice.

- Immune-related adverse events: Please see Section 5.7.2.1 below and the separate guidance document in the administrative binder regarding diagnosis and management of adverse experiences of a potential immunologic etiology.

- Management of Infusion Reactions: Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritus/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

Table 4 Treatment guidelines for subjects who experience an infusion reaction associated with administration of MK-3475. Additional guidance information can be found in Appendix G.

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS,	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with:

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For Further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

5.7.2.1 Supportive Care Guidelines for Events of Clinical Interest and Immune-related Adverse Events (irAEs)

Events of clinical interest of a potential immunologic etiology (irECIs) may be defined as an adverse event of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. irAEs may be predicted based on the nature of the MK-3475 compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of

potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAE. Information on how to identify and evaluate irAEs has been developed and is included in the Event of Clinical Interest and Immune-Related Adverse Event Guidance Document located in the Administrative Binder.

Further details on management of events of clinic interest and immune related adverse events can be found in Appendix G.

Recommendations to managing irAEs not detailed elsewhere in the protocol are detailed in Table 5.

Table 5 General Approach to Handling irAEs

irAE	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold MK-3475	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold MK-3475 Discontinue if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 12 weeks of toxicity	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks.

5.7.2.2 Supportive Care Guidelines for Pneumonitis

Subjects with symptomatic pneumonitis should immediately stop receiving MK-3475 and have an evaluation. The evaluation may include bronchoscopy and pulmonary function tests to rule out other causes such as infection. If the subject is determined to have study drug associated pneumonitis, the suggested treatment plan is detailed in Table 6. Additional guidance information can be found in Appendix G.

Table 6 Recommended Approach to Handling Pneumonitis

Study drug associated pneumonitis	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1 (asymptomatic)	No action	Intervention not indicated
Grade 2	Withhold MK-3475, may return to treatment if improves to Grade 1 or resolves within 12 weeks	Systemic corticosteroids are indicated. Taper if necessary.
Grade 3 and Grade 4	Discontinue MK-3475	Systemic corticosteroids are indicated. The use of infliximab may be indicated as appropriate. Refer to the Event of Clinical Interest and Immune-related Adverse Event Guidance Document for additional recommendations.

For Grade 2 pneumonitis that improves to ≤ Grade 1 within 12 weeks, the following rules should apply:

- First episode of pneumonitis
    - May increase dosing interval by one week in subsequent cycles
- Second episode of pneumonitis – permanently discontinue MK-3475 if upon rechallenge subject develops pneumonitis ≥ Grade 2

5.8 Diet/Activity/Other Considerations

5.8.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.8.2 Contraception

MK-3475 may have adverse effects on a fetus in utero. Furthermore, it is not known if MK-3475 has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom

(by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in Sections 7.4.3 and 7.4.4. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.8.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on this protocol, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the MD Anderson IND office without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the MD Anderson IND office. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the MD Anderson IND office as described above and in Section 7.4.7.

5.8.4 Use in Nursing Women

It is unknown whether MK-3475 is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.9 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.3.1 – Other Procedures. A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.

- Confirmed radiographic disease progression
  Note: A subject may be granted an exception to continue on treatment with

confirmed radiographic progression if clinically stable or clinically improved as per the investigator's discretion

- Unacceptable adverse experiences as described in Section 5.7.2.1
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reason

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.4 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.4.3-7.4.4. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.10 Subject Replacement Strategy

Subjects on this trial will be replaced if they are unable to receive lymphodepletion, TIL infusion, IL-2, or the first dose of MK-3475.

5.11 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 TRIAL FLOW CHART

	Turnstile I Screening	Turnstile II Screening (-28 to -1 days)	Inpatient Hospitalization for Lymphodepletion, TIL and IL-2	Day +3 (±24 hours)	Day +7 (±2 days)	Week 3 Day +21 (+7 days)	Week 6 Day +42 (±7 days)	Week 9 Day +63 (±7 days)	Week 12, 15, 18, 24, 27, 30, 36, 39, 42, 48, 51, 54, 60, 63, 66, 72, 75, 78, 84, 87, 90 (±7 days)	Week 21, 33, 45, 57, 69, 81, 93 (±7 days)	End of Treatment	Post Treatment (every 12 weeks) (±7 days)	Survivor Follow Up
Study Assessments													
Informed consent	x	x											
Eligibility Criteria	x	x											
Infectious Disease Panel [a]	x												
Demographics		x											
Medical History		x										x	
Concurrent Medications		x	x			x	x	x	x	x	x	x	
Review Adverse Events		x	x		x	x	x	x	x	x	x	x	
Vital Signs/ECOG		x	x			x	x	x	x	x	x	x	
Physical Exam		x	x			x	x	x	x	x	x	x	
CBC with Differential		x	x			x	x	x	x	x	x	x	
Urine or serum B-HCG [b]		x						x		x			
PT/INR, PTT		x											
Comprehensive Serum Chemistry		x	x			x	x	x	x	x	x	x	
Urinalysis		x						x					
TSH, fT4, T3		x						x		x		x	
CMV PCR [c]				x		x							
PFTs		x											
Stress Echocardiogram		x											
12 Lead EKG		x						x					
Tumor Imaging (CT or PET)	x	x						x		x	x	x	
CNS Imaging (CT or MRI)	x	x						x		x	x	x	
Medical Photography [d]		x						x		x	x	x	
FACT-G and FACT-Melanoma		x						x		x			
Tumor Biopsy		x				x	x	x		x			
Correlative Studies Blood Collection		x			x	x	x	x		x	x	x	

	Turnstile I Screening	Turnstile II Screening (-28 to -1 days)	Inpatient Hospitalization for Lymphodepletion, TIL and IL-2	Day +3 (±24 hours)	Day +7 (±2 days)	Week 3 Day +21 (+7 days)	Week 6 Day +42 (±7 days)	Week 9 Day +63 (±7 days)	Week 12, 15, 18, 24, 27, 30, 36, 39, 42, 48, 51, 54, 60, 63, 66, 72, 75, 78, 84, 87, 90 (±7 days)	Week 21, 33, 45, 57, 69, 81, 93 (±7 days)	End of Treatment	Post Treatment (every 12 weeks) (±7 days)	Survivor Follow Up
Study Assessments													
MK-3475 Administration						x	x	x	x	x			
Survival Follow-Up Review [e]													x

[a] Infectious disease panel: Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, HIV 1/HIV 2 Antibody, HTLV I/II Antibody, RPR Qual, CMV Antibody, West Nile Virus, Chagas Disease and EBV Panel
[b] Screening urine or serum B-HCG must be performed within 14 days of initiating lymphodepletion
[c] We will monitor all patients receiving TIL with serum CMV PCR testing at day 3 (+/-24 hours) post TIL infusion and 3 weeks (+/-7 days) after TIL infusion.
[d] Medical photography only if the patient has cutaneous disease
[e] The patient will be contacted by phone every 3 months.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the investigator for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

Feeder cells will be CMV negative if pre-treatment testing of the patient reveals absence of CMV seropositivity. If a patient is CMV positive at baseline, CMV positive feeder cells may be utilized. Pools of feeder cells from CMV seropositive patients will be tested by PCR to assess for the presence of CMV in the feeder cell population. Those feeder cells that have undetectable levels of CMV by PCR will be deemed eligible to be used as feeders in the TIL expansion process. We will monitor all patients receiving TIL with serum CMV PCR testing at day 3 (+/-24 hours) post TIL infusion and 3 weeks (+/-7 days) after TIL infusion. CMV PCR blood testing is drawn in a single 10mL purple top tube. The test will be performed at MDA through the molecular diagnostics lab. If there is evidence of CMV reactivation as evidenced by the CMV pcr, infectious disease specialist will be consulted and further management or therapy will be per expert recommendations.

7.1.2 Administrative Procedures

7.1.2.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.2.2 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable

representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and MDA IND office requirements.

7.1.2.3 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.2.4 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.3 Prior and Concomitant Medications Review

7.1.3.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial in subject's medical record.

7.1.3.2 Concomitant Medications

The investigator or qualified designee will document medication in medical record, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.4 Disease Details and Treatments

7.1.4.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.4.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.4.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment.

7.1.5 Assignment of Screening Number

Patients will be assigned a screening number which will be determined by the order in which the patients initiate screening procedures. 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 MD Anderson Cancer Center's Clinical Oncology Research System (CORe) utilizing a two-turnstile registration before any study specific tests are performed.

7.1.6 Assignment of Randomization Number

Subjects will be approved for treatment after fulfilling the screening evaluations as specified by Turnstile 1 and Turnstile 2 (section 5.1.1 and 5.1.2). Subjects will then be assigned a subject number and will be eligible for randomization. Subject randomization will be implemented by CORe. Once a subject number has been assigned, it cannot be reassigned to any other patient. If the subject is prematurely discontinued from the study without having received the prescribed treatment, an additional subject may be enrolled as a replacement subject.

7.1.7 Trial Compliance (Medication/Diet/Activity/Other)

Assurance of patient trial compliance will be assessed at each clinic visit by the clinical trial nurse and treatment staff.

7.2 Clinical Procedures/Assessments

7.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Appendix A). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with MK-3475 exposure should be evaluated to

determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irAE). See Section 5.7.2.1, Appendix G and the separate guidance document in the administrative binder regarding the identification, evaluation and management of AEs of a potential immunological etiology.

Please refer to section 7.4.5 for detailed information regarding the assessment and recording of AEs.

7.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

During the lymphodepleting chemotherapy regimen and high dose IL-2 therapy, patients will be monitored with vital signs at baseline, approximately every 15 minutes (+/- 10 minutes) during cell infusion and after cell infusion hourly (+/- 30 minutes) for 4 hours after the infusion.

7.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart. See Appendix B for ECOG Status definitions.

7.2.6 12 lead EKG

12-lead ECG will be obtained as indicated in the Times and Events Tables in Section 6.0. Each 12-lead ECG will be performed after the subject has rested at least five minutes in a semi-recumbent or supine position.

Those QTc values greater than 480msec as calculated by the machine must be confirmed manually using Bazett's formula given below:

$$QTc\ (Bazett) = \frac{QT}{\sqrt{RR}}$$

If there are any clinically significant abnormalities including but not limited to a QTcB > 500msec, confirm with two additional ECGs taken at least 5 minutes apart.

7.2.7 Tumor Imaging and Assessment of Disease

Complete evaluation of evaluable lesions with physical examination and appropriate CT scans (chest, abdomen, pelvis at least, ct neck if clinically warranted) or whole body PET/CT will be performed approximately at 9 weeks (+/- 7 days) after TIL infusion and then at 12 week intervals (+/- 7 days) for the duration of therapy. After patients have completed protocol therapy, repeat imaging evaluations will continue at 12 week intervals.
For patients with cutaneous disease, medical photography will be obtained at baseline and then at the time of each imaging evaluation.

Tumor response to therapy in this study will be done using RECIST 1.1 criteria. The *immune-related response criteria (irRC)* which is a modified version of WHO criteria will also be recorded but determination of the primary endpoint will be via RECIST 1.1 (Appendix D) [53]. Patient management will be based on irRC specifications. Tumor measurements for determining irRC response will be performed by each patient's individual attending physician. Tumor measurements will be documented in the patient's research chart and within the patient database maintained by the assigned data coordinator.

Measurable Lesions are lesions that can be accurately measured in two perpendicular diameters, with at least one diameter $\geq$ 10 mm as per irRC and RECIST 1.1 criteria. The area will be defined as the product of the largest diameter with its perpendicular. Skin lesions can be considered measurable. Cutaneous lesions that are $\geq$ 5 mm in diameter can be considered measurable.
Non-Measurable (evaluable) Lesions are all other lesions, including unidimensionally measurable disease and small lesions.

7.2.8 Tumor Tissue Collection and Correlative Studies Blood Sampling

Peripheral blood samples for immune monitoring including flow cytometry and tracking persistence of T cell clones will consist of 10cc in a red top tube and up to 50cc in green top tubes. These samples will be collected prior to tumor harvest and/or excision, prior to lymphodepletion, at day +7 (+/- 2 days) and just prior to first dose (+ 7 days) and second dose (+/- 7 days) of MK-3475. These samples will also be collected at the time of each restaging scan and at the time of documented progression.

Tumor biopsies will be performed during screening for Turnstile 2 and then at day +21 (+/-7 days) just prior to first dose of anti PD-1 antibody and at day + 42 (+/- 7 days) just prior to second dose of anti PD-1 antibody. Biopsy of residual disease will also be performed at week 9 at the time of restaging scans. Optional biopsies for persistent disease will then be performed every 12 weeks at the time of restaging scans. These biopsies will be critical to the immunologic correlative studies which are planned (see section 4.2.2.2). Potential biopsy types include punch, incisional, excisional or image-guided cores depending on the individual patient scenario.

7.2.9 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below in Table 7. During the preparative regimen and high dose IL-2 therapy, patients will have a complete blood count (CBC) and electrolytes every 1 to 2 days of treatment. Patients will have CBC, electrolytes and liver function test prior to each MK-3475 infusion.

Interval laboratories may be performed at a patient's local (non-MD Anderson) laboratory if this is deemed to be in the patient's best interest. These laboratory studies must be reviewed by the PI/treating MDACC physician to determine clinical significance of these studies.

Table 7 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
	(CO_2 or biocarbonate)	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

7.3 Other Procedures

7.3.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.4.5 - Assessing and Recording Adverse Events. Subjects who complete 12 months of treatment with MK-3475 may discontinue treatment. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.4.3) and then proceed to the Follow-Up Period of the study (described in Section 7.4.4).

7.4 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.4.2.1 Screening

All patients must sign an informed consent form and a negative pregnancy test (urine or serum) must be documented for women of childbearing potential before enrollment in Turnstile I and being registered in PDMS. Once enrolled into Turnstile I (2004-0069), patients will be screened for Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, HIV 1/HIV 2 Antibody, HTLV I/II Antibody, RPR Qual, CMV Antibody, West Nile Virus, Chagas Disease and EBV Panel, within 30 days of signing the informed consent**.** If patient's tests are found to be positive for prior (inactive) infection with Hepatitis A, Hepatitis B, CMV or EBV, patients will still be eligible for treatment on protocol 2014-0922. If patient's tests are found to be positive for all other tests, they will not be eligible for Turnstile II. If cultures come back positive after TIL Harvest, they will be terminated. These infectious disease tests will be drawn per the specifications in protocol 2004-0069 and won't be repeated for this specific treatment protocol.

While T-cells are being grown (up to 8 weeks), patients may be treated with alternative therapies. Investigational agents can be used as long as there is a 4 week wash-out period prior to initiation of lymphodepletion.

Other screening procedures will include:

1. Full physical exam including vital signs and documentation of ECOG performance status

2. Measurable disease as defined by irRC RECIST 1.1 on baseline CT chest, abdomen, pelvis (and neck if clinically indicated) or MRI of disease site

3. MRI brain or CT brain with contrast

4. Laboratory assessments as depicted in Section 7.2.2 to assess for adequate bone marrow and organ function

5. Dobutamine stress echocardiogram showing absence of inducible ischemia and LVEF over 50%

6. Pulmonary function tests with FEV1 > 65%

7. Biopsy of at least one site of metastatic disease that is separate from the target lesion

8. Peripheral blood sample for immune monitoring including flow cytometry and tracking the persistence of T cell clones will consist of 10cc in a red top tube and 50cc's in green top tubes

7.4.1.1 Screening Period

The screening period will last for up to 28 days after signing the informed consent document.

7.4.2 Treatment Period

Patients will be seen in clinic within 1 week of initiation of lymphodepleting chemotherapy to review screening evaluations including labs and scans, for updated documentation of concurrent medications and baseline AEs and for full physical exam.

Before the treatment starts and at every 3 month intervals, the patient will be asked to complete two quality of life questionnaires. It should take about 15 minutes to complete the questionnaires (FACT-G, FACT-Melanoma, Appendix E, F).

Patients will be admitted to the hospital seven days prior to the planned TIL infusion to initiate lymphodepleting chemotherapy. Patients will be seen daily in the hospital throughout the course of lymphodepletion, TIL infusion and IL-2 administration. Patients in Arm 1 must stay in the hospital throughout the course of HD-IL2 until they have recovered from the toxicity of IL-2 and bone marrow function has recovered (neutrophil count is >500/mm3, hemoglobin is >8g/dL and platelets are > 30,000/mm3). Patients on Arm 2 will be eligible for hospital discharge while still on the 14 days of low dose SC IL-2 if neutrophil count is >500/mm3, hemoglobin is >8g/dL and platelets are > 30,000/mm3.

Patients in both arms will initiate MK-3475 on day 21 (+ 7 days) after TIL infusion and subsequently will be seen in clinic every 3 weeks prior to each planned dose of MK-3475.

Complete evaluation of evaluable lesions with physical examination and appropriate CT scans (CT chest, abdomen, pelvis) or PET/CT will be performed approximately at 9 weeks (+/- 7 days) after TIL infusion and then at 12 week intervals (+/- 7 days) for the duration of therapy. After patients have completed protocol therapy, repeat imaging evaluations will continue at 12 week intervals. For patients with cutaneous disease, medical photography will be obtained at baseline and then at the time of each imaging evaluation.

Peripheral blood samples for immune monitoring including flow cytometry and tracking persistence of T cell clones will consist of 10cc in a red top tube and up to 50cc in green top tubes. These samples will be collected prior to tumor harvest and/or excision, prior to lymphodepletion, at day +7 (+/- 2 days) and just prior to first dose (+ 7 days) and second dose (+/- 7 days) of MK-3475. These samples will also be collected at the time of each restaging scan and at the time of documented progression.

7.4.3 Post-Treatment Visits

After completion of the one year duration of therapy, follow up examinations and tests will be determined by the treating physician. It is recommended that follow up visits and restaging scans be performed approximately every 12 weeks (± 7 days) for three years after completion of protocol therapy.

After that time, we will send the patient a questionnaire (FACT-G, FACT-Melanoma) (Appendix E, F) to get information regarding their quality of life for the next five years. For this reason, we will ask the patients to continue to provide us with a current address and telephone number, even after completion of this research study.

7.4.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8-12 weeks by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death or end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

7.4.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone approximately every 3 months to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.4.5 Assessing and Recording Adverse Events

This study will utilize the National Cancer Institute Common Terminology Criteria (CTC) for Adverse Events version 4.0 for toxicity and Adverse Event reporting. A copy of the CTCAE version 4.0 can be found in Appendix A. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.

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. 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. 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.

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

1. *Cyclophosphamide:* Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.

2. *Fludarabine:* Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

3. Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

4. High Dose IL-2: A variety of side effects have been associated with high-dose IL-2 administration in our experience at the NCI and a listing of these side effects in 283 patients who received 447 treatment courses are listed in Appendix H.

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or

within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

All adverse events suspected to be due to the combination of TIL with anti pd-1 (for both cohorts of patients) and low dose IL-2 (only for patients in Arm B) will be recorded from the time the consent form is signed through 90 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. Adverse events resulting from lymphodepletion and high dose IL-2 will not be recorded. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.4.8.1.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.4.6 Definition of an Overdose for This Protocol and Reporting of Overdose to the MDA IND office

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for MK-3475 by 20% over the prescribed dose. No specific information is available on the treatment of overdose of MK-3475. In the event of overdose, MK-3475 should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 48 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.4.7 Reporting of Pregnancy and Lactation to the MDA IND office

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner

that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.4.8 Immediate Reporting of Adverse Events to the MDA IND office

7.4.8.1 Serious Adverse Events

- 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:

- Results in death;
- 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.Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- Is a congenital anomaly/birth defect;

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

- 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.

- 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).

- 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.

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

Serious adverse events will be captured from the time of the first protocol-specific intervention, until 90 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.

Additionally, any serious adverse events that occur after the 90 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

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

7.4.8.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 48 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.4.6 - Definition of an Overdose for This Protocol and Reporting of Overdose to the IND office, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is greater than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

3. In the event a subject develops any of the following AEs, a detailed narrative of the event should be reported as an ECI to the MDA IND office within 48 hours

a. Grade ≥ 3 diarrhea
b. Grade ≥ 2 colitis
c. Grade ≥ 2 pneumonitis
d. Grade ≥ 3 hypo- or hyperthyroidism

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported within 48 hours to the IND office.

7.4.9 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0 (Appendix A). Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 8 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.4.10 Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

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 "University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy on Reporting Serious Adverse Events". 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).

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.

The MDACC "Internal SAE Report Form for Prompt Reporting" will be used for reporting to the IND Office.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Overview and Sample Size Justification

The primary objective of this trial is to assess the overall response rate (ORR) in each arm. A total of 36 patients will be randomized in a 1:1 ratio to the HD-IL2 and LD IL-2 arms. With 18 patients in an arm, half the width of a two-sided 95% confidence interval around the ORR will be a maximum of 23%. Secondary endpoints include safety, OS, PFS, and blood and tumor biomarkers. Secondary analyses will consist of comparing secondary endpoints between the treatment arms as well as assessing the association between endpoints and clinical and disease covariates of interest. As the trial proceeds, the ORR will be monitored separately in each arm to assure that if it is too low, accrual onto the arm will stop.

8.2 Primary Analyses

The ORR will be computed separately by arm and presented with exact 95% confidence intervals.

8.3 Secondary Analyses

The ORR will be compared between the two treatment arms by using Fisher's exact test. Safety parameters will be compared between the arms by using Wilcoxon rank-sum tests for continuous parameters and Fisher's exact tests for categorical parameters. The method of Kaplan and Meier will be used to estimate the distributions of OS and PFS, and distributions will be compared between arms by

using the log-rank test. Cox regression analysis will be used to assess the association between disease and clinical covariates of interest and OS and PFS. The association between ORR and the same covariates will be assessed by using logistic regression. For the blood and tumor biomarkers collected over time, we will use a generalized linear mixed model (GLMM) approach to account for intra-patient correlation.

8.4 Futility Monitoring

The primary ORR endpoint in this trial will be monitored using a Bayesian sequential monitoring rule (Thall et all, 1995). If in either arm, there is a high probability that the ORR is less than 40%, accrual onto the arm will be stopped. Patients will be monitored in cohorts of size 3. We will enroll a minimum of 6 patients in an arm before stopping. The following stopping rules and boundaries were developed using the multc99 application (version 2.0) from the Biostatistics department. Accrual will be stopped early in any arm if in that arm

$$\Pr[\text{100-day ORR} < 40\% \mid \text{data}] > 0.95$$

That is, if we determine that there is a greater than 95% chance that the ORR in an arm is less than 40%, accrual into the arm will be stopped. We assume a beta (0.8, 1.2) prior distribution for the ORR rate (historical prior distribution based on 1000 patients) in each arm, which has a mean of 0.40 corresponding to the target ORR. Stopping boundaries corresponding to this probability criterion are to terminate accrual in an arm if:

Number of Patients Evaluated for ORR	Stop the arm if this many responses
6	0
9	0-1
12	0-2
15	0-2
18	Always Stop (arm complete)

This stopping rule was chosen to assure that the probability that an arm will stop early would be between 10% and 15% if the true ORR rate is as low as 40%. The operating characteristics of this rule are shown in the table below.

Table 1. Operating Characteristics for ORR Monitoring Rule

If the true ORR in an arm is…	**Early Stopping Probability**	**Arm Sample Size 25^{th}, 50^{th}, 75^{th} percentiles**		
20%	0.605	6	12	18
30%	0.305	12	18	18
40%	0.119	18	18	18
50%	0.035	18	18	18
60%	0.008	18	18	18

The monitoring rules will be assessed by the study team with assistance as necessary from the Department of Biostatistics at MD Anderson Cancer Center.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized in Table 9.

Table 9 Product Descriptions

Product Name & Potency	**Dosage Form**
MK-3475 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, and the trial site personnel are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

The drug product is stored under refrigerated conditions (2ºC- 8ºC).

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

For All Studies

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all subinvestigators and study site personnel, may be used and disclosed for study management purposes, as part of a regulatory submissions, and as required by law. This information may include:

- name, address, telephone number, and email address;
- hospital or clinic address and telephone number;
- curriculum vitae or other summary of qualifications and credentials; and
- other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the IND office. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory agencies or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

10.2 Compliance with Financial Disclosure Requirements

10.3 By signing this protocol, the investigator agrees to provide to MD Anderson accurate financial information to allow MD Anderson to submit complete and accurate certification and disclosure statements as required by U.S. Food and Drug Administration regulations (21 CFR Part 54).Compliance with Law, Audit and Debarment

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

10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), MD Anderson is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.5 Quality Management System

By signing this protocol, MD Anderson agrees to be responsible for implementing and maintaining quality control and quality assurance systems with written SOPs to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

10.6 Data 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 utilizing a two-turnstile registration before any study specific tests are performed. The continual reassessment method (CRM) used in phase I will be implemented using the Biostatistics Department Clinical Trial Conduct Website.

11.0 REFERENCES

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7 Li Y, Liu S, Hernandez J, et al. MART-1-specific melanoma tumor-infiltrating lymphocytes maintaining CD28 expression have improved survival and expansion capability following antigenic restimulation in vitro. *J Immunol* 184, 452-465.

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12.0 APPENDICES A-N in PDOL

APPENDIX O. Events of Clinical Interest

Colitis (reported as ECI if ≥ Grade 2)		
Bowel Obstruction	Colitis	Colitis microscopic
Enterocolitis	Enterocolitis hemorrhagic	GI Perforation
Necrotizing Colitis		
Diarrhea (report as ECI if ≥ Grade 3 or any grade resulting in dose modification)		
Endocrine (reported as ECI if ≥ Grade 3 or any grade resulting in dose modification)		
Adrenal Insufficiency	Hyperthyroidism	Hypophysitis
Hypopituitarism	Hypothyroidism	Thyroid Disorder
Thyroiditis		
Eye		
Uveitis (report as ECI if ≥ Grade 2 or any grade resulting in dose modification)		
Hepatic (reported as ECI if ≥ Grade 2 or any grade requiring dose modification)		
Hepatitis	Hepatitis, Autoimmune	
Pneumonitis (reported as ECI if ≥ Grade 2)		
Acute Interstitial Pneumonitis	Interstitial Lung Disease	Pneumonitis
Renal (reported as ECI if ≥ Grade 2 or any grade resulting in dose modification)		
Nephritis	Nephritis Autoimmune	Renal Failure
Renal Failure, Acute		
Skin (always reported as ECI regardless of grade)		
Dermatitis Exfoliative	Erythema Multiforme	Stevens-Johnson Syndrome
Toxic Epidermal Necrolysis		
Skin (reported as ECI if ≥ Grade 3 or any grade resulting in dose modification)		
Pruritus	Rash	Rash generalized
Rash maculo-papular	Vitiligo	
Other (The following should always be reported as an ECI, regardless of grade)		
Autoimmune Neuropathy	Demyelinating Polyneuropathy	Guillain-Barre
Myasthenia Gravis like syndrome	Non-infectious myocarditis	Non-infectious pericarditis
Pancreatitis	Rapid onset of Grade 3 fatigue in the absence of disease progression	

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