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Phase I/Ib Study of Adoptive Cellular Therapy Using Autologous IL-21-Primed CD8+ Tumor Antigen-Specific T Cells in Combination with Utomilumab (PF-05082566) in Patients with Platinum Resistant Ovarian Cancer

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1. INTRODUCTION

This protocol proposes to examine the safety and duration of *in vivo* persistence and anti-tumor efficacy of adoptively transferred autologous tumor-reactive ovarian cancer antigen-specific $CD8^+$ T cells with or without utomilumab (PF-05082566), an agonistic anti-CD137 therapy in patients with advanced ovarian, fallopian tube, or primary peritoneal cancer.

Adoptive T cell therapy (ACT) is one form of personalized immune therapy involving the ex vivo selection and expansion of antigen-specific T cells for infusion into patients [1]. This approach overcomes many of the obstacles that can limit vaccine-elicited responses and provides rigorous control over the magnitude, specificity, avidity and phenotype of the effector T cells[1-5]. Studies using adoptively transferred antigen-specific T cells performed by our lab have demonstrated clinical benefit in time to progression and in some cases durable partial to complete responses for patients with metastatic melanoma [2,5]. Analysis of these previous clinical studies reveals that improved efficacy can be achieved by: 1) extending the in vivo persistence of transferred T cells, 2) broadening the repertoire of the immune response to limit the outgrowth of antigen-loss tumor variants, and 3) addressing mechanisms of tumor immune evasion [2,5].

We recently established a robust protocol for generating long-lasting central memory type T cells against a panel of cancer-testis antigens as well as several other shared tumor-associated antigens that will now allow us to target non-melanoma solid tumor malignancies, including ovarian cancer. [6-9] While the addition of immune checkpoint inhibitors to adoptive cellular therapy has been productive, the availability of agonist antibodies to costimulatory receptors such as CD137 provides an additional means of extending in vivo persistence and overcoming mechanism of immune evasion by recruiting endogenous T cell and NK cell responses to non-targeted tumor associated antigens.

CD137 (also known as 4-1BB) is a molecule that is up regulated on activated T cells and functions as a costimulatory receptor. When engaged to its ligand (CD137L/ 4-1BBL), CD137 delivers positive costimulatory signals that enhance TCR signaling, IL-2 transcription, and T cell survival. The use of anti-CD137 antibody can increase T-cell recruitment into tumor sites, enhance their cytotoxic activity, and protect T cells from activation-induced cell death (AICD)[10].

We propose a combined biologic strategy involving the use of adoptively transferred ovarian cancer antigen-specific CD8 T cells and the concomitant administration of anti-CD137 as a means of enhancing the transferred T cell response and promoting the generation of endogenous T cell responses against a broader panel of tumor-associated antigens that are released in a pro-inflammatory environment following lysis of ovarian $antigen^+$ tumors by transferred T cells.

This phase I/Ib study will evaluate the safety, in vivo persistence, and anti-tumor efficacy of adoptively transferred antigen-specific CTL alone and in combination with utomilumab (PF-05082566) in patients with advanced ovarian cancer. A maximum of 18 evaluable patients will be enrolled- in a dose escalation study of utomilumab. (Please note that throughout the protocol utomilumab, anti-CD137 and PF-05082566 will be used interchangeably and all refer to the same drug).

2. OBJECTIVES

2.1. PRIMARY

1. Assess the safety and toxicity of adoptively transferred central memory-type CTL targeting ovarian cancer antigens administered alone, and in combination with, utomilumab, an agonistic anti-CD137 antibody, in patients with platinum resistant ovarian cancer
2. Evaluate the functional and numeric in vivo persistence of adoptively transferred central memory-type CTL with and without utomilumab.

2.2. SECONDARY

Evaluate the anti tumor effect of adoptively transferred central memory-type CTL targeting ovarian cancer antigens as measured by best overall response rate (BORR) and progression free survival (PFS).

3. RATIONALE

3.1. IMMUNOTHERAPY FOR OVARIAN CANCER

Despite high rates of complete response to the combination of tumor reductive surgery and adjuvant platinum-taxane based chemotherapy, the vast majority of patients with stage III/IV epithelial ovarian cancer (EOC) recur with a median progression free survival of approximately 18 months[11]. With rare exceptions, relapsed ovarian cancer is considered incurable, and patients who progress on, or recur less than 6 months from the completion of platinum-based chemotherapy, are deemed "platinum resistant," and constitute a very poor prognostic group. Despite decades of clinical investigations, most therapies are associated with only modest response rates and a typical median progression free survival of 3-4 months across a wide variety of cytotoxic and biological agents[12]. Thus, development of new strategies that will ultimately improve the overall survival of patients with EOC represents a crucial unmet need.

The landmark success of immune checkpoint inhibitor drugs such as Ipilimumab and Nivolumab has rejuvenated the field of immune-oncology and has led to intense focus on other co-stimulatory and co-inhibitory immune pathways[13]. Immunogenicity of EOC has been well documented, and there is extensive literature demonstrating the presence of clonally activated $CD3^+CD8^+$ $TCR\alpha\beta^+$ T-cells in ovarian tumors and their prognostic significance [14-20]. TIL derived from EOC patients demonstrate tumor specificity and killing of autologous EOC tumor cells[20]. A meta-analysis of studies investigating the prognostic significance of TIL in ovarian cancer revealed an independent positive effect on survival associated with the presence of TIL[21]. There is strong evidence that EOC harbors an immune-suppressive environment that includes T-regs, CTLA4-and PD-1 expressing TILs, monocyte/macrophages, and elevated intratumoral and plasma levels of immunosuppressive cytokines such as IL10, IL6 and TGFβ[22-28]. Hence, the immune system represents a potentially paradigm changing and under explored area of therapeutic intervention for ovarian cancer.

3.2. TUMOR ANTIGENS RELEVANT TO OVARIAN CANCER

A primary obstacle to the development of antigen-specific T cell therapy for ovarian cancer has been the difficulty in identifying appropriate target antigens. The most well-defined ovarian tumor associated antigen (TAA), the HER2/neu proto-oncogene, is overexpressed in 10-15% of ovarian cancers, and has been targeted in vaccine trials for the treatment of patients with breast and ovarian cancers. Although HER2/neu-specific responses can be generated in vivo following repeated peptide or protein immunizations, such responses have not been particularly robust and HER2/neu-specific T cells generated in vitro using the predicted immunogenic peptides or HER2/neu proteins generally fail to recognize tumors expressing physiological levels of antigen. Efforts underway to augment HER2/neu specific responses in vivo through various immunization strategies (DNA vaccines, novel adjuvants) and the identification of additional potentially more immunogenic epitopes presented by Class I and Class II MHC may lead to the generation of more vigorous, high affinity HER2/neu-specific cellular responses in the future. Currently, however, the in vitro generation of HER2/neu-specific T cells is not sufficiently mature for clinical trials of adoptive T cell therapy.

In recent years, several groups, using methods to screen tumor-derived expression libraries with autologous tumor-reactive T cells or the sera of patients with cancer, have identified a number of novel immunogenic tumor proteins[29,30]. The identification of these antigens, and the mapping of specific epitopes recognized by CD4+ and CD8+ T cells, has facilitated the development of strategies designed to augment antigen-specific T-cell responses.

3.2.1. TUMOR ASSOCIATED ANTIGENS IN OVARIAN CANCER TARGETED IN THIS STUDY

Successful immunotherapy requires the characterization of TAA that are commonly expressed in ovarian tumors, but not in normal tissues. Moreover, the ideal antigen should exhibit a high frequency of expression in cancer and evidence of immunogenicity. We have evaluated two tumor antigens overexpressed or uniquely in ovarian cancer: PRAME and COL6A3, both of which have been extensively analyzed for prevalence of expression tumor and normal tissues and immunogenicity.

3.2.2. COL6A3

COL6A3 encodes the alpha-3 chain of type VI collagen, and facilitates cell anchoring by interacting with integrins, decorins, hyalurnon and proteoglycans. The antigen and the HLA-A*0201-restricted peptide is overexpressed on several solid tumor malignancies, including ovarian cancer (> 35%) and is up regulated in platinum-resistant ovarian cancer lines [31]. Among 280 normal tissue samples (including brain, heart, liver, cartilage, skin,) peptide presentation was detected in placenta only and 115 of 475 tumor samples, including 9/20 ovarian cancers. According to the TCGA database, exon 6 expression of COL6A3 is specifically higher in tumor samples compared to very low levels in normal tissue. Immunogenicity assays and function T cell data in our lab have demonstrated the ability to generate responses in 14/25 healthy donor PBMCs (56%); responses in COL6A3+ patients may be significantly higher. We have chosen to target COL6A3 because it likely represents not only a cancer cell target, but also a tumor-associated stromal/ fibroblast antigen and addresses the possibility that co-targeting of this compartment may enhance anti-tumor efficacy both directly and indirectly[32]. Immunogenicity assays and function T

cell data in our lab have demonstrated the ability to generate responses in 3.11 of 19 donors (unpublished data).

3.2.3. PRAME

PRAME, also known as preferentially expressed antigen in melanoma, is a germinal tissue-specific gene expressed in placenta and endometrium, and both hematological and solid tumor malignancies. Several HLA-A*0201 restricted epitopes have been identified, one of which, PRAME-004, has been shown to be presented in ovarian cancer of high immunogenicity[33]. This epitope has been found only on tumor samples, and not normal tissues. In the TCGA database, it is expressed at very high levels in melanoma, ovarian and uterine/endometrial cancers (> 40 RPKM) with no expression in normal tissues except at very low levels in adrenal gland. Its prevalence of expression in ovarian cancer is > 80%.

3.2.4. TARGETING PRAME, COL6A3, OR BOTH

When targeting two antigens, that do not appear to be linked in expression, we calculated the prevalence of both antigens being expressed. Among 262 high-grade ovarian cancers, COL6A3 was expressed in 50% of samples; PRAME being expressed 82%, yielding a combined targeting prevalence of 32%. Given the prevalence of HLA-A*0201 of 40-50%, we expect that approximately two-thirds of the potential subjects will have at least one targetable antigen, while 12-15% will be positive for both antigens. In this investigation a total of 10 billion CTL per meter squared, targeting either a single or both antigens, will be administered.

3.3. ADOPTIVE T CELL THERAPY: RATIONALE AS A TREATMENT MODALITY

In contrast to tumor vaccination strategies, adoptive T cell therapy allows more rigorous control over the magnitude and avidity of the targeted response by appropriate manipulation and selection in vitro of the T cells used for therapy[34,35]. Tumor-reactive effector cells of a desired specificity and phenotype can be identified in vitro, exposed to cytokines and immune-modulators that influence differentiation during priming and expanded to very large numbers – greater than 10^{10} T cells - producing frequencies of antigen-specific T cells in the peripheral blood that are over ten-fold higher than that possible by current immunization regimens alone.

Effector cells that have been used in adoptive therapy are generally represented by:

1. Tumor-infiltrating lymphocytes (TIL) that are harvested from tumor sites and expanded using supraphysiologic doses of IL-2 for adoptive transfer,
2. Antigen-specific T cells (CTL) generated from peripheral blood following cyclic in vitro stimulation with the antigenic epitope and expanded for adoptive transfer

Historically, TIL have been more commonly used for adoptive therapy due to their relatively lower labor- and resource- intensive requirements; however, their use is limited to patients with accessible tumor of sufficient cell yield from whom TIL cultures can be generated. The generation of antigen-specific CTL from the peripheral blood is technically more demanding and its use is restricted to a few specialized centers; however, for targeting PRAME, COL6A3 and other tumor-associated antigens, antigen-specific CTL can be routinely generated from HLA-A2+ patients using a strategy pioneered in our lab we have coined " endogenous T cell therapy (ETC), a form of adoptive cellular therapy involving the isolation and

expansion of tumor-reactive T cells from the peripheral blood. By developing enabling technologies using IL-21 priming to enrich for a long lasting central memory population of T cells and the use of a clinical grade cell sorter, we have been able to render this a feasible and broadly applicable strategy for patients not only with melanoma, but also patients with other solid tumor malignancies for which other forms of immune-based therapies, including other forms of adoptive cellular therapy has been limiting. Strategies to streamline the production of antigen-specific CTL and yield a product in 7 weeks from time of peripheral blood cell collection have been developed and conditioning regimens and associated toxicities have been minimized while maintaining similar anti-tumor efficacy[1-3,7,36].

3.4. ANTIGEN-LOSS VARIANTS AND ANTIGEN-SPREADING

Both murine and human studies have demonstrated tumor regression following immunologic targeting of only a single antigen or epitope in settings in which the antigen is known to be heterogeneously expressed[4,37-41]. In a vaccine study of melanoma patients, anti-tumor CTL recognizing non-targeted antigens MAGE-C2 and gp100 expanded several-fold *in vivo* after MAGE-3 vaccination, exceeded the frequency of anti-MAGE-3 CTL, and were associated with complete tumor regression. Using $CD4^+$ T cell clones to the single tumor-associated antigen, NY-ESO-1, we demonstrated the induction of a broader multivalent response appearing in a patient who developed a complete regression of his tumor[42]. Recent studies using CTL targeting MART-1 in combination with anti-CTLA4 demonstrate induction of endogenous T cell responses to non-targeted tumor-associated antigens, NY-ESO-1, gp100, MAGE-A1 and tyrosinase accompanied by tumor regression and in 2 of 10 patients a complete response suggesting a correlation between antigen-spreading and tumor regression[2].

3.5. CD137 AND CANCER

The expression of CD137 on tumor-reactive T cells and its ability to increase T-cell recruitment into tumor sites, protect T cells from activation-induced cell death, and enhance their cytotoxic activity make the use of agonistic antibodies against CD-137 an attractive strategy for cancer immunotherapy[43,44]. Utomilumab that will be used in this study is currently in several clinical trials and results of the phase I monotherapy study were reported at ASCO in 2014 (Abstract #3007). This study was an open-label, dose escalation conducted in patients with advanced malignancies for which no curative therapy was available. Cohorts of 3-6 patients were enrolled initially using a 3+3 design (0.006 to 0.3 mg/kg), then a Time-To-Event CRM design for higher doses (0.6 to 5 mg/kg). Among 27 treated patients, the best clinical response was disease stabilization, in 6 patients (22%). Importantly, there were no dose-limiting toxicities and all discontinuations were due to disease progression, not adverse events.

Furthermore, recently the preliminary results of the phase I study of the combination of utomilumab and Pembrolizumab in 23 patients with a range of advanced or metastatic solid cancers (ASCO Abstract #3002, 2016). Six patients achieved an objective response to utomilumab plus pembrolizumab, including two complete responses in small cell lung cancer and RCC. Treatment emergent adverse events were mostly grade 1-2 and no subject discontinued study medication due to treatment-related adverse events. The study demonstrated the safety and efficacy profile of utomilumab in doses up to 5.0 mg/kg in combination with pembrolizumab supports further investigation in patients with advanced solid tumors.

In a preclinical translational investigation of ovarian cancer-derived TIL, our group has shown that the use of anti-CD137 ex vivo results in higher success rates and proportion of CD8+ TIL (Sakellarious-Thompson et al., SITC 2015 Abstract).

3.6. REGULATORY T CELLS

$CD4^+$ regulatory T cells (Treg) comprise 5-10% of $CD4^+$ cells in human peripheral blood and have been phenotypically characterized as $CD4^+$ cells constitutively expressing surface CD25. Treg $CD4^+$ cells, in contrast to recently activated $CD4^+$ T cells that are $CD25^{med}$ or resting $CD4^+$ T cells that are $CD25^{lo}$, can be isolated based on being phenotypically $CD25^{hi}$, and further distinguished by expression of the forkhead transcription factor, Foxp3. Cyclophosphamide has been shown to deplete Treg cells, and in murine studies cyclophosphamide-mediated Treg depletion enhanced anti-tumor immunity[45-48], suggesting this may in part be the basis for the immuno-potentiating effect of cyclophosphamide in tumor therapy models. In patients, circumstantial evidence suggests that the levels of Treg cells may correlate with disease stage and prognosis[49], and selective depletion of $CD25^+$ cells using an IL-2-diphtheria-toxin conjugate is the subject of several ongoing clinical trials[50,51].

3.7. THE USE OF INTERLEUKIN-2 IN ADOPTIVE T CELL THERAPY

In our clinical studies, adoptively transferred CTL clones had a median survival of 6.6 +/- 0.8 days in the absence of IL-2. The addition of a 14-day course of low-dose IL-2 (250,000 U/m^2 twice daily) following T cell infusion significantly prolonged median CTL survival to 16.8 +/- 1.6 days without associated toxicity 4. The frequency of transferred CTL in vivo began to fall before the end of the 14-day course, suggesting limited benefit might be expected with continued administration of IL-2 at this dose beyond 14 days.

High-dose IL-2 (720,000 U/kg three times daily) has been administered following adoptive transfer of CD8+ T cells[52,53]. In one study, clonal T cells were promulgated to very high numbers and clinical responses were observed; however, due to the design of the study, no conclusions could be drawn as to whether high-dose IL-2 was required for the in vivo survival or expansion of transferred T cells. In addition to a direct effect on T cell expansion, it could be speculated that increase vascular permeability induced by high-dose IL-2 provides improved tissue access to tumor sites. Additionally, our own studies and that of others[54] have shown that exposure to high-dose IL-2 (1000 U/ml) leads to rapid, antigen-independent expansion of CTL clones in vitro (in a manner similar to IL-15) while exposure to low-dose IL-2 had little effect. Of note, responses to high-dose IL-2 were only observed with antigen-specific CTL clones generated by cyclical re-stimulation using PBMCs of vaccine-naïve donors. CTL clones generated from G209M-vaccinated donors and exposed to high doses of IL-2 in vitro failed to respond to either high-dose IL-2 or IL-15, thus concurring with the hypothesis that the manner and source from which T cells are generated can influence their in vivo behavior.

3.8. ADOPTIVE T CELL THERAPY USING ANTIGEN-SPECIFIC CD8+ T CELLS IN LYMPHODEPLETED RECIPIENTS

We initially evaluated the use fludarabine alone as a lymphodepleting-conditioning reagent due to its predictable kinetics and relatively low toxicity. By comparing an infusion of T cell clones given first without, and then a second infusion given following fludarabine lymphodepletion we could directly evaluate the influence of fludarabine lymphodepletion on a uniform CD8+ T cell population. Following fludarabine lymphodepletion, induction of lymphopenia led to an increase in serum levels of homeostatic cytokines, IL-7 and IL-15, and an average 3-fold increase in the median T cell survival compared to that achieved by an infusion of the identical T cell clone infused without prior lymphodepletion[55]. In this

study, we also determined that apart from the extrinsic influence of prior conditioning, the duration of in vivo persistence was associated with an intrinsic property of a given T cell clone – this property being the proliferative capacity of a T cell clone as measured in vitro by the number of cell divisions (CFSE dilution) or absolute cell number following exposure to low-dose IL-15.

One insight borne out of the clinical studies performed by the NCI and our above studies of adoptive cellular therapy is that the in vivo clinical response is related to in vivo persistence and that methods to modulate and/or identify tumor-reactive T cells with enhanced replicative potential would lead to more effective therapy. Indeed, work in murine models demonstrate the superior anti-tumor effect of "early effector" T cells and in non-human primates, it was recently demonstrated that methods to enrich for T cells harboring a central memory program are more likely to experience an extended period of in vivo persistence even when driven along an effector pathway. However, the endogenous frequency of tumor antigen-specific T cells with central memory properties is relatively rare. To this end, we explored an alternative approach to generating a population of tumor-antigen-specific CD8 T cells with enhanced self-renewal capacity.

3.9. IL-21 EXPOSURE LEADS TO GENERATION OF CD28HI HELPER-INDEPENDENT CD8 T CELLS IN VITRO

We discovered that among the γ-chain receptor cytokines, IL-21 alone, was unique in not only increasing the frequency of antigen-specific CTL recognizing a tumor-associated antigens that could be generated but also in arresting differentiation at a stage that conferred helper-independence to the population of tumor-reactive CTL. Antigen-specific CTL exposed to IL-21 during priming expressed CD45RO, very little CCR7 or CD62L, but retained high levels of CD28, which is rarely expressed by memory effector tumor-reactive T cells following in vitro stimulation. This unique phenotype was stable for several weeks during in vitro expansion without the requirement for further IL-21 exposure suggesting that imprinting occurred during priming. Engagement of these CTL with the tumor ligand alone mediated IL-2 production-which was blocked with CTLA4-Ig, demonstrating that IL-2 production/helper-independence was mediated through CD28[8,56]. In subsequent studies, we demonstrated that pretreating the T cell culture by CD25 depletion, led to elimination of 50% of FoxP3+ Treg cells and enhancement of antigen-specific CTL frequency by ten-fold. However, combined CD25 depletion, and IL-21 exposure during in vitro stimulation, led to elimination of > 99.9% of Treg cells (more than one log- greater decrease in Treg frequency than what could previously be achievable by non-invasive means), and a greater than 200-fold increase in the numbers of tumor-reactive antigen-specific CTL that could be elicited [8,56]. When expanded for adoptive therapy, 25-65% of the total T cell culture was represented by tumor antigen-specific CTL, while retaining CD28 expression.

3.10. SAFETY CONSIDERATIONS

When targeting tumor-associated antigens that are also self proteins, transferred T cells may mediate autoimmune toxicity. In some cases, the target antigen is expressed by normal tissues without significant physiologic consequence. However, unexpected toxicities due to adoptive T cell therapy will be addressed by therapy with steroids, which provide a lympholytic and anti-inflammatory effect. We have conducted in vitro studies demonstrating that a concentration of methylprednisolone at levels about one-log below that attained in vivo (100 ng/ml) is sufficient to induce apoptosis in our T cell clones resulting

in >90% cell death within 48 hours. Steroids may additionally suppress nonspecific inflammatory mediators (such as macrophages) that may be precipitated by T cell activation[57,58].

Our experience in patients receiving T cell infusions who were started on steroid therapy for reasons not related to treatment-related toxicity, have demonstrated that the administration of steroids is effective in eliminating infused clones from the peripheral blood. Two patients in the initial study receiving melanoma-specific T cell clones were given emergent steroid therapy for an impending obstruction of the superior vena cava by enlarging tumor mass. Analysis of the peripheral blood in these patients demonstrated rapid decrease in circulating T cell clones from a frequency of > 1/750 PBMCs to undetectable levels within 48 hours after beginning steroid therapy consistent with in vitro findings. In another ongoing clinical trial of adoptive therapy, we are conducting to evaluate reconstitution of CMV immunity post marrow transplantation, two patients received infusions of CMV-specific CD4+ T cell clones similarly demonstrated rapid disappearance of the infused clones following steroid therapy for graft-versus-host disease.

3.11. RATIONALE AND PROPOSED STUDY OF ADOPTIVE T CELL THERAPY USING IL-21 MODULATED CD8 CTL FOR THE TREATMENT OF PATIENTS WITH ADVANCED OVARIAN CANCER

Current strategies that we and others have evaluated to extend the in vivo persistence of transferred T cells include the administration of IL-2 and the use of pre-infusion conditioning regimens to inhibit the influence of regulatory cells limiting expansion and to induce lymphopenia and up regulation of homeostatic cytokines such as IL-7 and IL-15. These extrinsic approaches have proven effective; however, the development of methods to isolate early stage effector CTL with superior self-renewal capacity would be highly desirable in light of animal studies demonstrating enhanced anti-tumor efficacy.

We recently developed, for the first time, a strategy to generate, helper-independent early effector human CD8 T cells which exhibit enhanced self-renewal capacity and high affinity for tumor-associated antigen targets. In vitro priming in the presence of the gamma-chain receptor cytokine, IL-21 and rapamycin, yielded CTL with an optimal balance of high replicative capacity and enhanced effector function. In murine studies evaluating the use of early effector/central memory T cells, a T cell dose that was 100 fold less than the standard dose was sufficient to mediate tumor eradication and in some cases, long term survival[59,60]. For this reason, we plan a starting dose approximately 10 to 100 fold less than that used in TIL studies (up to 200 billion cells) where no selection or enrichment was made to generate helper-independent CTL.

In this study, we propose a first-in-human study evaluating the use of IL-21 modulated antigen-specific CTL for the treatment of patients with advanced ovarian cancer following cyclophosphamide conditioning.

3.12. GENERATION OF PRAME AND COL6A-SPECIFIC CTL

We will use the A2-restricted epitope of PRAME and COL6A3 to generate autologous antigen specific, CTL from the PBMCs of leukapheresis products. Dendritic cells will be pulsed with the A2-restricted epitope peptide of PRAME or COL6A3, and individually co-cultivated with autologous PBMC in 48-well cultures supplemented with IL-21 according to results of preclinical studies; restimulation after 7 days will be supplemented with IL-2 and IL-7. Following 1-2 cycles of in vitro stimulation, individual wells

will be screened with the respective tetramer and wells with highest frequency of ovarian cancer antigen-specific CTL will be pooled, stained with tetramer and sorted using a clinical grade cell sorter. We have routinely achieved sort enrichment of antigen-specific CTL to 80% purity starting with a population containing as low as 0.3% tetramer+ T cells. In vitro expansion of these cells achieves 3000 to 5000-fold expansion, reaching numbers sufficient for adoptive transfer (10^{10} cells/m^2) within 6-8 weeks from leukapheresis.

4. STUDY DESIGN

All patients will receive low-dose cyclophosphamide conditioning (300 mg/m^2) 2 days prior to infusion, and low dose IL-2 (250,000 U/m^2 s.c. q12h for 14 days) after infusion. For Dose Level 1 cohorts, an infusion of ovarian cancer antigen-specific CTL will be administered on Day 0 at a total dose of 1 x 10^{10}/m^2 total, if both antigens are present (0.5 x 10^{10} for each antigen); if only one antigen is present then CTL will be administered on Day 0 at a total dose of 1 x 10^{10}. In the absence of any dose-limiting toxicity, the subsequent cohorts will be treated with the same number of ovarian cancer antigen -specific CTL in combination with two dose levels of utomilumab as described in Section 4.2. Utomilumab will be administered within 24 hours of T cell infusion and continued for a total of 6 doses 4 weeks apart. Subjects for whom at least 10 billion/m^2 antigen specific T cells cannot be generated will be deemed inappropriate candidates for treatment and will be removed from the study and replaced. If Dose Level 1 is found to be too toxic, de-escalation to Dose Level -1 consisting of a total of 2X10^9/m^2 (1 billion T cells/m^2 per each antigen if both antigens present).

4.1. TARGET POPULATION

Patients with platinum resistant recurrent or persistent high grade ovarian, fallopian tube, or primary peritoneal cancer and measurable disease by radiographic criteria, expressing HLA-A*0201 and whose tumor expresses either or both of the target antigens PRAME and COL6A3 will be eligible. We anticipate that patient accrual will be achieved within 18 months, with treatment, follow-up and analysis completed after an additional 6 months.

4.2. STUDY DESIGN

A maximum of 18 evaluable patients will be enrolled. Patients will be enrolled in cohorts of 3 patients to evaluate the safety of using ovarian cancer antigen specific T_{CM} without (Dose Level 1) and with escalating doses of utomilumab (Dose Level 2 and 3 at 0.3 and 1.2 mg/kg, respectively). Subjects are considered evaluable for response based on treatment cohort. For the cohort assigned to T-cells alone, subjects must have received outpatient cyclophosphamide and T-cell infusion to be considered evaluable. For cohorts assigned to T-cell plus Utomilumab, subjects are considered evaluable for response if they receive T-cells plus at least one dose of Utomilumab. Additional details including the dose escalation rule is provided in **Section 11**. Subjects who receive T cells alone will serve as a baseline comparison for the immunobiological effect of utomilumab on the transferred ovarian cancer antigen-specific CTL, which can be tracked and analyzed directly ex vivo.

4.3. ENDPOINTS

4.3.1. PRIMARY

1. Safety and toxicity will be assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Patients will be monitored closely following therapy as described in the Schedule of Treatment and Evaluation (**Table 1**). Dose limiting toxicities are defined in **Section 9**. We have not observed any serious adverse events related to ovarian cancer antigen specific T cell therapy in our patients with leukemia nor have there been any reported serious toxicities in clinical trials targeting PRAME and COL6A3 to our knowledge. However, the combination with anti-CD137 warrants closer monitoring in light of potential immune-related AEs. Patients with pre-existing lung disease and autoimmune disease will be excluded.
2. Duration of in vivo persistence. Transferred antigen-specific T cells will be tracked by tetramer analysis as well as by TCR tracking (Immunoseq assay, Adaptive Biotech), both assays, have been established in our lab as reliable measures of T cell persistence. Duration of in vivo persistence will be defined as the time between T cell infusion and last time point at which the transferred T cell DNA signature is detectable in peripheral blood. Multiparameter analysis can be performed on the tetramer+ T cells to evaluate expression of memory/differentiation and exhaustion/coinhibitory markers in vivo following adoptive transfer.

4.3.2. SECONDARY

PFS and BORR. Patients with recurrent ovarian cancer experience progressively shorter periods of disease control.

5. PATIENT ELIGIBILITY

Patients with recurrent or persistent platinum resistant high grade epithelial ovarian cancer will be considered for this study. Patients with platinum resistant constitute a very poor prognostic group[12]. Despite decades of clinical investigations targeting this population, most cytotoxic and biological agents result in only modest rates of response and have a typical median PFS of 3-4 months across a wide variety of cytotoxic and biological agents[12,61]. **Given the timeline for screening and T cell manufacturing, subjects are eligible for enrollment and leukapheresis while still platinum-sensitive. However, they must have developed platinum resistant disease (progression on a platinum-containing regimen or recurrence within 180 days of last dose of platinum-containing chemotherapy) prior to treatment.**

Eligibility will also require a tumor biopsy to determine the expression of COL6A3 and/or PRAME by quantifiable RT-PCR as performed by Immatics Inc. (CLIA-certified conditions – already established). Peripheral blood mononuclear cells for T cell culture will be collected from these patients by leukapheresis. Leukocyte collection can occur at any point in their disease course and will be at least three weeks after previous course of chemotherapy to allow for recovery of leucocytes. Approximately 2-3 months are required to isolate, expand and complete quality control testing on T cells for infusion. Once isolated, T cells can be cryopreserved for later use. In this interval patient may proceed with other clinically used chemotherapy regimens.

5.1. INCLUSION CRITERIA FOR ENROLLMENT AND LEUKAPHERESIS (TURNSTILE 1)

a. Histopathologic documentation (must be performed or reviewed at MD Anderson) of recurrent high grade epithelial ovarian cancer.
b. At least one prior line of platinum-based chemotherapy (subjects are eligible for enrollment and leukapheresis while still platinum-sensitive, however, they must have developed platinum resistant disease for treatment (turnstile 2)).
c. 18 to 75 years of age.
d. Tumor biopsy reveals expression PRAME and/or COL6A3 by Immatics assay.
e. Expression of HLA-A*0201.
f. ECOG performance status of 0-1 (Appendix G) and an expected survival of greater than 16 weeks.
g. Willing and able to give informed consent.
h. Adequate normal organ and marrow function as defined below:
   - Hemoglobin ≥ 9.0 g/dL,
   - Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/L (≥ 1000 per mm^3),
   - Platelet count ≥ 75 x 10^9/L (≥100,000 per mm^3),
   - Serum bilirubin ≤ 1.5 x institutional upper limit of normal (ULN) unless diagnosed with Gilbert's syndrome,
   - AST/ALT ≤ 2.5 x institutional upper limit of normal unless liver metastases are present, in which case it must be ≤ 5x ULN, and
   - Serum creatinine CL >40 mL/min by the Cockcroft-Gault formula (Cockcroft and Gault 1976) or by 24-hour urine collection for determination of creatinine clearance:

$$\text{Creatinine CL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age}) \times 0.85}{72 \times \text{serum creatinine (mg/dL)}}$$

i. Subjects must either be of non-reproductive potential (ie, post-menopausal by history: ≥50 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry.
j. Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study in such a manner that the risk of pregnancy is minimized. Suggested precautions should be used to minimize the risk or pregnancy for at least 1 month before start of therapy, and while women are on study for up to 3 months after T cell infusion, and at least 8 weeks after the study drug is stopped.

5.2. INCLUSION CRITERIA FOR TREATMENT (TURNSTILE 2)

Note: Evaluate within one week of planned start of cyclophosphamide pre-infusion.

a. ECOG performance status of '0-1' (Appendix G).
b. Subjects must have platinum resistant disease (progression on a platinum-containing regimen or recurrence within 180 days of last dose of platinum-containing chemotherapy). Subjects that are

not platinum resistant but are deemed not to be candidates for platinum-based chemotherapy due to prior significant allergic reaction may participate with PI approval.

c. Bi-dimensionally measurable disease by radiographic imaging (MRI or CT scan).
d. At least 4 Weeks must have elapsed since the last chemotherapy, immunotherapy, radiotherapy or major surgery. At least 6 Weeks for bevacizumab.
e. Toxicity related to prior therapy must either have returned to ≤ grade 1, baseline, or been deemed irreversible.
f. Persons of reproductive potential must agree to use and utilize an adequate method of contraception throughout treatment and for at least 8 weeks after study drug is stopped.
g. Willing and able to give informed consent.

5.3. EXCLUSION CRITERIA FOR ENROLLMENT

Note: Exclusion criteria must be met by all subjects prior to proceeding with leukapheresis.

Exclusion criteria will be reassessed and confirmed within one week of planned start of cyclophosphamide infusion. Complete pulmonary function test and/or dobutamine stress echocardiography may be repeated at this time if deemed necessary by the Principal Investigator:

a. Clinically significant pulmonary dysfunction, as determined by medical history and physical exam. All patients will undergo pulmonary functions testing. Results of this evaluation will be considered before excluding patients, and all patients with FEV1 < 60% of normal or DLco (corr for Hgb) < 55% will be excluded.
b. Significant cardiovascular abnormalities including any one of the following:
    i. Congestive heart failure,
    ii. Clinically significant hypotension,
    iii. Symptoms of coronary artery disease,
    iv. Presence of cardiac arrhythmias on EKG requiring drug therapy, or
    v. Patients with a history of cardiovascular disease.

    All patients will have a dobutamine stress echocardiography before beginning treatment. Results of this evaluation will be considered before excluding patients on the basis of cardiovascular abnormalities. Subjects with evidence of stress-induced cardiac ischemia or ejection fraction less than 55% will be excluded.
c. History of central nervous system (CNS) metastasis.
d. Autoimmune disease: Patients with a history of Inflammatory Bowel Disease are excluded from this study, as are patients with a history of autoimmune disease (e.g. Systemic Lupus Erythematous, vasculitis, infiltrating lung disease) whose possible progression during treatment would be considered by the Investigator to be unacceptable.
e. Participation in another clinical study with an investigational product administered during the last 28 days.
f. Receipt of the last dose of previous chemotherapy, hormonal, or biologic treatment for ovarian, fallopian tube, or primary peritoneal cancer in the last 28 days (in the last 6 weeks for bevacizumab).

g. Current or prior use of immunosuppressive medication within 28 days before enrollment, with the exceptions of intranasal and inhaled corticosteroids or systemic corticosteroids at physiological doses, which are not to exceed 10 mg/day of prednisone, or an equivalent corticosteroid.
h. Any prior Grade ≥3 immune-related adverse event (irAE) while receiving any previous immunotherapy agent, or any unresolved irAE >Grade 1.
i. History of allogeneic organ transplant.
j. Unresolved partial or complete small or large bowel obstruction.
k. Receipt of live attenuated vaccination within 30 days prior to enrollment or within 30 days of planned lymphodepletion
l. Any underlying medical or psychiatric condition, which in the opinion of the Investigator, will make the administration of study drug hazardous or obscure the interpretation of adverse events.
m. Active viral hepatitis.
n. Confirmed HIV infection.
o. Confirmed diagnosis of Ehler-Danlos Syndrome.

6. STUDY AGENTS

6.1. ANTIGEN-SPECIFIC $CD8^+$ T CELLS

All T cells administered will be autologous T cells derived from the subject's peripheral blood lymphocytes. Methods employed to generate and qualify $CD8^+$ antigen-specific T cells for infusions are detailed in Appendix I Briefly, T cells demonstrating antigen-specific cytolytic function will be tested for:

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

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

6.2. CYCLOPHOSPHAMIDE

Cyclophosphamide (CY) at 300 mg/m^2 will be administered intravenously (IV) as an outpatient procedure. CY administration will be scheduled 2 days prior to prior to T cell infusion (Day -2).

6.3. INTERLEUKIN-2

IL-2 will be initiated within 6 hours of T cell infusion. Low-dose IL-2 will be administered at 250,000 U/m^2 s.c. q12h x 14 days. Patients will receive at least their first dose while inpatient. Prior to discharge, patients or their caregiver will be instructed on s.c. self-administration.

6.4. UTOMILUMAB ADMINISTRATION

Utomilumab will be administered per manufacturer's instructions (Pfizer).

7. PLAN OF TREATMENT/STUDY SCHEDULE

Table 1. Schedule of Treatment and Evaluation

Visit	Screen	Leuka-pheresis	Pre-infusion	CY	T Cells	On Treatment													EOT [a]
Visit Day	-28 to -1 before Leu-kapheresis	Variable	-28 to -2	-2	0[b]	1[c]	3	7	14	21	28	35	42	56	70	84	112	140	168[d]
Visit Window			-	-	-	-	±3 Days												
Written Informed Consent	X[e]		X[f]																
Medical History	X																		
Concomitant Medications	X		X	X[m]	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[g]	X	X	X	X[m]	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[h]	X	X[i]	X	X[m]	X[j]	X		X	X	X	X	X	X	X	X	X	X	X	X
O2 Saturation	X		X	X[m]	X[j]	X		X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X		X	X[m]	X	X		X	X	X	X	X	X	X	X	X	X	X	X
ECOG performance status	X		X																
CBC with diff and platelets	X	X[k]	X[l]	X[m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistry Panel[n]	X	X	X[m]	X[m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation[o]	X																		
Thyroid function[p]	X		X								X			X		X	X	X	
ß-HCG Serum Pregnancy Test[q]	X	X		X															
HLA typing[r]	X																		
Dobutamine Stress Echocardiography	X		X[s]																
Pulmonary Function Test	X		X[s]																
Infection testing[t]	X																		
Leukapharesis		X																	
EKG			X		X[u]														
Staging for measurable disease[v]			X									X[w]					X		X[x]
CA125			X									X					X		X[y]
Tumor Biopsy[z]	X[aa]																		
Cyclophosphamide[bb]				X															
T Cells[cc]					X														
Low Dose IL-2					X[dd]														
Anti-CD137[ee]						X					X			X		X	X	X	
Immune Monitoring[ff]			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[a] If a subject leaves the study prior to reaching Day 168, the End-of-treatment visit is defined as the last visit where the decision is made to discontinue protocol directed treatment.
[b] Subject to be admitted to in-patient service ahead of T cell infusion.
[c] Subject to be discharged from in-patient service after Anti-CD137 treatment and when they clinically meet discharge criteria.
[d] Patients who have detectable transferred cells >0.5% of CD8+ T cells at the end of monitoring, show tumor response (refer to **Section 8.2** for definitions) or show antigen spreading, will continue to be monitored on study with visits every 8 weeks or as often as clinically indicated unless the disease gets worse, patients start another therapy, or patient is taken off study.
[e] Turnstile 1/Leukapheresis Consent
[f] Turnstile 2/Treatment Consent. Eligibility Criteria must be met. Steroids are not permitted 3 days prior to T cell infusion and concurrently during therapy. Patients may not be on any other treatments for their cancer aside from those included in the protocol. Patients may not undergo another form of treatment concurrently with this study. If patients agree to proceed with T cell therapy and all criteria are met, then a cyclophosphamide infusion visit (outpatient) and T cell infusion visit (inpatient) appointment will be scheduled.
[g] Full physical examination is required at screening. Targeted physical examinations are to be performed at all other time points.
[h] Vital signs will include temperature, pulse, breathing, and blood pressure. See **Section 8.1**.
[i] Leukapheresis may be deferred for patients with active infections or oral temperature > 38.2° C within 72 hours prior to planned Leukapheresis.
[j] Performed before infusion, every 15 minutes during infusion, every 30 minutes for 2 hours after completion of infusion, and then every 6 hours over night.
[k] Leukapheresis may be deferred for patients with Hct < 28%, lymphocytes <1000/uL and platelets <50,000 immediately prior to Leukapheresis.
[l] Patients will be excluded from treatment if CBC and Chemistry profile prior to cyclophosphamide and T cell infusion includes any of the following: WBC ≤ 2000/uL, Hct ≤ 24% or Hb ≤ 8 g/dL, ANC ≤ 1000, Platelets ≤ 50,000, Creatinine ≥ 3.0 x ULN, AST/ALT ≥ 2.5 x ULN, and Bilirubin ≥ 3 x ULN.
[m] If not performed within previous 7 days. Confirmation that subjects meet the Turnstile 2 inclusion and exclusion criteria must be performed within one week of planned start of Cyclophosphamide pre-infusion.
[n] Comprehensive chemistry panel to include electrolytes, BUN, Cr, Liver function tests (AST, alkaline phosphatase, total bilirubin, and direct bilirubin), and LDH. .
[o] Coagulation testing to include PT, PTT, and INR and will be checked prior to biopsy and as clinically indicated (e.g. prior to a planned procedure, if taking anti-coagulation medication or if evidence of coagulation disorder).
[p] Thyroid function tests are required prior to start of outpatient visits of utomilumab administration, unless abnormal in which case they are followed as clinically indicated.
[q] Only for subjects of child-bearing potential.
[r] HLA typing will only be performed if HLA status is unknown.
[s] Only will be repeated at the discretion of the Investigator.
[t] Patients will be screened for Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, and HIV 1/HIV 2 Antibody. Positive screenings will not be enrolled.
[u] If not performed within the previous 30 days.
[v] Patients must have bi-dimensional measureable disease by radiographic imaging (MRI or CT scan).
[w] To be performed between Day 35 and 42.
[x] Not required if the assessment has been performed within 6 weeks.
[y] Not required if the assessment has been performed within 6 weeks.
[z] Subjects are required to have a biopsy at screening to determine the expression of COL6A3 and/or PRAME. As an optional procedure, subjects may provide extra tissue at the time of clinically-indicated biopsies (on treatment or at the time of progression).
[aa] To be transferred to Immatics for COL6A3 and PRAME expression determination.
[bb] Cyclophosphamide will be administered at 300 mg/m^2 intravenously 2 days prior to T cell infusion as an outpatient procedure.
[cc] Administer T cells at a dose of 10^{10} cells/m^2. T cells from in vitro cultures will be washed three times with RPMI-Hepes solution and re-suspended in 250-500 ml of 0.9% NaCl in preparation for infusion.
[dd] Low-dose IL-2 (250,000 U/m^2 s.c. q12h) will begin within 6 hours of T cell infusion and continue for a total of 14 days (28 doses).
[ee] Dose level 1 patients will receive no anti-CD137. Dose level 2 and 3 patients will receive intravenous anti-CD137 at 0.3mg/kg and 1.2 mg/kg, respectively, within 24 hours of T cell infusion.
[ff] 60 ml in tubes containing heparin (green top) and 5 ml in a tube containing Potassium Oxalate/Sodium Fluoride (gray top). See **Section 8.3**.

8. SCHEDULE OF EVALUATIONS

8.1. GENERAL TOXICITY ASSESSMENT

Vital signs (temperature, pulse, breathing, blood pressure), Physical exams, oxygen saturation, Comprehensive chemistry panel, Complete blood counts, differentials and platelet counts will be completed according to the schedule as outlined in Table 1. Of note, the dates listed on the study calendar are approximate as many patients reside out of the area and cannot always follow the time points as dictated by the protocol.

8.2. EFFICACY ASSESSMENT

8.2.1. CLINICAL RESPONSE

Radiographic imaging and clinical assessment of residual disease will be compared with pre-infusion baseline assessment according to modified RECIST 1.1 (see **Section 8.2.2**). A complete response (CR) will be defined as total regression of all tumor, a partial response (PR) as 30% or greater decrease in the sum of the longest diameter of target lesions and progressive disease (PD) as 20% increase in the sum of the longest diameter of target lesions (modified world health organization criteria or mWHO). This assessment will be performed at 6 and 12 weeks following T cell infusion and then every 8 weeks (± 1 week) until disease progression or intervening therapy. The overall response rate (OR) will be after 1 cycle (12) weeks.

8.2.2. IMMUNE RELATED RESPONSE EVALUATION

The response to immunotherapy may differ from the typical responses observed with cytotoxic chemotherapy including the following[62,63]:

- Response to immunotherapy may be delayed,
- Response to immunotherapy may occur after PD by conventional criteria,
- The appearance of new lesions may not represent PD with immunotherapy, and
- SD while on immunotherapy may be durable and represent clinical benefit.

Based on the above-described unique response to immunotherapy and based on guidelines from regulatory agencies, e.g., European Medicines Agency's "Guideline on the evaluation of anti-cancer medicinal products in man" (EMA/CHMP/205/95/Rev.4) for immune modulating anti-cancer compounds, this study will include the following in addition to standard RECIST 1.1 criteria:

RECIST will be modified so that PD must be confirmed with a second radiologic assessment, preferably at least 5 weeks (+/- 7 days) after the initial assessment of PD in the absence of clinically significant deterioration. Treatment with utomilumab should continue between the initial assessment of progression and confirmation of progression at outlined in the protocol.

Modification of RECIST as described is meant to discourage the early discontinuation of the study regimen and provide a more complete evaluation of its anti-tumor activity than would be seen with conventional response criteria. Nonetheless, the efficacy analysis will be conducted by programmatically deriving each efficacy endpoint based on the first evidence of progression (by modified RECIST 1.1 criteria described below) when progression is confirmed by a second scan.

Of note, clinically significant deterioration is considered to be a rapid tumor progression that necessitates treatment with anti-cancer therapy other than the study regimen or other symptomatic progression that requires urgent medical intervention (e.g., central nervous system metastasis, respiratory failure due to increasing malignant pleural effusion, or development of mechanical bowel obstruction due to carcinomatosis or tumor compression).

8.2.3. BEST OVERALL RESPONSE RATE (BORR)

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

8.2.4. DURATION OF RESPONSE

A patient's Duration of Response is defined as the time between the date measurement criteria are first met for subsequently-confirmed PR or CR (whichever status is recorded first) and the date of disease progression or death. Duration of Response will be calculated for all patients with CR or PR using the Kaplan-Meier product limit method. For those patients who remain alive and have not progressed, Duration of Response will be censored on the date of last tumor assessment.

8.2.5. PROGRESSION FREE SURVIVAL (PFS)

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

8.3. IMMUNE MONITORING

Patients will have 60 mL of heparinized blood (green top tube) drawn at the pre-T cell infusion visit, immediately prior to the $CD8^+$ T cell infusion, and on days +1, +3 and +7 and then weekly through week 6, then every 2 weeks through week 12, and then followed by every 4 weeks until the patient comes off the protocol.

Patients will also have 5 mL gray top tube drawn at the pre-infusion visit, immediately prior to the $CD8^+$ T cell infusion, and on day +1, +7, +14, +28, +42, +56, +84, +112, +140 and +168 after the T cell infusion.

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

Immune monitoring will be carried out in the Yee Laboratory, with the support of the Core Immune Monitoring Lab.

For the primary translational endpoint, the numeric and functional persistence of transferred CTL will be performed on peripheral blood obtained from patients prior to T cell infusion (baseline sample) on Days $^+1$, $^+3$, $^+7$, and weekly thereafter. To prevent inter-test bias, all samples will be cryo-preserved, and then

thawed and assayed simultaneously. The numeric frequency of transferred T cells will be determined using peptide MHC-tetramer analysis or specific CDR3 TCR quantitative PCR of transferred CTL if necessary. The function of transferred CTL will be determined by intracellular cytokine staining of tetramer$^+$ CD8$^+$ cells following in vitro stimulation as previously described.

8.4. TUMOR IMMUNOPHENOTYPING

Patients undergoing clinically indicated biopsies (on treatment or at the time of progression) will be asked to provide consent for optional additional biopsy cores for research purposes at the time of the clinically indicated biopsy. These biopsies will be used to assess the outgrowth of antigen or MHC-loss tumor variants.

In addition, when possible biopsies will be used to investigate localization of transferred antigen-specific CTL to tumor sites will be evaluated. Single cell suspensions will be prepared and stained to identify antigen-specific CTL by tetramer analysis. Tetramer positive cells will be sorted and analyzed for identity to the original infused CTL by PCR of a cell-specific CDR3 region of the T cell receptor. Where possible, these results will be compared with a pre-infusion tumor sample and the peripheral blood. A T cell frequency at least five-fold higher than that found in the peripheral blood or pre-infusion sample will be considered evidence of T cell localization/accumulation in disease sites.

9. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

9.1. DOSE LIMITING TOXICITY (DLT)

- Toxicities will be counted as a DLT only if deemed to be at least possibly related to the experiment therapy.
- Any ≥ Grade 3 bronchospasm or other hypersensitivity reaction;
- Any adverse event, laboratory abnormality or inter current illness which, in the judgment of the clinical team, presents a substantial clinical risk to the patient with continued dosing
- Any other ≥ Grade 3 non-skin related adverse event that does not return to baseline levels within 7 days, with the exception of events listed below in **Section 9.2**.

9.2. EXCEPTIONS

- Grade 1-2 Cytokine Release Syndrome (CRS) that, in the opinion of the investigators, is attributable to T cell infusions: Including but not limited to asthenia, flu-like symptoms, myalgia, lymphopenia, neutropenia that returns to a Grade 2 toxicity on 7 days from day of onset.
- Hospitalization for ≤ Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the Investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy.
  - Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy. Note: Utomilumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.
- Fever > 40C for less than 24 hours.
- WBC < 1000 for less than 7 days.

- Platelets < 50,000 for less than 7 days.
- ANC < 500 for less than 7 days.

9.3. CRITERIA FOR DISCONTINUATION OF THERAPY

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

9.4. TRIAL STOPPING CRITERIA

We will early stop the trial if at the lowest dose level (i.e., dose -1), (i) there is one or more death within 30 days after the investigational treatment at least possibly related to the study agent; or/and (ii) the number of patients experienced DLT reach the following stopping boundary, where the definition of DLT is provided in **Section 9.1**.

Table 2. Stopping Criteria

The number of patients treated at **dose level -1**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Stop the trial if # of DLT >=	NA	NA	2	3	3	4	4	4	5	5	6	6	6	7	7	8	8	8

9.5. MANAGEMENT OF SYMPTOMS DURING T CELL INFUSION

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

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

- Hypoxemia will initially be managed with supplemental oxygen and further measures as dictated by standard medical practice.
- Management of cytokine release syndrome (CRS) is an evolving field and hence will be conducted according to the most up to date institutional guidelines. Management will include transfer to ICU and use of Tocilizumab as clinically indicated. Patients who do not respond to Tocilizumab and other supportive measures will undergo ablation of adoptively transferred T cells as described in **Section 9.5**.

9.6. TOXICITIES WARRANTING ABLATION OF ADOPTIVELY TRANSFERRED T CELLS

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

Examples of such scenarios include:

- Hypotension (systolic BP <90 mmHg and > 20 mmHg below baseline), tachycardia (HR > 130), tachypnea (RR > 32) and/or hypoxemia (arterial O2 saturation < 90%), not responsive to supportive care.
- Grade 2 or greater allergic reaction, consisting of bronchospasm or generalized urticaria.
- Grade 3 or greater toxicity occurring in any other organ system following administration of antigen-specific cytotoxic T cells, and not attributable to a different cause. Exceptions to this are:
  - Fever of >40˚C which lasts less than 48 hours following infusions
  - Lymphopenia of Grade 3 that does not revert to Grade 2 within 96 hours
- Cytokine Release Syndrome (grade 3-4) unresponsive to Tocilizumab and other non-steroid interventions.

Patients will receive corticosteroids if treatment-related toxicity warranting ablation of T cells is observed and the following dose schedule is suggested however may be modified according to institutional guidelines:

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

10. REPORTING SERIOUS ADVERSE EVENTS

10.1. DEFINITION OF 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**:**

- 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.
- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- 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 WIRB.

Serious adverse events will be captured from the time of the first protocol-specific intervention until 30 days after the last study treatment/intervention, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

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

10.2. REPORTING TO THE FDA

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

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

All related grade 2 and all Grade 3 or higher adverse events, regardless of the attribution, will be captured and entered into the CRF. Unrelated grade 1 and grade 2 will not be entered into the CRF. Adverse events will be captured from the time of the first protocol-specific intervention until 30 days after the last dose of study drug, with the following exception: For all subjects (whether or not intervening therapy is used), the reporting window for AEs will start from the time of the first protocol-specific intervention to 7 days after protocol-directed apheresis or until the start of intervening therapy, whichever occurs first, except in the case of a Serious Adverse Event, which must be captured, regardless of grade. . Adverse events will also be captured from the time of the first protocol-specific intervention after the patient is enrolled in Turnstile 2 until 30 days after the last dose of study drug.

Table 3. Capturing Adverse Events

Grade and Attribution	Capture in CRF?
1 and Unrelated	No
1 and Related	No
2 and Unrelated	No
2 and Related	Yes
3 or higher and Unrelated	Yes
3 or higher and Related	Yes

All adverse events will be noted and graded according to the NCI CTCAE version 5.0. 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.

10.3. REPORTING TO PFIZER

Once patients receive utomilumab, the study staff will forward to Pfizer the AE or SAE reports at the same time they are sent to the FDA. These submissions to Pfizer will include the MD Anderson AE reporting form and the Pfizer "Reportable Event Fax Cover Sheet (US)." These reports should be sent to Pfizer via facsimile at 1-866-997-8322.

10.3.1. HY'S LAW

Cases of potential drug-induced liver injury as assessed by laboratory test values ("Hy's Law Cases") are also reportable to Pfizer. If a Study subject develops abnormal values in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or both, concurrent with abnormal elevations in total bilirubin and no other known cause of liver injury, that event would be classified as a Hy's Law Case. As such, the term SAE will be understood to also include Hy's Law Cases.

10.3.2. EXPOSURE OR LACK OF EFFECT

Even though there may not be an associated SAE, exposure to the Pfizer Product during pregnancy, exposure to the Pfizer Product during lactation, and occupational exposure to the Pfizer Product are reportable, and lack of effect of the Pfizer Product may also be reportable. As such, the term SAE will be understood to include exposure during pregnancy, exposure during lactation, occupational exposure, and reportable instances of lack of effect. Reports submitted to Pfizer should include the "Exposure During Pregnancy (EDP) Supplemental Form."

11. STATISTICAL CONSIDERATIONS

11.1. TRIAL DESIGN

We will employ the Bayesian optimal interval (BOIN) design (Liu and Yuan, 2015) to find the MTD. The BOIN design is implemented in a simple way similar to the traditional 3+3 design, but is more flexible and possesses superior operating characteristics that are comparable to those of the more complex model-based designs, such as the continual reassessment method (CRM).

The target toxicity rate for the MTD is 0.3 and the maximum sample size is 18. We will enroll and treat patients in cohorts of size 3. For the goal of determining the MTD, the dose-limiting toxicity (DLT) assessment periods are 6 weeks for T cells (i.e., dose 1 and dose -1) and 8 weeks for T cells + utomilumab (i.e., doses 2 and 3), respectively. The trial design is described as follows:

1. Patients in the first cohort are treated at dose level 1.
2. To assign a dose to the next cohort of patients, we conduct dose escalation/de-escalation according to the rule displayed in Table 4. When using Table 4, please note the following
    a. "Eliminate" means that we eliminate the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.
    b. When we eliminate a dose, we automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, we stop the trial for safety. In this case, no dose should be selected as the MTD.
    c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, we treat the new patients at the current dose.
    d. If the current dose is the lowest dose and the rule indicates dose de-escalation, we will treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point we will terminate the trial for safety.
    e. If the current dose is the highest dose and the rule indicates dose escalation, we will treat the new patients at the highest dose.
3. Repeat step 2 until the maximum sample size of 18 is reached or the trial is stopped.

Table 4. Dose escalation/de-escalation rule for the BOIN design

	The number of patients treated **at the current dose**																	
Actions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Escalate if # of DLT <=	0	0	0	0	1	1	1	1	2	2	2	2	3	3	3	3	4	4
De-escalate if # of DLT >=	1	1	2	2	2	3	3	3	4	4	4	5	5	6	6	6	7	7
Eliminate if # of DLT >=	NA	NA	3	3	4	4	5	5	5	6	6	7	7	8	8	8	9	9

To safeguard patients from the case that the lowest dose (i.e., dose -1) is overly toxic, we will early stop the trial if at the lowest dose level, (i) there is one or more death within 30 days after the investigational treatment at least possibly related to the study agent; or/and (ii) the number of patients experienced DLT reach the following stopping boundary, where the definition of DLT is provided in **Section 9.1**.

Table 5. Stopping rule for the BOIN design

	The number of patients treated at **dose level -1**																	
Action	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Stop the trial if # of DLT >=	NA	NA	2	3	3	4	4	4	5	5	6	6	6	7	7	8	8	8

After the trial is completed, we select the MTD based on isotonic regression as specified by Liu and Yuan [64]. Specifically, we select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, we select the higher dose level when the isotonic estimate is lower than the target toxicity rate; and we select the lower dose level when the isotonic estimate is greater than the target toxicity rate.

11.2. OPERATING CHARACTERISTICS

Table 6 shows the operating characteristics of the trial design based on 1000 simulations of the trial. The operating characteristics show that the design selects the true MTD with high probabilities and allocates more patients to the dose levels with the DLT rate closest to the target of 0.3.

Table 6. Operating Characteristics of the BOIN design

	Dose Level				Number of Patients	% Early Stopping
	-1	1	2	3		
Scenario 1						
True DLT rate	0.03	0.10	0.30	0.45		
Selection %	0.2	18.3	57.9	23.6		0.0
# Pts Treated	0.2	6.3	8.0	3.6	18.0	
Scenario 2						
True DLT rate	0.02	0.06	0.13	0.30		0.0
Selection %	0.0	1.1	27.2	71.7	18.0	

# Pts Treated	0.0	3.9	5.9	8.1		
Scenario_3						
True DLT rate	0.01	0.02	0.08	0.15		
Selection %	0.0	0.1	4.3	95.6		0.0
# Pts Treated	0.0	3.3	4.2	10.5	18.0	
Scenario_4						
True DLT rate	0.50	0.70	0.80	0.90		
Selection %	23.9	0.8	0.0	0.0		75.3
# Pts Treated	7.0	4.2	0.1	0.0	11.4	

11.3. ANALYSIS PLAN

The vivo persistence of adoptively transferred central memory-type CTL will be summarized using the Kaplan-Meier curve at each dose level. Summary statistics, including mean, standard deviation and 95% confidence interval, will be used to describe the response rate. Kaplan-Meier curve will be used to describe the PFS and duration of the response.

A toxicity summary will be submitted to the IND Office Medical Monitor and Safety Group, after the first 3 evaluable subjects complete 6 weeks of T-Cell only treatment, or 8 weeks of T-Cell plus Utomilumab combination treatment, and every 3 evaluable subjects thereafter. Reviewer's approval must be obtained prior to advancing/changing dose levels. An efficacy summary will be submitted to the IND Medical Monitor and Safety Group,after every six evaluable subjects have completed 12 weeks of treatment.

12. DATA ENTRY AND PROTOCOL MANAGEMENT

For the purposes of this study at M. D. Anderson Cancer Center, the Prometheus data management system will be employed. All patients will be registered in CORe before any study specific tests are performed.

13. ADMINISTRATIVE CONSIDERATIONS

13.1. PROTOCOL MONITORING

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

The principal investigator will monitor the data and toxicities to identify trends. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The 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 will submit toxicity data summary for each set of 3 evaluable patients enrolled to the IND Office Medical Monitor and the EDSMB.

13.2. CHANGES TO THE PROTOCOL

Any change or addition to this protocol requires a written protocol amendment that must be approved by the IND Office and the WIRB. A copy of the written approval of the WIRB must be received by the IND

Office and the principal investigator before implementation of any changes. The WIRB must review and approve all amendments to the protocol.

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