

*Abbreviated Title: Anti-mesothelin CAR PBL*

*Version Date: April 12, 2018*

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## **PROTOCOL TITLE**

A Phase I/II Study of Metastatic Cancer Using Lymphodepleting Conditioning Followed by Infusion of Anti-mesothelin Gene Engineered Lymphocytes

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### **Investigational Agents:**

Drug Name:	Anti-mesothelin CAR (coSS1scFv-CD28Z) transduced peripheral blood lymphocytes (PBL)
IND Number:	14989
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility

**Commercial Agents:** Cyclophosphamide, Fludarabine, and Aldesleukin

## PRÉCIS

### **Background:**

- We have constructed a single retroviral vector that contains a chimeric T cell receptor (CAR) that recognizes mesothelin, which can be used to mediate genetic transfer of this CAR with high efficiency (> 50%) without the need to perform any selection.
- In co-cultures with mesothelin expressing cells, anti-mesothelin transduced T cells secreted significant amounts of IFN- $\gamma$  (with high specificity).

### **Objectives:**

Primary objectives:

- To evaluate the safety of the administration of anti-mesothelin CAR engineered peripheral blood lymphocytes in patients receiving a non- myeloablative conditioning regimen, and aldesleukin.
- Determine if the administration anti-mesothelin CAR engineered peripheral blood lymphocytes and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic cancer.

### **Eligibility:**

Patients who are 18 years of age or older must have

- metastatic or unresectable cancer that expresses mesothelin;
- previously received and have been a non-responder to or recurred after standard care;
- Patients may not have contraindications for low dose aldesleukin administration.

### **Design:**

- PBMC obtained by leukapheresis will be cultured in order to stimulate T-cell growth.
- Transduction is initiated by exposure of approximately  $10^8$  to  $5 \times 10^8$  cells to retroviral vector supernatant containing the anti-mesothelin CAR.
- Patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed by intravenous infusion of ex vivo CAR gene-transduced PBMC plus low dose IV aldesleukin
- Patients will undergo complete evaluation of tumor with physical examination, CT of the chest, abdomen and pelvis and clinical laboratory evaluation four to six weeks after treatment. If the patient has SD or tumor shrinkage, repeat complete evaluations will be performed every 1-3 months. After the first year, patients continuing to respond will continue to be followed with this evaluation every 3-4 months until off study criteria are met.
- The study will be conducted using a Phase I/II optimal design. The protocol will proceed in a phase 1 dose escalation design. Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be entered into two cohorts based on histology: cohort 1 will include patients with mesothelioma, and cohort 2 will include patients with other types of cancer that express mesothelin.
- For each of the 2 strata evaluated, the study will be conducted using a phase II optimal design where initially 21 evaluable patients will be enrolled. For each of these two arms of

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the trial, if 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled but if 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled in that stratum.

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## 1 INTRODUCTION

### 1.1 STUDY OBJECTIVES:

#### 1.1.1 Primary objectives:

- To evaluate the safety of the administration of anti-mesothelin CAR engineered peripheral blood lymphocytes in patients receiving the non- myeloablative conditioning regimen, and aldesleukin
- Determine if the administration of anti-mesothelin CAR engineered peripheral blood lymphocytes and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen will result in clinical tumor regression in patients with metastatic or unresectable cancer that expresses mesothelin.

#### 1.1.2 Secondary objective:

- Determine the in vivo survival of CAR gene-engineered cells.

### 1.2 BACKGROUND AND RATIONALE:

Studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments, the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T cell immune reactions against tumor-associated antigens.

In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules and it has been shown that tumor antigens are presented and recognized by T cells in this fashion. The molecule that recognizes these peptide fragments is the T-cell receptor (TCR). The TCR is analogous to the antibody immunoglobulin molecule in that, two separate proteins (the TCR alpha and beta chains) are brought together to form the functional TCR molecule. An alternate approach to enable T cells to recognize targets is to utilize a chimeric T cell receptor which is constructed by using the variable regions of the heavy and light chains of an antibody connected by a linker sequence and fused to CD28, CD3 zeta and 41BB signaling chains. Thus, the T cell can recognize and signal based on the non-MHC restricted recognition of the target. The goal of this protocol is to transfer genes encoding a chimeric receptor that recognizes mesothelin into normal peripheral blood lymphocytes (PBL) derived from cancer patients and to return these engineered cells to patients aimed at mediating regression of their tumors. This trial is similar to previous Surgery Branch TCR gene transfer adoptive immunotherapy protocols except that we will use a receptor that recognizes targets based on the recognition of an antibody reactive with the mesothelin molecule. This has the potential to treat patients with common epithelial malignancies of multiple histologies.

### 1.2.1 Prior Surgery Branch Trials of Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes in Patients with Metastatic Melanoma

Studies in the Surgery Branch, National Cancer Institute, identified genes that encode melanoma tumor associated antigens (TAA) recognized by tumor infiltrating lymphocytes (TIL) in the context of multiple MHC class I molecules<sup>1</sup>. These TAA appeared to be clinically relevant antigens responsible for mediating tumor regression in patients with advanced melanoma since the TIL used to identify these antigens were often capable of mediating in vivo anti-tumor regression. Two antigens, which were present in virtually all fresh and cultured melanomas, were called MART-1 (Melanoma Antigen Recognized by T Cells - 1), and gp100. The genes encoding these two antigens have been cloned and sequenced. The MART-1 gene encodes a 118 amino acid protein of 13 kd. The gp100 gene encodes a protein identical to that recognized by monoclonal antibody HMB-45. These antigens were thus the original targets of our gene therapy cell transfer protocols in patients with metastatic melanoma.

In the great majority of murine models demonstrating the therapeutic effectiveness of the adoptive transfer of lymphocytes mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required. North and colleagues demonstrated that adoptive transfer of lymphocytes was not effective unless the mouse was immunosuppressed by total body irradiation or chemotherapy prior to adoptive transfer of lymphocytes<sup>2</sup>. Very similar observations were made in other rodent cancer models<sup>3</sup>. We thus incorporated a lymphodepleting chemotherapy into our human cell transfer studies.

In the Surgery Branch, NCI, we developed a protocol to rapidly expand heterogeneous TILs for adoptive transfer<sup>4</sup>. TILs were expanded using the rapid expansion protocol (REP) in the presence of OKT3, irradiated allogeneic feeder cells and IL-2. These REPed TILs retained highly specific in vitro anti-tumor activity, often contained reactivities against several antigenic epitopes and contained both CD8+ and CD4+ lymphocytes. These autologous bulk TIL were re-infused to patients following a nonmyeloablative chemotherapy with cyclophosphamide and fludarabine. These patients subsequently received high-dose IL-2 and some received peptide immunization when the TIL reactivity was against known MART-1 and gp100 peptides (protocol 99-C-0158). A total of 43 patients received this treatment<sup>5,6</sup>. This regimen resulted in objective cancer regressions in 49% of patients (21 of 43) with metastatic melanoma (**Table 1**).

Murine models predicted that increasing the extent of lymphodepletion could increase the effectiveness of the cell transfer therapy<sup>7</sup>. Thus, we performed two additional sequential trials of ACT with autologous anti-tumor lymphocytes (TIL) in patients with metastatic melanoma. Increasing intensity of host preparative lymphodepletion consisting of cyclophosphamide and fludarabine with either 200cGy (25 patients) or 1200 cGy (25 patients) total body irradiation (TBI) was administered prior to cell transfer. While non-myeloablative chemotherapy alone showed an objective response rate of 49%, when 200cGy or 1200cGy TBI was added the response rates were 52% and 72% respectively (**Table 1**). TBI appeared to result in increased patient survival. Responses were seen in all visceral sites including brain. Host lymphodepletion was associated with increased serum levels of the lymphocyte homeostatic

cytokines IL-7 and IL-15<sup>7</sup>. Objective responses were correlated with the telomere length of the transferred cells<sup>8</sup>.

Patients exhibited the expected hematological toxicities associated with the cyclophosphamide, fludarabine and TBI preparative regimens. Other toxicities are shown in **Table 2**. Patients recovered marrow function rapidly after cell infusion with absolute neutrophil counts greater than 500 per mm<sup>3</sup> by day 12 and sustained platelet counts above 20,000 per mm<sup>3</sup> by day 14 (except 4 patients on the TBI 1200 protocol with platelet recovery on days 16, 17, 20, and 22).

### 1.2.2 Surgery Branch Trials of Cell Transfer Therapy Using Transduction of Anti-TAA TCR Genes into Non-reactive TIL or PBL

It is often not possible to isolate sufficient tumor samples from melanoma patients and even when tumor is available, only about 60 to 70% generate melanoma reactive TIL cultures. As a potential alternative to the requirement to establish TIL cultures from melanoma patients, we sought methods that could be used to easily obtain a polyclonal population of T cells with anti-TAA properties. Transfer of antigen specific TCR genes to PBL is a potential method generating large numbers of reactive anti-cancer T cells<sup>9</sup>. In a murine model of this approach, TCR gene transfer into murine splenocyte T-cells was performed using a retroviral vector<sup>10</sup>. The engineered T cells were shown to expand in vivo upon viral challenge and efficiently homed to effector sites. In addition, small numbers of TCR-transduced T cells promoted the rejection of antigen-expressing tumors in the mice. Retroviral vector mediated gene transfer can be used to engineer human T cells with high efficiency. In published work, the Surgery Branch was among the first to demonstrate that retroviral vector-mediated transfer of TCR genes could endow human PBL with anti-tumor reactivity<sup>11,12</sup>.

To expand on and potentially improve on these early results, we next isolated TCR genes from both gp100 and MART-1 reactive T cells. These studies have been published and are presented briefly here<sup>11,12</sup>. To test the in vivo efficacy of these MART-1 TCR engineered T cells, 31 HLA-A\*0201 patients with progressive metastatic melanoma were treated. Results in the first 17 patients were published in 2006<sup>13</sup>. All patients were refractory to prior therapy with IL-2. T cell cultures from all patients were biologically reactive, with specific secretion of interferon- $\gamma$  following co-culture with either MART-1 peptide pulsed T2 cells and/or melanoma cell lines expressing the MART-1 antigen. Gene transfer efficiencies measured by staining for V $\beta$ 12 expression in these lymphocytes ranged from 17% to 67% (mean value 42%). Four of the 31 patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria.

There were no toxicities in any patient attributed to the gene-marked cells. We thus demonstrated for the first time in humans, that normal autologous T lymphocytes, transduced *ex vivo* with anti-TAA TCR genes and reinfused in cancer patients can persist and express the transgene long-term *in vivo* and mediate the durable regression of large established tumors.

A similar study was conducted using gp100 TCR gene marked cells, however this retroviral vector had a low titer when produced under GMP conditions. Fourteen patients were treated

on this study. No antitumor responses have been seen. There have been no grade 5 toxicities observed on this study, and all grade 3 and 4 toxicities observed were expected toxicities associated with the non-myeloablative chemotherapy regimen or IL-2.

The low response rate in our prior MART-1 TCR gene transfer protocol led us to identify MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial<sup>14,15</sup>. We treated 24 patients with metastatic melanoma using autologous PBL transduced with an improved MART-1 F5 TCR following a non-myeloablative chemotherapy. Six patients (25%) have achieved an objective partial response. Toxicities were similar to those seen in the prior TCR gene therapy trial except that 15 patients developed a transient mild anterior uveitis easily reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. Transient rashes have also been seen. There were no treatment related deaths.

We have also conducted a clinical trial with a TCR that recognizes the gp100:154-162 melanoma peptide. This TCR was raised in an HLA-A2 transgenic mouse immunized with this peptide<sup>15</sup>. We have now treated 21 patients with metastatic melanoma using autologous PBL transduced with this improved gp100 TCR following a non-myeloablative chemotherapy. Four patients (19%), have achieved an objective partial response. Seven patients developed a transient mild anterior uveitis reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. There were no treatment related deaths.

In addition to the studies listed above in patients with metastatic melanoma, we have recently initiated several studies for patients with other metastatic cancers using PBL transduced with TCR genes or chimeric antigen receptor (CAR) genes. We have studied PBL transduced with TCR genes targeting p53, CEA, NY-ESO-1, TRAIL bound to the DR4 Receptor, and MAGE A3/12 and PBL transduced with CAR targeting Her-2, CD19 and VEGFR2. Patients on these studies also received a non-myeloablative chemotherapy regimen consisting of cyclophosphamide and fludarabine, and high dose IL-2. In two studies, 08-C-0121 (anti-ESO-1 TCR) and 09-C-0082 (anti-CD19 CAR), we have seen impressive clinical responses. In 08-C-0121, in patients with melanoma, we have observed two confirmed complete responses and three confirmed partial responses in the twelve evaluable patients who did not receive vaccination with the ALVAC vaccine, and one confirmed complete response and one confirmed partial response were observed in the 6 evaluable patients that received the vaccine. In the eight evaluable patients with other cancers, there have been 5 confirmed partial responses out of the 7 treated patients with synovial sarcoma who did not receive vaccination with the ALVAC vaccine, and there have been 3 confirmed partial responses in the 5 evaluable patients who received the vaccine. Therefore, to date the response rate for this study is 50%. In study 09-C-0082, eight patients have been treated and are evaluable on this study including six patients with CLL, one patient with follicular lymphoma, and one patient with splenic marginal zone lymphoma. We have observed a clinical response rate of 75%, which includes 5 patients with a partial response (PR) and 1 patient with a complete response (CR) to treatment. Toxicity information regarding TCR and CAR transduced cell therapy studies conducted in the Surgery Branch is included in Section **1.2.6** below.

### 1.2.3 Mesothelin as a Target for Cell Transfer Studies

As discussed above, adoptive cell transfer (ACT) immunotherapy strategies designed to directly target antigens expressed on tumor cells can result in durable objective regression in patients with metastatic melanoma. Despite its demonstrated effectiveness in experimental mouse tumor models and in the human, the application of ACT is limited because of the need to identify antigens with highly selective expression in cancer. Mesothelin as a target for ACT can potentially have wide application for the treatment mesothelin-positive cancer histologies including, but not limited to, mesothelioma, ovarian and pancreatic cancer. Redirecting immune cells to target mesothelin offers an alternative to overcome the obstacles confronting conventional palliative surgery and chemotherapy as well as current tumor-specific immunotherapeutic approaches.

Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in mesothelioma, ovarian, pancreatic and some other cancers<sup>16-18</sup>. Small amounts of mesothelin shed into the circulation may be detected by sandwich ELISA. It has been suggested that elevated soluble mesothelin levels may correlate with advanced disease stage and total tumor burden. The biological function of mesothelin is unknown. A trans-intracellular binding activity with CA125, a tumor antigen routinely used in the diagnosis and monitoring of ovarian cancer has been noted<sup>19</sup>. Among normal human tissues mesothelin is expressed on mesothelial cells of the pleura, pericardium, and peritoneum, as well as the basal cells of the trachea, tonsil epithelium, and cells in the Fallopian tubes but is absent from vital organs including heart, liver, lung, kidney and nervous tissue<sup>20,21</sup>. There is limited evidence that mesothelin is also expressed to some extent on the corneal epithelium<sup>22</sup>.

Initial immunotherapy efforts to target mesothelin on epithelial cancer cells involved the use of an immunotoxin, SS1P. SS1P is a high affinity anti-mesothelin disulfide-stabilized murine-antibody Fv genetically combined with PE38<sup>23</sup>. PE38 is a fragment of the potently cytotoxic *Pseudomonas* exotoxin from which the native cell-binding domain and other unnecessary sequences have been removed. The chimeric recombinant immunotoxin, SS1(dsFv)PE38 (referred to as SS1P), kills mesothelin-expressing cells but not similar cells that do not express detectable levels of mesothelin. As an alternate approach to an immunotoxin, a chimeric antibody has been developed. MORab-009 is a chimeric IgG/κ antibody in which the murine SS1single chain variable fragment (scFv) is fused in frame with the human IgG1 and kappa constant regions. This monoclonal antibody has been shown *in vitro* to elicit antibody-dependent cellular cytotoxicity (ADCC) and inhibit tumor growth in a mouse xenograft model<sup>24</sup>.

In order to extend the current immunotherapy approaches, we have developed a chimeric T cell receptor (CAR) utilizing SS1scFv fused in frame to the intracellular signaling chains of a conventional T cell receptor. This SS1 CAR, when transduced into lymphocytes, enables the lymphocyte to recognize mesothelin-positive targets based on the antigen recognition of the antibody scFv. This CAR combined with the antitumor potency of ACT suggested to us that the transduction of a chimeric receptor recognizing mesothelin into lymphocytes could produce self-replicating T cells capable of selectively destroying mesothelin-expressing tumors.

Cancer histologies such as lung, pancreatic, ovarian and esophageal are some of the leading causes of cancer deaths among both men and women today. These cancer histologies, in addition to pleural mesothelioma, have also been shown to express mesothelin. The incidence of mesothelin-positive tumors has been determined by RT-PCR and/or immunohistochemistry (IHC). In many studies, all tumors from all patients with mesothelioma and pancreatic cancer express mesothelin (**Table 3**). Thus, in all clinical studies targeting mesothelin at the NCI (R. Hassan and I. Pastan) no confirmation of mesothelin expression is necessary for patients with mesothelioma or pancreatic cancer. IHC evidence of mesothelin expression will be required for patients with other cancer types. It has been shown that 10/12 (83%) lung adenocarcinomas were positive for mesothelin based on IHC data. Additionally, in the same study, 7/9 (78%) of NCI-60 cell lines evaluated were also positive<sup>25</sup>. Similarly, Chang *et al.* (1992) demonstrated by IHC that 15/15 (100%) of pleural mesotheliomas, 0/4 sarcomatous mesotheliomas, 10/15 (66%) of nonmucinous ovarian cancers and 0/4 mucinous ovarian cancers stained positive for mesothelin<sup>20,26</sup>. In addition, it was determined by RT-PCR and IHC that 4/4 (100%) and 60/60 (100%), respectively, of primary pancreatic adenocarcinomas expressed mesothelin<sup>27,28</sup>. While the incidence is much lower, mesothelin has been shown to be expressed on a variety of other cancer histologies including esophageal, cervical and head and neck<sup>27</sup>. Based on the data presented herein, mesothelin can have wide application as an immunotherapy target.

#### 1.2.4 Preclinical Studies to Provide the Rationale for this Clinical Protocol

SS1P has been used in two Phase I clinical trials. In the first trial, the SS1P immunotoxin was administered as an I.V. bolus. The primary objectives of the trial were to determine the safety, toxicity, maximum tolerated dose (MTD) and pharmacokinetics of SS1P with secondary objectives of evaluating effects on tumor growth and disease progression<sup>29</sup>. In terms of the clinical response, of the 33 evaluable patients, 4 had minor responses, 19 patients had stable disease and 10 patients had progressive disease. The dose limiting toxicity (DLT) was grade 3 pleuritis in 3/33 patients<sup>29</sup>. In a second Phase I trial, the administration of the drug was changed from I.V. bolus to continuous infusion. Of the 24 patients treated, 1 patient had a partial response. Five patients experienced pleuritic chest pain<sup>30</sup>.

Alternatively, the chimeric anti-mesothelin monoclonal antibody (MORab-009) was evaluated in a Phase I clinical trial<sup>31</sup>. MORab-009 was determined to be well tolerated with 11/24 patients treated exhibiting stable disease. It was reported that 2 patients experienced grade 4 transaminitis and grade 3 serum sickness. No other grade 3/4 toxicities were reported. MORab-009 is now being evaluated in a multi-center Phase II clinical trial.

Previously, Carpenito *et. al.* (2009) cloned the murine SS1 CAR directed against mesothelin into a lentiviral vector<sup>32</sup>. The authors demonstrated that coupling the SS1 scFv to human CD28 and CD3 $\zeta$  (CD28Z) or CD28, 4-1BB and CD3 $\zeta$  (CD28BBZ) resulted in greater than 80% CAR $^+$  lymphocytes that specifically recognized mesothelin-positive tumor targets. When tested in a mouse xenograft model, both constructs were able to effectively treat large established mesothelin-positive tumors when CAR $^+$  lymphocytes were administered

intravenously<sup>32</sup>. Subsequently, this same group was able to demonstrate similar tumor treatment efficacy using RNA-electroporated lymphocytes in the same xenograft model<sup>33</sup>.

Based on the previously published data, we generated a MSGV-based recombinant retroviral vector encoding a second-generation CAR comprised of the codon-optimized anti-human SS1 single chain variable regions (ScFv) linked to the intracellular human T cell signaling sequences derived from CD28 and the CD3 $\zeta$  chain of the T cell receptor via the human CD8 $\alpha$  hinge and transmembrane regions (referred to as coSS1scFv-CD28Z). The recombinant retroviral construct is described schematically in **Figure 1A**. Surface expression of the retrovirally encoded transgene product in transduced CD3 $^+$  primary human T cells was determined by flow cytometry. As shown in **Figure 1B**, the coSS1scFv CAR-expressing vector efficiently transduced OKT3-activated human peripheral blood lymphocytes (PBL, range 63-82% after correcting for background staining). When co-cultured with mesothelin-positive tumor cell lines, the CAR-transduced PBL showed mesothelin-specific recognition of the target cells as measured by gamma interferon release.

#### 1.2.5 Construction and testing of retroviral vectors expressing CAR against human mesothelin

The ability of the T cell based anti-mesothelin targeting strategy to treat established mesothelin-positive tumors in a mouse xenograft model led us to explore the translation of these preclinical findings to the treatment of human tumors<sup>32,33</sup>. We constructed a recombinant retroviral vector expressing CAR against human mesothelin using the sequences from the SS1 scFv<sup>34</sup>.

The anti-human mesothelin CAR vector termed coSS1scFv-CD28Z was comprised of a scFv derived from a murine antibody fragment, SS1, linked to the hinge and transmembrane sequences from the human CD8 $\alpha$ , which was in turn fused to the intracellular sequences derived from human CD28 and the CD3 $\zeta$  chain of TCR (**Figure 1A**).

The functional integrity of the SS1 CAR-encoding retroviral vector was tested *in vitro* in several assays. Supernatants from gibbon ape leukemia virus-pseudotyped high titer virus producer cells were used to transduce OKT3 activated human PBLs from 3 different donors. The SS1 CAR-encoding retroviral vector transduced human PBLs at a high frequency resulting in 63 to 82% of the CD3 $^+$  T cells expressing the CAR on the cell surface (**Figure 1B**). Next, we tested the ability of SS1 CAR-modified T cells to recognize mesothelin positive human cancer cells as measured by specific IFN- $\gamma$  secretion. coSS1scFv-CD28Z CAR-transduced PBLs from 3 different donors, 8 days post transduction were cocultured for 24 hours with mesothelin-negative cells (A431) or mesothelin-positive (A431-H9, a stable transfectant expressing mesothelin; OVCAR3 and Panc10.05, **Figure 1C**). The surface expression on mesothelin-positive and negative cancer cell lines was determined by flow cytometry using the MN antibody for mesothelin detection (**Figure 2**). CAR-transduced PBL were subjected to a secondary or rapid expansion (REP) by exposure to soluble OKT3 antibody, high-dose Interleukin 2 (IL-2) and irradiated feeders. Following a REP, the average cell expansion from the 3 patient PBL tested was approximately 180-fold (**Figure 3A**). In addition, the CAR-

transduced cells maintained both CAR surface expression (**Figure 3B**) and specific recognition of mesothelin-positive tumor targets (**Figure 3C**).

Thus based on the previously published preclinical studies, which provide the rationale for the translation of SS1 CAR-transduced cells for the treatment of human cancer and data from the experiments presented herein, we selected the coSS1scFv-CD28Z vector for use in our human clinical protocol. A cGMP master cell bank was manufactured and used for the production of cGMP Gammaretroviral vector supernatant. Each of the vector harvests was titered on human PBL (**Table 4**) and then subsequently tested for specific recognition of mesothelin-positive tumor targets as measured by specific IFN $\gamma$  release (**Table 5**). All cGMP reagents were subjected to and passed all biosafety testing as stipulated by the FDA guidance documents.

### 1.2.6 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose IL-2 have expected toxicities discussed earlier. The non-myeloablative chemotherapy used in this protocol has been administered to over 150 patients and all have reconstituted their hematopoietic systems.

CAR transduced human T cells have been administered to humans in several clinical trials. We treated 14 patients with metastatic ovarian cancer utilizing autologous T cells transduced with a retroviral vector encoding a single chain antibody that recognized the ovarian cancer-associated antigen alpha-folate receptor. The single chain antibody was linked to the signaling domain of the Fc receptor gamma chain. These 14 patients received up to  $1.7 \times 10^{11}$  cells intravenously and except for mild malaise and fevers there were no toxicities attributed to the cell administration. Lamers et al. reported on the treatment of three patients who received autologous T cells transduced with a retrovirus encoding an antibody against the carbonic anhydrase IX molecule expressed on renal cell cancers<sup>35</sup>. Three patients were treated and mild transaminase elevations precluded the treatment of additional patients. This was probably due to the expression of carbonic anhydrase IX expression on biliary ductal epithelium.

Pule, et al. engineered human T cells to express a CAR directed to the diasialoganglioside GD2, a tumor associated antigen expressed by human neuroblastoma cells<sup>36</sup>. These CAR transduced cells were administered to eleven children between the ages of 3 and 10, all of whom had metastatic neuroblastoma. There were no adverse effects attributable to the genetically modified CAR T cells in the eleven subjects that were followed for up to two years following cell infusion. Two patients exhibited an objective response and several additional patients exhibited necrosis in established tumors. Persistence of the transduced cells was greater when the CAR were inserted into EBV specific cytolytic T lymphocytes than when the CAR were inserted into T cells that were activated by an anti-CD3 monoclonal antibody.

Till et al. reported the adoptive immunotherapy of patients with indolent non-Hodgkin's lymphomas and mantle cell lymphoma using specific T cells transfected with CAR recognizing the CD20 molecule<sup>37</sup>. Seven patients were treated and one achieved a partial response. These

cells were administered in conjunction with low dose subcutaneous IL-2. There were no toxicities associated with the cell transfer. Brentjens et al. recently reported on the administration of autologous T cells transduced with a CAR recognizing the CD19 molecule in patients with chronic lymphocytic leukemia<sup>38</sup>. Three patients were treated with modified T cells alone without dose limiting toxicities. However, the first patient who was enrolled in a second cohort of T cell administration following a cyclophosphamide lymphodepleting regimen developed a syndrome of fever and hypotension and died four days later. Following extensive analysis of this patient it was concluded that the adverse event may have been due to an idiosyncratic reaction to the combination of the cyclophosphamide and cell administration.

Most toxicities observed in these studies were expected toxicities of the chemotherapy and aldesleukin administration. However, in several studies, we have observed serious adverse events related to the transduced cells. In 08-C-0121 (anti-ESO-1 TCR transduced PBL), one patient experienced grade 3 dyspnea and grade 4 hypoxia resulting in intubation. These events were attributed to the cells, and aldesleukin as well as the extensive pulmonary disease in this patient. In 09-C-0041 (anti-Her2 CAR transduced PBL), the first patient, with metastatic colorectal cancer, was treated with  $10^{10}$  autologous T cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later. This toxicity was apparently due to a previously unrecognized ability of this CAR to recognize Her-2 expressed on lung epithelial cells although the exact explanation for the toxicity is not clear<sup>39</sup>. In 09-C-0047 (anti-CEA TCR transduced PBL), all three patients treated experienced a variety of gastrointestinal events which were attributed to the gene/cell therapy including diarrhea, and colitis<sup>21</sup>. All gastrointestinal events have since resolved in these patients, and the patients' colonic mucosa has returned to normal, and the patients have normal bowel function. Grade 3 diarrhea lasting longer than 72 hours is considered a DLT per protocol and this event was observed in two of three patients enrolled in protocol 09-C-0047, meeting the criteria for stopping protocol accrual. One patient treated on this study experienced a partial response. In 09-C-0082 (anti-CD19 CAR transduced PBL) SAEs related to the cell therapy include dose-limiting toxicities (DLT) of grade 4 somnolence in two patients, grade 3 creatinine in one patient and grade 3 confusion (aphasia) and grade 3 neuropathy (right facial droop) in another patient. In 11-C-0062 (anti-MAGE A3/12 TCR transduced PBL), out of the nine patients treated, four patients were intubated, and three of these intubations were the result of toxicities attributed to the cells. One of these patients experienced hypoxia, seizures, and encephalopathy resulting in confusion, dysarthria, ataxia, short-term memory loss, non-fluent aphasia, and action myoclonus in the face and limbs. This patient recovered from these toxicities and was discharged. Two other patients had somnolence lasting 3 and 5 months, respectively, before dying from the effects of the treatment. Both patients have had brain biopsies which indicated significant leukomalacia, sparing the cortex. This IND has been placed on clinical hold by the FDA while we investigate these SAEs. In 11-C-0013 (anti-VEGFR2 CAR, two patients experienced elevated liver function tests which were attributed to the cell therapy. One of these patients also had an elevated EBV level, increased triglycerides, and bilirubin, hypoxia and hypotension. After consultation with Medical Oncology, this patient was treated with rituximab and prednisone as it was suspected that he had hemophagocytic syndrome or lymphoma. This patient experienced worsening mental status and was intubated. Shortly thereafter, this patient died of respiratory failure. In our most recent study, 12-C-0006 (IL-12/anti-ESO-1 TCR transduced PBL), the

first patient received  $1 \times 10^7$  transduced cells and did well for the first two weeks following cell administration. However, she then began to experience shortness of breath, elevated liver enzymes, hypotension and acute renal failure. She was transferred to the ICU and was intubated and underwent CVVH. Also at this time, skin breakdown was observed, and serum cytokine levels, interferon-gamma and IL-12, were extremely elevated. The patient received a total of 5 doses of alemtuzumab, methylprednisolone, and a single dose of ustekinumab to decrease levels of interferon gamma and IL-12. She also experienced a gastric bleed and extensive lung infiltration. The patient's condition continued to deteriorate and she expired approximately one month after receiving the cells.

In other protocols we have administered over  $3 \times 10^{11}$  TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. As discussed above, the expansion of tumor reactive cells is a desirable outcome following the infusion of antigen reactive T-cells. Some patients receiving gp100 or MART-1 reactive cells have developed vitiligo or uveitis probably due to destruction of normal melanocytes though these toxicities have been manageable. We do not believe the transfer of these gene modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

## **2 ELIGIBILITY ASSESSMENT AND ENROLLMENT**

### **2.1 ELIGIBILITY CRITERIA**

#### **2.1.1 Inclusion Criteria**

- a. Metastatic or unresectable measurable cancers that express mesothelin. As in other protocols conducted by Dr. Hassan in the NCI, epithelial mesotheliomas and pancreatic cancers do not need to be assessed for mesothelin expression since all of these tumors have been shown to express mesothelin. Other metastatic or unresectable cancers must be shown to express mesothelin as assessed by RT-PCR or immunohistochemistry on tumor tissue. Bi-phasic mesotheliomas must express mesothelin on greater than 50% of the cells in the epithelial component. Diagnosis will be confirmed by the Laboratory of Pathology, NCI.
- b. Patients must have previously received at least one systemic standard care (or effective salvage chemotherapy regimens) for metastatic or unresectable disease, if known to be effective for that disease, and have been either non-responders (progressive disease) or have recurred.
- c. Greater than or equal to 18 years of age and less than or equal to 70 years of age.
- d. Willing to sign a durable power of attorney
- e. Able to understand and sign the Informed Consent Document

- f. Clinical performance status of ECOG 0 or 1.
- g. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after treatment.
- h. Serology:
  - o Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)
  - o Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.
- i. Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.
- j. Hematology:
  - o Absolute neutrophil count greater than 1000/mm<sup>3</sup> without the support of filgrastim.
  - o WBC (> 3000/mm<sup>3</sup>).
  - o Platelet count greater than 100,000/mm<sup>3</sup>.
  - o Hemoglobin greater than 8.0 g/dl.
- k. Chemistry:
  - o Serum ALT/AST less or equal to 2.5 times the upper limit of normal.
  - o Serum creatinine less than or equal to 1.6 mg/dl.
  - o Total bilirubin less than or equal to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.
- l. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).  
**Note:** Patients may have undergone minor surgical procedures within the past 3 weeks, as long as all toxicities have recovered to grade 1 or less.
- m. Subject's must be co-enrolled in protocol 03-C-0277.

### 2.1.2 Exclusion Criteria

- a. Patients with sarcomatoid mesothelioma as mesothelin is not expressed in this type of mesothelioma.
- b. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.
- c. Patients with known brain metastases.
- d. Patients receiving full dose anticoagulative therapy.
- e. Active systemic infections (e.g.: requiring anti-infective treatment), coagulation disorders or any other major medical illnesses.
- f. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- g. Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

- h. Patients with diabetic retinopathy.
- i. Concurrent Systemic steroid therapy.
- j. History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- k. History of coronary revascularization or ischemic symptoms.
- l. Documented LVEF of less than or equal to 45% tested in patients with:
  - Clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block, chest pain, or ischemic heart disease
  - Age  $\geq$  65 years old
- m. Documented FEV1 less than or equal to 60% predicted tested in patients with:
  - A prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years).
  - Symptoms of respiratory dysfunction
- n. Patients who are receiving any other investigational agents.

## 2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under our companion protocol, 99-C-0128.

### 2.2.1 Within 3 months of prior enrollment

- a) HIV antibody titer and HBsAg determination, anti HCV
- b) Anti CMV antibody titer, HSV serology, and EBV panel, (Note: patients who are known to be positive do not need to be retested)
- c) Immunohistochemistry or PCR of tumor tissue for expression of mesothelin (testing is permitted to be conducted at any time prior to this point).
- d) Confirmation of diagnosis of metastatic cancer by the Laboratory of Pathology of the NCI. (Note: Testing is permitted to be conducted at any time prior to enrollment.)

### 2.2.2 Within 8 weeks prior to enrollment

- a) Pulmonary Function Testing will be conducted in all patients.
- b) Cardiac Evaluation (stress thallium, echocardiogram, MUGA, etc.) for patients who are greater than or equal to age 60, or who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart block. Patients with a LVEF of less than or equal to 45% will not be eligible. Patients under the age of 60 who have cardiac risk factors may also undergo cardiac evaluation as noted above (e.g., diabetes, hypertension, and obesity).

### 2.2.3 Within 4 weeks prior to enrollment:

- a. Complete history and physical examination, including weight and vital signs, and noting any organ system involvement and any allergies/sensitivities to antibiotics. (Note: patient history may be obtained within 8 weeks)

- b. Baseline imaging to determine the status of disease. This may include CT, MRI, PET, or Photography.

**2.2.4 Within 14 days prior to enrollment:**

- a) Screening blood test
  - Chemistry: Creatinine, ALT/GPT, AST/GOT, and Total Bilirubin
  - CBC with differential and platelet count
  - Coagulation profile including INR, PT/APTT, and fibrinogen.
- b) Urinalysis and culture, if indicated

**2.2.5 Within 7 days prior to enrollment:**

- a.  $\beta$ -HCG pregnancy (serum or urine) test on all women of child-bearing potential
- b. ECOG performance status of 0 or 1

**2.3 PROTOCOL REGISTRATION**

**2.3.1 Prior to registration for this protocol:**

Patients will initially be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) for apheresis to obtain PBL prior to transduction of PBL cells, by the clinical fellow or research nurse.

Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (CoA), patients will sign the consent document for this protocol.

**2.3.2 Registration Procedure:**

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

**3 STUDY IMPLEMENTATION**

**3.1 STUDY DESIGN**

**3.1.1 Treatment Phase:**

Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation.

### 3.1.1.1 Performed on 03-C-0277

PBL will be grown, transduced with the anti-mesothelin CAR genes and expanded for this trial according to standard operating procedures submitted in the IND. PBMC will be obtained by leukapheresis (approximately  $1 \times 10^{10}$  cells). PBL will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately  $1 \times 10^7$  to  $5 \times 10^8$  cells to supernatant containing the anti-mesothelin CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful CAR gene transfer will be determined by FACS analysis for the CAR protein and anti-mesothelin reactivity will be tested by cytokine release as measured on transfected cells. Successful CAR gene transfer for each transduced PBL population will be defined as >30% CAR positive cells and for biological activity, gamma-interferon secretion must be at least 200pg/ml, and greater than twice background.

### 3.1.2 Phase 1 - Dose Escalation:

Initially, the protocol will enroll 3 patients in each dose cohort unless a patient experiences a dose limiting toxicity (DLT). If dosing is escalated to cohort 10, 6 patients will be accrued to this cohort in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion.

The total number of anti-mesothelin engineered cells transferred for each cohort will be:

- Cohort 1       $10^6$  cells
  - De-escalation cohorts:
    - Cohort -1:  $3 \times 10^5$  cells
    - Cohort -2:  $10^5$  cells
- Cohort 2       $3 \times 10^6$  cells
- Cohort 3       $10^7$  cells
- Cohort 4       $3 \times 10^7$  cells
- Cohort 5       $10^8$  cells
- Cohort 6       $3 \times 10^8$  cells
- Cohort 7       $10^9$  cells
- Cohort 8       $3 \times 10^9$  cells
- Cohort 9       $10^{10}$  cells
- Cohort 10       $3 \times 10^{10}$  cells

Dosing is based on total cell number rather than % transduced cells since our transduction efficiency with the anti-mesothelin CAR genes is between 70-90% in PBL.

In each cohort, if one of the first three patients treated experiences a DLT, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three additional patients will be accrued at the next-lowest dose, for a total of 6, in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion. If a dose limiting toxicity occurs in the first cohort, that cohort will be expanded to n=6 patients. If there are 1 or fewer DLTs in the first cohort, the study will

proceed to the second cohort. If two DLTs occur in the first cohort, the dose will be de-escalated as described above in cohort -1. If 2 DLTs occur in cohort -1, the dose of cells will be de-escalated as described above in cohort -2. If two DLTs occur in cohort -2, the study will be terminated.

Patients will be enrolled sequentially, therefore enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort. If sufficient cells cannot be grown to meet the criteria for the assigned cohort, the patient will be enrolled in the appropriate cohort for the number of cells infused.

The maximum tolerated cell dose is the highest dose at which  $\leq 1$  of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

Prior to receiving the engineered PBL cells, patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, CAR gene-transduced PBL plus IV aldesleukin (72,000 IU/kg q8h for a maximum of 15 doses).

### 3.1.2.1 Safety Assessments:

A staggering period of 21 days following regimen completion (defined as the last dose of aldesleukin) will be conducted for the first 3 patients enrolled in this study. We will proceed with a 2-week inter-cohort staggering period following regimen completion (defined as the last dose of aldesleukin) if the cytokine profiles from the first 3 patients support safety.

A two-week safety assessment period following regimen completion (defined as the last dose of aldesleukin) will be conducted between each patient in every cohort.

If any unexpected grade 4 or greater toxicity occurs in a patient treated in either the phase 1 or phase 2 portions of this study, a thorough evaluation of the available safety information will be conducted to justify a decision to continue enrolling new subjects into the study. The study will be halted until this evaluation has occurred and results discussed with FDA.

### 3.1.3 Phase 2 Portion:

Similar to the Phase 1 portion, prior to receiving the engineered PBL cells, patients in the phase 2 portion will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed in one to four days by intravenous infusion of in vitro tumor reactive, CAR gene-transduced PBL plus IV aldesleukin (72,000 IU/kg q8h for a maximum of 15 doses).

The phase 2 portion of the protocol will proceed utilizing the MTD of anti-mesothelin CAR engineered cells as determined in the phase 1 portion. Patients will be entered into two cohorts based on histology: cohort 1 will include patients with mesothelioma, and cohort 2 will include all other cancer types that express mesothelin.

See section **3.1.2.1** for the safety assessment to be conducted for any incidence of unexpected grade 4 or greater toxicity.

### 3.1.4 Definition of Dose Limiting Toxicity (DLT):

Dose-limiting toxicity is defined as follows:

- Grade 2 or greater allergic reaction or reaction that involves bronchospasm or generalized urticaria
- All grade 3 or greater toxicities with the exception of:
  - Grade 3 or 4 myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin and thrombocytopenia,
  - Expected chemotherapy toxicities as defined in the Pharmaceutical Information section **11**.
  - Aldesleukin expected toxicities as defined in **Appendix 1** and **Appendix 2**.
  - Immediate hypersensitivity reactions occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.
  - Grade 3 Fever
  - Grade 3 Metabolic Laboratory abnormalities without significant clinical sequel that resolve to grade 2 within 7 days
  - Grade 3 autoimmunity, that resolves to less than or equal to a grade 2 autoimmune toxicity within 10 days

### 3.1.5 Protocol Stopping Rules:

The study will be halted pending discussions with the FDA and NCI IRB if the following conditions are met:

- If two DLTs occur in the first cohort of the Phase 1 portion of this study.
- During the phase 1 portion of the study - if two or more patients develop a grade 3 or greater toxicity related to the cell product, with the exception of:
  - Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 7 days
  - Grade 3 fever
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.
- If EBV lymphoma in another EBV negative subject occurs in this or any other SB adoptive cell therapy study, accrual of EBV negative subjects to all such studies must halt pending review of the event by the IRB and FDA
- If one or more treatment related deaths occur due to the cell infusion, we will promptly discuss this with the NCI IRB and FDA.
- During the phase 2 portion of the study - once five or more patients have been enrolled, if 20% or more patients cumulatively enrolled develop a DLT, as described in Section **3.1.4**

### 3.2 DRUG ADMINISTRATION

#### 3.2.1 Preparative Regimen with Cyclophosphamide and Fludarabine

(Starting on day-6, study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. Administration of diuretics, electrolyte replacement and hydration and monitoring of electrolytes should all be performed as clinically indicated. Chemotherapy infusions maybe slowed or delayed as medically indicated.)

##### Day -7 and -6

###### Approximately 6 hours Prior to Cyclophosphamide

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 1.5 – 2.6 ml/kg/hr (starting approximately 6 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). At any time during the preparative regimen, if urine output is <1 ml/kg/hr or if body weight is >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV maybe administered. The hydration rate will be capped at 250mL/hr.

###### Approximately 1 hour pre-Cyclophosphamide

Ondansetron (0.15 mg/kg/dose [rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight] IV every 8 hours X 3 days) will be given for nausea.

Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day X 2 days over 1 hr. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

A decreased dose of cyclophosphamide at 30mg/kg/day (x2 days) will be considered for patients who have a history of prolonged hematologic recovery from prior chemotherapy treatments.

###### Immediately following the end of Cyclophosphamide

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section **11**) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

##### Day -7 to Day -3:

Fludarabine 25 mg/m<sup>2</sup>/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**. (*The fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6*)

### 3.2.2 Cell Infusion and Aldesleukin Administration

**The following criteria must be met for the subject to receive the cell infusion:  $T \leq 38.3^{\circ}\text{C}$ , Pulse  $\leq 100$  BPM, O<sub>2</sub> Sat  $>92\%$  and systolic BP either  $>90$  mm Hg systolic or within 20 mm Hg of pre-treatment.**

The anti-mesothelin CAR PBLs are delivered to the patient care unit by a staff member from the Tumor Immunology Cell Processing Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. Please note, aldesleukin is being administered at the lower dose of 72,000 IU/kg/dose.

Aldesleukin (based on total body weight) will be administered at the lower dose of 72,000 IU/kg as an intravenous bolus over a 15-minute period approximately every 8 hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered by supportive measures within 24 hours of the last dose of aldesleukin. Dosing will be delayed or stopped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in [Appendix 1](#). Toxicities will be managed as outlined in [Appendix 2](#). Dosing may be held or stopped at the discretion of the treating investigator. ([Appendix 3](#) lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center).

Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

Day 0 (two to four days after the last dose of fludarabine):

- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes or as clinically determined by an investigator for patient safety (between one and four days after the last dose of fludarabine).
- Aldesleukin will be given as described above.

Day 0-4 (Day 0 is the day of cell infusion):

- Beginning on day 1 or 2, filgrastim will be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count  $> 1.0 \times 10^9/\text{L}$  X 3 days or  $> 5.0 \times 10^9/\text{L}$ .
- Aldesleukin will be given as described above.

### 3.2.3 Treatment Schedule

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Therapy												
Cyclophosphamide (60 mg/kg)	X	X										
Fludarabine (25 mg/m <sup>2</sup> )	X	X	X	X	X							
Anti-mesothelin CAR PBL								X <sup>1</sup>				
Aldesleukin								X <sup>2</sup>	X	X	X	X
Filgrastim <sup>3</sup> (5 mcg/kg/day)									X	X	X	X
TMP/SMX <sup>4</sup>								X		X		X
160mg/800mg (example)												
Fluconazole <sup>5</sup> (400 mg po)								X	X	X	X	X
Valacyclovir po or Acyclovir IV <sup>6</sup>								X	X	X	X	X

<sup>1</sup>One to four days after the last dose of fludarabine

<sup>2</sup>Initiate within 24 hours after cell infusion

<sup>3</sup>Continue until neutrophils count > 1X10<sup>9</sup>/L for 3 consecutive days or > 5x10<sup>9</sup>/L.

<sup>4</sup>The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2, starting day 0 or within one week of anticipated lymphopenia

<sup>5</sup>Continue until ANC > 1000/mm<sup>3</sup>

<sup>6</sup>In patients positive for HSV or VZV continue until CD4 >200 X 2

### 3.3 ON-STUDY EVALUATION

#### Note: Please refer to section 5 for research evaluations.

##### 3.3.1 Within 28 days prior to starting the preparative regimen

- EKG
- coagulation profile including INR, APTT, platelets and fibrinogen.

##### 3.3.2 Within 14 days prior to starting the preparative regimen

- Apheresis as indicated.
- Baseline blood test
  - Complete Blood Count with differential
  - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
  - PT/PTT
  - TBNK
  - Thyroid Panel
  - Urinalysis
- Anti CMV antibody titer, HSV serology, and EBV panel. (may be performed within 3 months of chemotherapy)
- Chest x-ray

- EKG

3.3.3 During the preparative regimen: DAILY

- Complete Blood Count with differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Urinalysis as needed
- Daily weight as indicated
- PT/PTT (every 3 days)

3.3.4 After Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
- Blood samples will be collected at approximately 1 hour, 4 hours, 24 hours, and 1 week following infusion for analysis of the cytokines IL-6, TNF- $\alpha$ , IFN- $\gamma$  and GM-CSF, and circulating mesothelin.
- EKGs and a coagulation profile including INR, APTT, platelets and fibrinogen will be conducted 7 days (+/- 2 days) and 14 days (+/- 2 days) after cell administration. If the patient is discharged prior to 14 days following cell infusion, an EKG and coagulation profile will be conducted prior to discharge.
- Once total lymphocyte count is greater than 200/mm<sup>3</sup>, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized).

3.3.5 During Hospitalization (following aldesleukin administration):

**Every 1-2 days**

- A review of systems and physical exam as clinically indicated
- CBC with differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Other tests will be performed as clinically indicated.
- Vital signs will be monitored as clinically indicated.

**3.4 POST TREATMENT (FOLLOW-UP) EVALUATION**

All patients will return to the NIH Clinical Center for their 1<sup>st</sup> evaluation for response 6 weeks (+/- 2 weeks) following the administration of the cell product.

**Please note:** Patients who have received multiple transfusions during the treatment phase or discharged with grade 3 or greater significant adverse events should be evaluated by referring physician and repeat labs as appropriate within 2 weeks of discharge. Labs drawn should be faxed to the study coordinator.

### 3.4.1 Time period of evaluations

Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated as noted below:

- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x3
- Every 6 months (+/- 1 month) x 2 years
- As per PI discretion for subsequent years

**Note:** Patients may be seen more frequently as clinically indicated

### 3.4.2 At each evaluation, patients will undergo:

- Physical examination, including weight and vital signs
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Protein, total
- Complete blood count with differential
- Urinalysis as indicated
- Thyroid panel as clinically indicated
- TBNK, until CD4 > 200 X 2
- EKG and a coagulation profile including INR, PTT/APTT, platelets and fibrinogen.
- Toxicity assessment, including review of systems
- Imaging (CT, MRI, and/or PET) as performed at baseline. This end of course evaluation will be used to determine tumor response.
- A 5 liter apheresis may be performed. If patient is unable to undergo apheresis, approximately 96 ml of blood may be obtained at the first follow up visit. Subsequently, approximately 60 ml of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed. This will be performed on 03-C-0277.
- Detection of RCR and persistence of CAR gene transduced cells: (see section 5.7). This will be performed on 03-C-0277.
- Long-term follow up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long term follow up period for retroviral vectors is 15 years. This will be performed on 09-C-0161.
- Note: Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or email contact. A request will be made to send laboratory, imaging and physician exam reports performed by their treating physician; and any outstanding toxicities will be reviewed with the patient.

## 3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

### 3.5.1 Criteria for removal from protocol therapy:

Patients will be taken off treatment (and followed until progression of disease) for the following:

- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Investigator discretion.
- Positive Pregnancy Test

### 3.5.2 Off Study Criteria:

Patients will be taken off study for the following:

- Completion of study follow up period.
- The participant requests to be withdrawn from study
- Radiographic or clinical disease progression.
- Lost to follow-up
- Death

Note: Once a subject is taken off study, no further data can be collected.

**Note:** Patients who are taken off study for progressive disease or study closure may be followed on protocol 09-C-0161 “Follow up Protocol for subjects Previously Enrolled in Surgery Branch Studies.”.

### 3.5.3 Off Protocol Therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office <ncicentralregistration-l@mail.nih.gov>.

## 4 CONCOMITANT MEDICATIONS/MEASURES

### 4.1 INFECTION PROPHYLAXIS:

*Note: Other anti-infective agents may be substituted at the discretion of the treating physician.*

#### 4.1.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/SMX) as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days, beginning day 0 or within 1 week of anticipated lymphopenia.

Dapsone (in G6PD sufficient patient), Atovaquone or Pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

#### 4.1.2 Herpes and Varicella Zoster Virus Prophylaxis

Patients with positive HSV or VZV serology will be given valacyclovir orally at a dose of 500 mg daily starting the day of cell infusion, or acyclovir, 250 mg/m<sup>2</sup> IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for Pneumocystis Varicella Zoster and Herpes will continue for 6 months post chemotherapy. If the CD4 count is less than 200 at 6 months post chemotherapy, prophylaxis will continue until CD4 count is greater than 200 X 2.

#### 4.1.3 Fungal Prophylaxis

Patients will start Fluconazole 400 mg p.o. the day of cell infusion and continue until the absolute neutrophil count is greater than 1000/mm<sup>3</sup>. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

### 4.2 BLOOD PRODUCT SUPPORT

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >10,000/mm<sup>3</sup>. All blood products will be irradiated. 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.

### 4.3 EMPIRIC ANTIBIOTICS

Patients will start on broad-spectrum antibiotics in accordance with current institutional guidelines for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm<sup>3</sup>. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

### 4.4 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and ranitidine (150 mg g12h). If patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products.

Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional anti-emetics will be administered as needed for nausea and vomiting uncontrolled

by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

## **5 BIOSPECIMEN COLLECTION**

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long term research efforts within the Surgery Branch and with collaborators as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

### **5.1 SAMPLES SENT TO DR. FIGG'S LAB**

- Venous blood samples will be collected in either a 4ml or an 8ml SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pickup.
- For sample pickup, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.
- The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

### **5.2 SAMPLES SENT TO THE SURGERY BRANCH CELL PROCESSING LABORATORY**

- Venous blood samples will be collected in 8ml CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pickup.
- Samples will be pick-up by the research nurse or designee and transported to the SB Cell Processing Laboratory within 24 hours of blood draw.
- The samples will be processed, barcoded, and stored in SB Cell Processing Laboratory.

### **5.3 PRIOR TO CHEMOTHERAPY ADMINISTRATION**

- 5 CPT tubes (8ml each) – send to SB lab
- 1 SST tube (8ml) – send to Figg's lab
- 1 SST tube (4 ml) daily; starting day of chemotherapy – send to Figg's lab

### **5.4 PRIOR TO CELL INFUSION (1-8ML SST)**

- Blood samples for analysis of the cytokines IL-6, TNF- $\alpha$ , IFN- $\gamma$  and GM-CSF. – send to Figg's lab

## 5.5 POST CELL INFUSION EVALUATIONS

- Once total lymphocyte count is greater than 200/mm<sup>3</sup>, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday and Friday x 5 days, then weekly (while the patient is hospitalized):
  - 5 CPT tubes (8ml each) – send to SB lab
  - 1 SST tube (8ml) - send to Figg's lab

## 5.6 IMMUNOLOGICAL TESTING

- Apheresis may be performed, prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be:
  - Cryopreserved for immunological monitoring of cell function,
  - Subjected to DNA and RNA extraction for PCR analysis of TCR and vector copy number estimation
- Lymphocytes will be tested directly and following in vitro culture. Direct immunological monitoring will consist of quantifying T cells reactive with mesothelin by FACS analysis using tetramer staining. Ex vivo immunological assays will consist of cytokine release by bulk PBL (+/- peptide stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the engineered PBL cryopreserved at the time of infusion. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences. Foxp3 levels will be analyzed by semiquantitative RT-PCR to evaluate for mRNA on PBL samples obtained prior to cell infusion and at the follow up time point.

## 5.7 MONITORING GENE THERAPY TRIALS: PERSISTENCE AND RCR

- Engineered cell survival. TCR and vector presence will be quantitated in PBMC samples using established PCR techniques. Immunological monitoring using both tetramer analysis and staining for the TCR will be used to augment PCR-based analysis. This will provide data to estimate the in vivo survival of lymphocytes derived from the infused cells. In addition, measurement of CD4 and CD8 T-cells will be conducted and studies of these T-cell subsets in the circulation will be determined by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell. Note: samples will be batched and assayed at the conclusion of the study.
- All patients will be co-enrolled on protocol 09-C-0161 “Follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies”. Patients’ blood samples will be obtained and undergo analysis for detection of Replication Competent Retroviruses (RCR) by PCR prior to cell infusion and RCR PCR will be performed at 3 and 6 months, and at one year post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA.

RCR PCR assays detect the GaLV envelop gene and are performed under contract. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.

## **5.8 SAMPLE, STORAGE, TRACKING AND DISPOSITION FOR SB CELL PROCESSING LABORATORY**

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Blood Processing Core (BPC) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. All samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Note: Blood and tissue collected during the course of this study will be stored, tracked and disposed of as specified in our companion protocol 03-C-0277, (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

## **5.9 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR DR. FIGG'S LAB**

### **5.9.1 Sample Data Collection**

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the Surgery Branch central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes

the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

### 5.9.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Note: Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

## 6 DATA COLLECTION AND EVALUATION

### 6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal

investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts.

All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events until their first week 6 follow-up evaluation or until off-study, whichever comes first.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

**End of study procedures:** Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

#### 6.1.1 Exclusions to Routine Adverse Event Reporting:

Patients will be receiving multiple agents which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin and supportive medications) in combination with the investigational agents; therefore, grade 1 events not related to the cell product will not be reported/recorded.

## 6.2 DATA SHARING PLANS

### 6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long term research efforts within the Surgery Branch and de-identified data may also be shared

with collaborators as specified in our companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols).

### 6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

## 6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response at 6 and 12 weeks (+/- 2 weeks), then every 3 months (+/- 1 month) x3, then every 6 months' (+/- 1 month) x 2 years, then as per PI discretion. In addition to a baseline scan, confirmatory scans may be obtained at least 4 weeks (not less than 4) following initial documentation of objective response.

Clinical Response will be determined using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.0).

### 6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with Cyclophosphamide.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of course 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

### 6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

**Non-measurable disease:** All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with  $\geq 10$  to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

**Target lesions:** All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

**Non-target lesions:** All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

### 6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

**Clinical lesions:** Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and  $\geq 10$  mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

**Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as

closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

**FDG-PET:** While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

### 6.3.4 Response Criteria

#### 6.3.4.1 Evaluation of target lesions<sup>1</sup>

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.
- Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

#### 6.3.4.2 Evaluation of non-target lesions<sup>2</sup>

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

- 1** All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.
- 2** All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as “present” or “absent.”

#### 6.3.4.3 Evaluation of best overall response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

#### 6.3.4.4 Confirmatory Measurement/Duration of Response

##### **Confirmation**

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

##### **Duration of Overall Response**

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

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

##### **Duration of Stable Disease**

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

## **6.4 TOXICITY CRITERIA**

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm#ctc\\_40](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40)).

## **7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN**

### **7.1 DEFINITIONS**

#### **7.1.1 Adverse Event**

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

#### **7.1.2 Suspected Adverse Reaction**

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

#### **7.1.3 Unexpected Adverse Reaction**

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

#### **7.1.4 Serious**

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

#### **7.1.5 Serious Adverse Event**

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

- Death,
- A life-threatening adverse drug experience

- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

#### 7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

#### 7.1.7 Life threatening Adverse Drug Experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

#### 7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

#### 7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

#### 7.1.10 Unanticipated Problems

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggest that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized

### 7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

#### 7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

#### 7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
  - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
  - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
  - All Grade 5 events regardless of attribution;
  - All Serious Events regardless of attribution.

**NOTE:** Grade 1 events are not required to be reported.

#### 7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

### 7.3 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

#### 7.3.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the anti-mesothelin CAR PBL as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-mesothelin CAR PBL, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

#### 7.3.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

##### 7.3.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site

- the Principal Investigator
- clinical protocol identifiers
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

#### 7.3.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

### 7.4 IND SPONSOR REPORTING CRITERIA

Up until the first follow-up evaluation (6 weeks ( $\pm$  2 weeks) following the administration of the cell product), the investigator must immediately report to the sponsor, using the mandatory MedWatch FDA Form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur after the first follow-up evaluation, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

Required timing for reporting per the above guidelines:

- Death (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the Center for Cancer Research (CCR) at: [CCRsafety@mail.nih.gov](mailto:CCRsafety@mail.nih.gov) and to the CCR PI and study coordinator.

#### 7.4.1 Waiver of expedited reporting to CCR

The investigators are requesting a waiver from reporting specific events in an expedited manner to the CCR. Patients will be receiving commercially available agents, such as fludarabine, cyclophosphamide, and aldesleukin. The majority of toxicities observed on Surgery Branch Adoptive Cell Therapy protocols are expected toxicities of the non-myeloablative chemotherapy regimen or IL-2 and occur in approximately 95% of the patients enrolled, therefore, we are requesting a waiver from reporting the following events in an expedited manner to the CCR.

- Grade 3 or greater myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Grade 3 or greater nausea, vomiting, mucositis - oral, anorexia, diarrhea, fever, chills, fatigue, and rash maculo-papular.
- Grade 3 hematuria, hypotension, sinus tachycardia, urine output decreased, confusion, infections, and febrile neutropenia.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of patients treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at:

[CCRsafety@mail.nih.gov](mailto:CCRsafety@mail.nih.gov)

#### 7.4.2 Reporting Pregnancy

##### 7.4.2.1 Maternal exposure

If a patient becomes pregnant during the first four months following treatment the pregnancy should be reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the Medwatch form “Describe Event or Problem”.

Pregnancy itself is not regarded as a SAE unless there is a suspicion that the study treatment under study may have interfered with the effectiveness of a contraceptive medication. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 “Pregnancy, puerperium and perinatal conditions - Other (pregnancy)” under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

#### 7.4.2.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of aldesleukin.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

### 7.5 DATA AND SAFETY MONITORING PLAN

#### 7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

#### 7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation

- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

#### 7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

### 8 STATISTICAL CONSIDERATIONS

The primary objectives of this trial is to determine safety and also to determine whether the combination of low-dose aldesleukin, lymphocyte-depleting chemotherapy, and an infusion of anti-mesothelin CAR-gene engineered lymphocytes is able to be associated with a modest fraction of patients that can experience a clinical response (PR +CR) to therapy. The duration of the clinical response will be followed to assess the efficacy of the regimen. A secondary objective is to have sufficient patients in order to do exploratory evaluations of survival of cells.

Initially, the protocol will enroll 3 patients in each cohort unless that patient experiences a dose limiting toxicity (DLT). If dosing is escalated to cohort 10, 6 patients will be accrued to this cohort in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion. The total number of anti-mesothelin engineered cells transferred for each cohort will be:

- Cohort 1       $10^6$  cells
  - De-escalation cohorts:
    - Cohort -1:  $3 \times 10^5$  cells
    - Cohort -2:  $10^5$  cells
- Cohort 2       $3 \times 10^6$  cells
- Cohort 3       $10^7$  cells
- Cohort 4       $3 \times 10^7$  cells
- Cohort 5       $10^8$  cells
- Cohort 6       $3 \times 10^8$  cells
- Cohort 7       $10^9$  cells
- Cohort 8       $3 \times 10^9$  cells
- Cohort 9       $10^{10}$  cells

- Cohort 10       $3 \times 10^{10}$  cells

In each cohort, if one of the first three patients treated experiences a DLT, three more patients would be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three additional patients will be accrued at the next-lowest dose, for a total of 6, in order to further characterize the safety of the maximum tolerated dose prior to starting the phase II portion. If a dose limiting toxicity occurs in the first cohort, that cohort will be expanded to n=6 patients. If there are 1 or fewer DLTs in the first cohort, the study will proceed to the second cohort. If two DLTs occur in the first cohort, the dose will be de-escalated as described above in cohort -1. If 2 DLTs occur in cohort -1, the dose of cells will be de-escalated as described above in cohort -2. If two DLTs occur in cohort -2, the study will be terminated.

Once the MTD has been determined, the study then would proceed to the phase II portion. Patients will be enrolled into individual strata depending on their specific histology. This stratification is being used to separate patients who have mesothelioma, and patients with all other histologies. The six patients accrued at the MTD in the phase I portion of this study will be included in the strata for the appropriate histology in the phase II portion.

For each of the two strata, the phase II portion of the study will be conducted using a phase II optimal design (Simon R, Controlled Clinical Trials 10:1-10, 1989). For the two strata, the objective will be to determine if the combination of low dose aldesleukin, lymphocyte depleting chemotherapy, and anti-mesothelin CAR-gene engineered lymphocytes is able to be associated with a clinical response rate that can rule out 5% ( $p_0=0.05$ ) in favor of a modest 20% PR + CR rate ( $p_1=0.20$ ).

In patients in each of the two strata, the following design will be used. For each strata, with  $\alpha=0.05$  (5% probability of accepting a poor therapy) and  $\beta=0.10$  (10% probability of rejecting a good therapy,), initially 21 evaluable patients will be enrolled. If 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled. If 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 2 to 4 of the 41 have a clinical response, then this will be considered inadequate for further investigation. If 5 or more of 41 patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration. Under the null hypothesis (5% response rate), the probability of early termination is 72%.

Further, to help ensure that maldistribution of patients who are either particularly responsive or unresponsive in the first stage does not materially interfere with the intended use of the two-stage design, we will aim to enroll 4-5 patients of each allowed histology among the first 21 enrolled in the 'other histology' arm. Although this has its own inherent issues due to limited sample size, since we believe that these 'other histologies' will behave the same clinically, it will permit us to evaluate the different response rates in a limited number of subjects and

determine if they differ markedly or not. Since this would merely be an exploratory analysis, we will also look at minor response as well to help evaluate for hints of efficacy. If the response rates do seem to potentially differ markedly by histology, despite our hypothesis that this will not happen, we may consider amending the protocol when appropriate to try to restrict enrollment to those histologies with stronger evidence of responsiveness. For patients with chemotherapy-sensitive tumors (i.e. sarcoma), only responses seen at day 28 and maintained at 4 months will be considered a positive response for accrual to the second phase of this study.

To complete the phase 1 dose-escalation stage, and both cohorts in the phase 2 portion of the study, a total of up to 136 patients may be required (10 possible escalations in the phase 1 portion with a maximum of 60 patients (but likely 20-30 patients will be accrued depending on the toxicity observed), and 2 phase 2 cohorts with a combined total of 76 patients, assuming 41 patients per cohort, but that 6 of the 82 will be those patients treated at the MTD). Provided that about 1-2 patients per month will be able to be enrolled onto this trial, approximately 6 years may be needed to accrual the maximum number of required patients.

## **9 COLLABORATIVE AGREEMENTS**

We have established a Cooperative Research and Development Agreement (CRADA #02716) with Kite Pharma, Inc., and will be sharing data with them.

## **10 HUMAN SUBJECTS PROTECTIONS**

### **10.1 RATIONALE FOR PATIENT SELECTION**

The patients to be entered in this protocol have metastatic cancer which is refractory to standard therapy, and limited life expectancies.

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

### **10.2 PARTICIPATION OF CHILDREN**

The use of the nonmyeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

### **10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT**

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **10.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

### **10.4 EVALUATION OF BENEFITS AND RISKS**

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in section **11**. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using patients own transduced T-cells without the need to identify anti-tumor T cells uniquely from each patient as was required in several prior protocols. Aldesleukin is an approved and effective treatment in some patients with metastatic melanoma and renal cancer. In this study, aldesleukin is administered after cell infusion to promote growth and survival of the infused cells. Although aldesleukin can cause serious adverse events, it's use in this protocol is essential to keep the cells alive. The success of this effort cannot be predicted at this time. Because all patients in this protocol have metastatic cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

### **10.5 CONSENT PROCESS AND DOCUMENTATION**

Patients initially signs a consent when they agree to have PBMC obtained for study and growth on 03-C-0277, Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

#### 10.5.1 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

## 11 PHARMACEUTICAL INFORMATION

Cyclophosphamide, fludarabine, and aldesleukin, the commercial drugs used in this study will not alter labelling of the FDA approved drugs. The investigation is not intended to support a new indication for use or any other significant changes to labeling or advertising in either Cyclophosphamide, Fludarabine, or Aldesleukin. The investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug products.

### 11.1 INVESTIGATIONAL REGIMEN

#### 11.1.1 Anti-Mesothelin CAR Transduced PBL

The procedure for expanding the human PBL and the Certificate of Analysis (CoA) are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the Surgery Branch. The CoA is included in Appendix 4 for this protocol, and in the IND submission for these cells along with the Standard Operating Procedures for the growth of the PBL, and transduction with retroviral supernatant containing the chimeric anti-mesothelin CAR genes. Note: Penicillin, Streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

#### 11.1.1.1 Retroviral Vector Containing the Anti-mesothelin CAR Gene

The retroviral vector supernatant [PG13-coSS1scFv-CD28Z (D6)] encoding a chimeric antigen receptor (CAR) directed against mesothelin was prepared and preserved following cGMP in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector utilizes the MSGV retroviral vector backbone and consists of 7024 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor (SA) sites, SS1 anti-mesothelin-based CAR protein (coSS1scFv-CD28Z) containing a signal peptide (GMCSFR2), codon-optimized SS1 light chain variable region (SS1 VL), linker peptide, codon-optimized SS1 heavy chain (SS1 VH), CD8 (hinge and transmembrane regions), CD28 (cytoplasmic region) and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer was determined by retroviral transduction of human peripheral blood lymphocytes followed CAR detection by FACS according to sponsor certificate. The supernate will be stored at SBVPF upon the completion of production at -80° C or shipped on dry ice and stored at Fisher Bioservices, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in ex vivo PBL transduction. There will be no re-use of the same unit of supernate for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at <http://bmbi.od.nih.gov/sect3bsl2.htm>.

#### 11.1.2 Aldesleukin (Interleukin-2, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Aldesleukin will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, is provided as single-use vials containing 22 million IU (-1.3 mg) aldesleukin as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

Storage: Intact vials are stored in the refrigerator (2° - 8°C) protected from light. Each vial bears an expiration date.

Dilution/Stability: Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of aldesleukin. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2°– 30°C.

Administration: The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin

will be administered as an inpatient.

**Toxicities:** Expected toxicities of aldesleukin are listed in the product label and in **Appendix 1** and 2. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix 1**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 2**.

#### 11.1.3 Fludarabine

**Description:** (Please refer to package insert for complete product Information) Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

**How Supplied:** It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

**Stability:** Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

**Storage:** Intact vials should be stored refrigerated (2-8°C).

**Administration:** Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

**Toxicities:** At doses of 25 mg/m<sup>2</sup>/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. 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, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the

current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

#### 11.1.4 Cyclophosphamide

**(Refer to FDA-approved package insert for complete product information)**

**Description:** Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

**How Supplied:** Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

**Stability:** Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

**Administration:** It will be diluted in 250 ml D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**.

**Toxicities:** Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. 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-mercaptopethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis 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 result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse

hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptopoethanesulphonate; given by IV injection) is a synthetic sulphydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

## 11.2 SUPPORTIVE MEDICATIONS

### 11.2.1 Mesna (Sodium 2-mercaptopoethanesulfonate, Mesnum, Mesnex)

(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 6**. Toxicities include nausea, vomiting and diarrhea.

### 11.2.2 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

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

### 11.2.3 Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days) beginning day 0 or within one week of anticipated lymphopenia and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can

cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur. Should allergies develop, the following medications may be used in place of TMP/SMX DS.

#### 11.2.3.1 Dapsone:

Dapsone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of *Pneumocystis pneumonia*. The dose is 100mg by mouth daily, starting on day 0 ( $\pm$  7 days) and continuing at least 6 months and until the CD4+ count is  $> 200$  on two consecutive lab studies. It is supplied as 25mg and 100mg tablets. Dapsone contains a sulfa group, although the cross reactivity in patients with sulfa allergies is quite low. Dapsone may be considered in patients with mild to moderate sulfa allergies. Dapsone should be avoided in patients with severe (i.e., a history of anaphylaxis or other equally serious reaction) reactions to sulfa drugs. Additionally, dapsone has been reported to cause hemolytic anemia in patients with G6PD deficiency. It is recommended that patients be tested for G6PD deficiency prior to the initiation of dapsone therapy. Dapsone is generally well tolerated, but may cause a number of hematologic adverse reactions, including increased reticulocyte counts, hemolysis, decreased hemoglobin, methemoglobinemia, agranulocytosis, anemia, and leukopenia. Other rare but serious adverse reactions include bullous exfoliative dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, pancreatitis, interstitial pneumonitis, and pulmonary eosinophilia. For more detailed information about adverse reactions, consult the package insert.

#### 11.2.3.2 Atovaquone:

Atovaquone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of *Pneumocystis pneumonia* in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or pentamidine.

Atovaquone may be given as a single daily dose of 1500mg orally or the dose may be split into 750mg given orally twice daily. Atovaquone will be started on day 0 ( $\pm$  7 days), and will continue for at least 6 months and until the CD4+ count is  $> 200$  on two consecutive lab studies. Atovaquone is supplied as an oral suspension containing 150mg/mL. Common adverse reactions to atovaquone include: headache, rash, diarrhea, nausea, vomiting, abdominal pain, cough, and fever. Rare but serious adverse reactions include acute renal failure, hepatitis and hepatic failure, angioedema, pancreatitis, and Stevens-Johnson syndrome. For more detailed information about adverse reactions, consult the package insert.

#### 11.2.3.3 Aerosolized Pentamidine:

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer with one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and

asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

#### 11.2.4 Herpes and Varicella Zoster Virus Prophylaxis

##### 11.2.4.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

##### 11.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials.

Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL.

Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

#### 11.2.5 Fluconazole

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

#### **11.2.6 Ondansetron hydrochloride**

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritus, constipation and urinary retention. Consult the package insert for specific dosing instructions.

#### **11.2.7 Furosemide**

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritus. Consult the package insert for a complete list of all side effects.

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**13 TABLES, FIGURES, AND APPENDICES:**

**Table 1**

Frequency and duration of objective responses in melanoma patients treated with standard Tumor Infiltrating Lymphocytes (TIL) in the Surgery Branch, NCI (9/1/11)

<u>Treatment*</u>	<u>Total</u>	<u>PR</u>	<u>CR</u>	<u>OR (%)</u>
<b>No TBI#</b>	<b>43</b>	<b>16</b>	<b>5</b>	<b>21 (49%)</b>
		α (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	(94+, 92+, 91+ 82+, 77+)	
<b>200cG TBI</b>	<b>25</b>	<b>8</b>	<b>5</b>	<b>13 (52.0%)</b>
		(14, 9, 6, 6, 5, 4, 3, 3)	(81+, 77+, 73+, 70+, 67+)	
<b>1200cG TBI</b>	<b>25</b>	<b>8</b>	<b>10</b>	<b>18 (72%)</b>
		(21, 13, 7, 6, 6, 5, 3, 2)	(61+, 58+, 57+, 52+, 51+, 51+, 51+, 50+, 19)	

\*All patients received cyclophosphamide (60mg/kg x 2d) and fludarabine (25mg/m<sup>2</sup> x 5d).

#Patients who received Rapidly Expanded TIL plus the full preparative regimen as a first TIL treatment

⊕Durations of response in months are in parenthesis under the number of responders.

“+” indicates on ongoing response.

52 responding patients: 42 had prior IL-2, 22 had prior IL-2 + chemotherapy

20 complete responses: 19 ongoing at 50 to 94 months

**Table 2**

Time in Hospital and Non-hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital <sup>1</sup>	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2 (6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

<sup>1</sup>Measured from the day of cell administration to discharge

**Table 3**

Mesothelin expression in human cancers by immunohistochemistry			
Cancer histology	Mesothelin Expression (%)	Comments	References
Mesothelioma	100	Present in all pleural mesotheliomas; absent in sarcomatous type	(Chang et al., 1992a; Or Ordóñez, 2003)
Pancreatic	100	Absent in normal pancreas and chronic pancreatitis	(Argani et al., 2001; Hassan et al., 2005b; Ordóñez, 2003)
Ovarian	67-100	Present in non-mucinous ovarian cancers; absent from mucinous type	(Chang et al., 1992b; Frierson et al., 2003; Hassan et al., 2005a; Ordóñez, 2003)
Lung adenocarcinoma	41-53	Present in lung adenocarcinomas and some expression in squamous and large cell lung cancer; absent in small cell lung cancer	(Ho et al., 2007; Miettinen and Sarlomo-Rikala, 2003; Ordóñez, 2003)
Esophageal	25, adenocarcinoma 75, squamous carcinoma		(Ordóñez, 2003)
Colorectal adenocarcinoma	28		(Ordóñez, 2003)
Cervical	25	Uterine, squamous carcinoma	(Ordóñez, 2003)

**Table 4**

**Table 4. Determination of coSS1scFv-CD28Z retroviral vector titers.**

Harvest	Titer (TU/mL, $\times 10^6$ )
1	$1.2 \pm 0.2$
2	$1.8 \pm 0.1$
3	$2.1 \pm 0.2$
4	$2.2 \pm 0.2$
5	$1.8 \pm 0.2$
6	$1.8 \pm 0.1$

**Table 5****Table 5. Specific cytokine release by CAR-transduced PBL.**

<b>Patient</b>	<b>Sample</b>	<b>None</b>	<b>Tumor Cell Line<sup>a</sup></b>			
			<b>Meso-</b>	<b>Meso+</b>		
			<b>A431</b>	<b>A431-H9</b>	<b>HAY</b>	<b>OVCAR3</b>
<b>Pt. K</b>	Harvest 1	634	174	<b>16269</b>	<b>13476</b>	<b>21020</b>
	Harvest 2	583	162	<b>30225</b>	<b>26808</b>	<b>37398</b>
	Harvest 3	705	194	<b>32773</b>	<b>30111</b>	<b>40249</b>
	Harvest 4	642	188	<b>32580</b>	<b>25345</b>	<b>32130</b>
	Harvest 5	657	193	<b>23620</b>	<b>19965</b>	<b>27088</b>
	Harvest 6	451	132	<b>20308</b>	<b>16231</b>	<b>21800</b>
<b>Pt. L</b>	Harvest 1	112	118	<b>32775</b>	<b>3958</b>	<b>2721</b>
	Harvest 2	113	108	<b>28481</b>	<b>8256</b>	<b>4486</b>
	Harvest 3	98	104	<b>36661</b>	<b>9200</b>	<b>4656</b>
	Harvest 4	101	100	<b>36893</b>	<b>9903</b>	<b>4700</b>
	Harvest 5	87	135	<b>36635</b>	<b>5100</b>	<b>2718</b>
	Harvest 6	102	128	<b>29288</b>	<b>4314</b>	<b>2597</b>
<b>Pt. M</b>	Harvest 1	102	87	<b>5686</b>	<b>20680</b>	<b>26210</b>
	Harvest 2	94	94	<b>10049</b>	<b>23260</b>	<b>16337</b>
	Harvest 3	91	80	<b>15035</b>	<b>26672</b>	<b>20740</b>
	Harvest 4	86	84	<b>14103</b>	<b>24536</b>	<b>22784</b>
	Harvest 5	116	75	<b>9357</b>	<b>25168</b>	<b>21669</b>
	Harvest 6	108	84	<b>9831</b>	<b>18134</b>	<b>16410</b>

<sup>a</sup> Cytokine release was measured following the incubation of 10<sup>5</sup> transduced T lymphocytes with 10<sup>5</sup> tumor target cells in 200 mL for 18h at 37°C. Negative tumor target, A431 and mesothelin-positive tumor targets include A431-H9 (transfected), HAY (mesothelioma cancer cell line) and OVCAR3 (ovarian cancer cell line). All cell lines were cultured in R10 medium consisting of RPMI 1640 containing 10% fetal bovine serum. Dilutions of culture supernatant were then tested for IFNg  $\delta$  enzyme-linked immunosorbent assay (ELISA). Data represented from 3 separate patient's PBL.

**Table 6**

**Modification of Dose Calculations\* in patients whose BMI is greater than 35**

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

$$\text{BMI} = \text{weight (kg)} / [\text{height (m)}]^2$$

2. Calculation of ideal body weight

$$\text{Male} = 50 \text{ kg} + 2.3 \text{ (number of inches over 60 inches)}$$

Example: ideal body weight of 5'10" male

$$50 + 2.3 (10) = 73 \text{ kg}$$

$$\text{Female} = 45.5 \text{ kg} + 2.3 \text{ (number of inches over 60 inches)}$$

Example: ideal body weight of 5'3" female

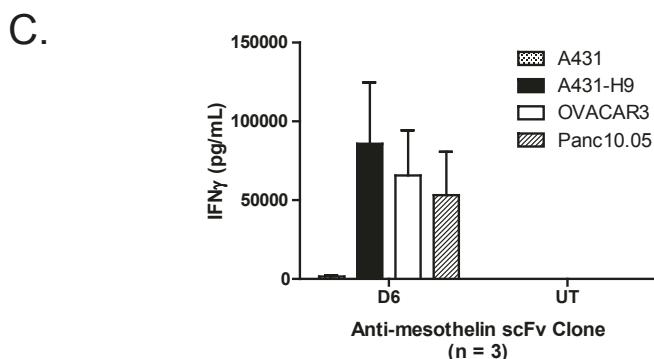
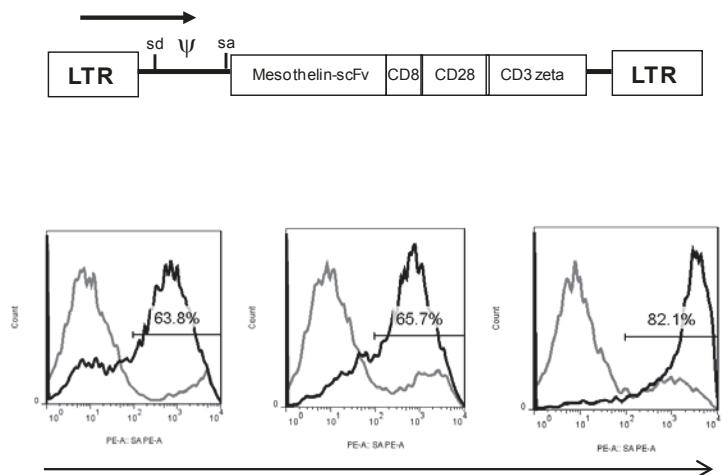
$$45.5 + 2.3 (3) = 57 \text{ kg}$$

3. Calculation of “practical weight”

Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

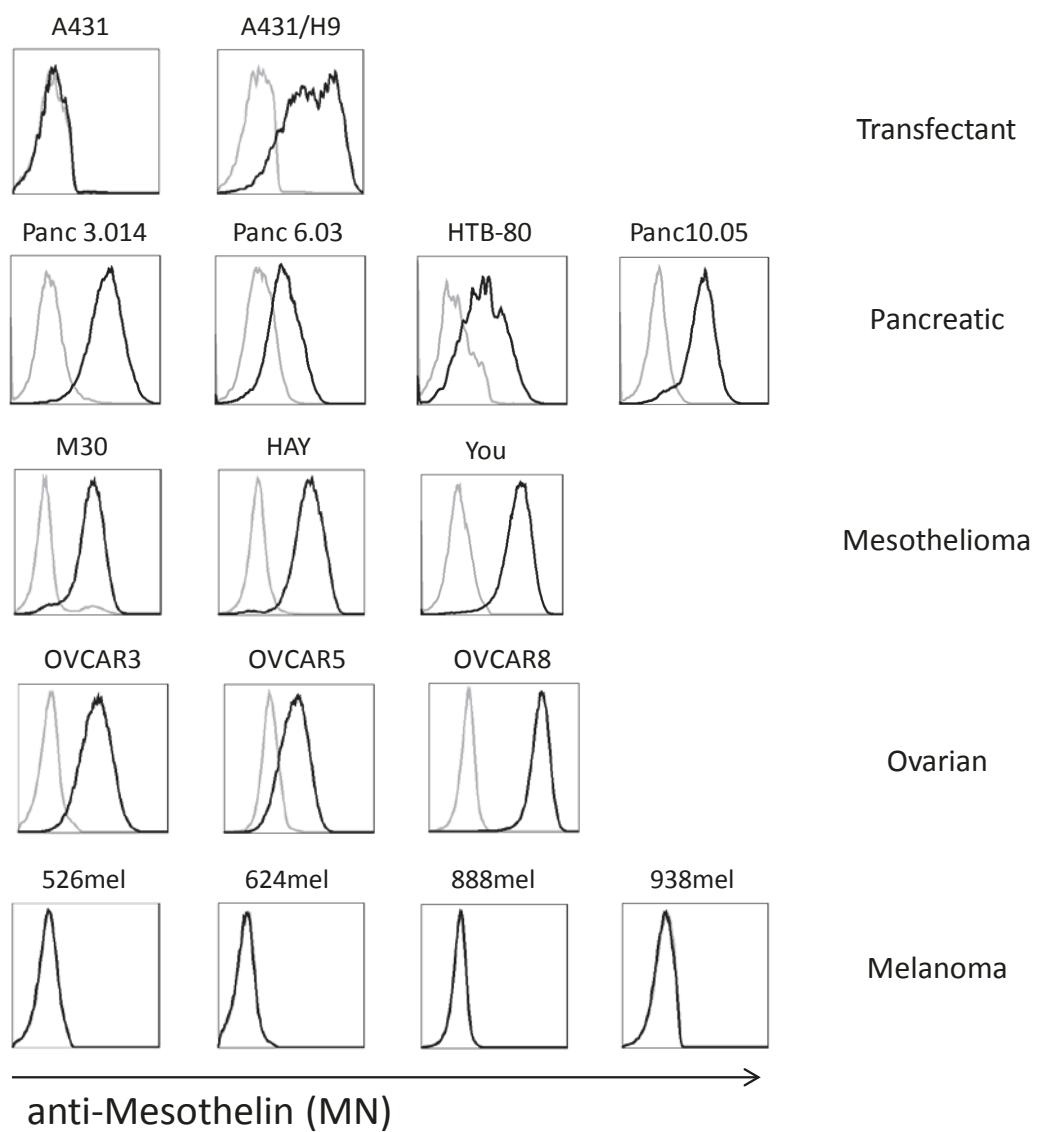
\*Practical weight will NOT be used in the calculation of dose for aldesleukin.

**Figure 1**



**Combinant retroviral vector encoding a bispecific antigen receptors (CAR) used in this study.** A) Schematic representation of the CAR construct. The CAR construct consists of a human CD8α, hinge and extracellular T cell signaling domains. B) Cell lines were transduced with the SS1 CAR-encoding vector. PBL were stained for CAR<sup>+</sup> cells using a streptavidin-PE. Transduction efficiency in CAR<sup>+</sup> cells. Data are from three separate experiments. C) Secreted IFNγ in response to mesothelin (1 x 10<sup>5</sup>) and mesothelin-positive target cell (A431) at 37°C and 5% CO<sub>2</sub>. Supernatants were assayed (SA) to detect IFNγ. Data representative of 3 independent experiments (data not shown).

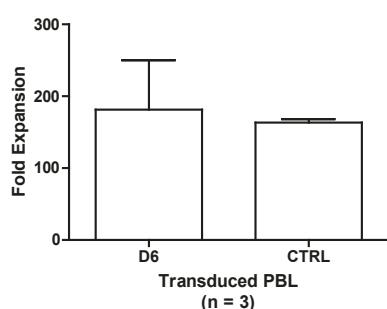
**Figure 2**



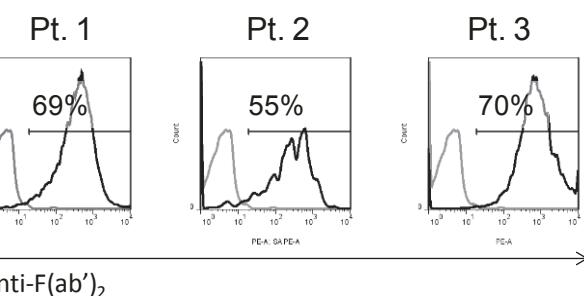
**Figure 2. Expression of mesothelin on human tumor cell lines.** Indicated cell lines were incubated with recombinant biotinylated MN monoclonal antibody (a mouse anti-human IgG specific for human mesothelin) or isotype control antibody (mouse IgG) followed by streptavidin-PE. Samples were analyzed by FACS. Black histograms indicated staining with the MN antibody and grey histograms, staining with isotype control.

**Figure 3**

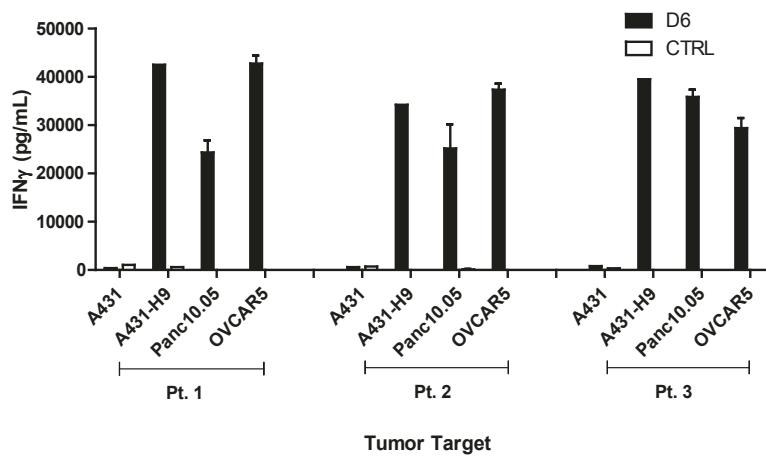
**A.**



**B.**



**C.**



**ace expression and functional analysis of CAR-transduced PBL following a REP**

**A) Expansion of CAR-transduced PBL.** CAR-transduced PBL (see Figure 1B) were transduced with a CAR construct that consists of a second stimulation with OKT3 antibody and cultured at a density of 1 x 10<sup>6</sup> mL in the presence of IL2 and irradiated feeders. On day 5 post-REP, fold expansion was measured. **B) Cell surface expression of SS1 CAR-transduced PBL following a REP.** CAR-transduced PBL were stained for CAR<sup>+</sup> cells using a biotinylated mouse anti-F(ab')<sub>2</sub> antibody and streptavidin-PE. Transduction efficiency in transduced PBL was represented as the percentage of CAR<sup>+</sup> cells. Data are from the same three separate patient's PBL as in Figure 1B. **C) Functional analysis of CAR-transduced PBL specifically secreted IFN $\gamma$  in response to mesothelin-positive tumor cell lines.** CAR-transduced effector cells (1 x 10<sup>5</sup>) and mesothelin-positive target cell lines (1 x 10<sup>5</sup>) were co-cultured in a 24-well plate (200 $\mu$ L) at 37°C and 5% CO<sub>2</sub>. Supernatants were harvested for enzyme-linked immunosorbent assay (ELISA) to detect IFN $\gamma$ . Data representative of 3 patient's PBL. CD19<sup>+</sup> B cells were used as controls in all experiments (data not shown).

**Appendix 1****ADVERSE EVENTS OCCURRING IN  $\geq 10\%$  OF PATIENTS TREATED WITH ALDESLEUKIN (n=525)<sup>1</sup>**

<b>Body System</b>	<b>% Patients</b>	<b>Body System</b>	<b>% Patients</b>
<i>Body as a Whole</i>			
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
<i>Cardiovascular</i>			
Hypotension	71	Hypocalcemia	11
Tachycardia	23	Alkaline phosphatase incr	10
Vasodilation	13	<i>Nervous</i>	
Supraventricular tachycardia	12	Confusion	34
Cardiovascular disorder <sup>a</sup>	11	Somnolence	22
Arrhythmia	10	Anxiety	12
<i>Digestive</i>			
Diarrhea	67	Dizziness	11
Vomiting	50	<i>Respiratory</i>	
Nausea	35	Dyspnea	43
Stomatitis	22	Lung disorder <sup>b</sup>	24
Anorexia	20	Respiratory disorder <sup>c</sup>	11
Nausea and vomiting	19	Cough increase	11
<i>Hemic and Lymphatic</i>			
Thrombocytopenia	37	Rhinitis	10
Anemia	29	<i>Skin and Appendages</i>	
Leukopenia	16	Rash	42
		Pruritus	24
		Exfoliative dermatitis	18
<i>Urogenital</i>			
		Oliguria	63

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

<sup>1</sup>Source: Proleukin® Prescribing Information - June 2007

## Appendix 2

### Expected IL-2 Toxicities and their Management

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

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

\*Unless the toxicity is not reversed within 12 hours

\*\* Unless the toxicity is not reversed to grade 2 or less by next treatment.

## Appendix 3

### Interleukin-2 toxicities observed in patients treated at the NIH Clinical Center

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	*	19	214	66
Number of Courses	236	85	210	35	*	30	348	95
Number of Courses								1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1-6.0	126	49	97	21	18	190	46	547
6.1-10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19	—	2	3	1	12	5	42
Weight gain (% body weight)								
0.0-5.0	106	23	65	8	9	117	49	377
5.1-10.0	78	41	111	22	10	148	26	436
10.1-15.0	43	17	26	3	9	62	15	175
15.1-20.0	7	3	8	1	1	15	3	38
20.1+	2	1	—	1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1-6.0	148	43	121	20	14	237	54	637
6.1-10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrhythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1-15	77	16	53	9	6	176	40	377
6-10	22	1	5	3	2	53	9	95
11-15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm <sup>3</sup> )								
<20,000	28	1	2	4	6	71	19	131
20,001-60,000	82	11	62	14	12	150	30	361
60,001-100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

\* Eleven patients are in two protocols.

## Appendix 4

### **Certificate of Analysis:** **Infused T cells transduced with anti-mesothelin CAR**

Date of preparation of final product:

Patient:

Tests performed on final product:

<i>Test</i>	<i>Method</i>	<i>Limits</i>	<i>Result</i>	<i>Tests Performed by</i>	<i>Initials /Date</i>
Cell viability <sup>1</sup>	trypan blue exclusion	>70%			
Total viable cell number <sup>1</sup>	visual microscopic count	Between 10 <sup>6</sup> and 5 x 10 <sup>10</sup> cells			
Identity	FACs analysis on final expanded cells <sup>7</sup>	> 80 % CD3+ after transduction			
Tumor reactivity <sup>2</sup>	γ-IFN release vs. cell line expressing mesothelin	>200 pg/ml and > 2 times background			
CAR expression <sup>2</sup>	FACS analysis of the transduced cells	PBL, >30%			
Microbiological studies	gram stain <sup>1,3</sup> ,	no micro-organisms seen			
	aerobic culture <sup>3,4</sup>	no growth			
	fungus culture <sup>3,4</sup>	no growth			
	anaerobic culture <sup>3,4</sup>	no growth			
	mycoplasma test <sup>5</sup>	negative			
Endotoxin	limulus assay <sup>1</sup>	≤ 5 E.U./kg			
RCR	S+L- Assay <sup>4</sup> RCR-PCR <sup>6</sup>	negative			

<sup>1</sup> Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.

<sup>2</sup> Performed 2-10 post transduction. Results are available at the time of infusion.

<sup>3</sup> Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

<sup>4</sup> Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.

<sup>5</sup> Performed 2-10 days prior to infusion. Results are available at the time of infusion.

<sup>6</sup> Performed on sample approximately 1-4 days prior to infusion. Results are available at the time of infusion.

<sup>7</sup> Performed 2-10 days prior to infusion. Results are available at the time of infusion. REP cells will be tested if intended for infusion.

Prepared by: \_\_\_\_\_ Date: \_\_\_\_\_

QC sign-off: \_\_\_\_\_ Date: \_\_\_\_\_  
Qualified Laboratory or Clinical Supervisor