Abbreviated Title: Anti-MAGE-A3-DP4 TCR PBL
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PROTOCOL TITLE

A Phase I/II Study of the Treatment of Metastatic Cancer that Expresses MAGE-A3 Using Lymphodepleting Conditioning Followed by Infusion of HLA-DP0401/0402 Restricted Anti-MAGE-A3 TCR-Gene Engineered Lymphocytes and Aldesleukin

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Investigational Agent:

Drug Name:	Anti-MAGE-A3-DP4 TCR PBL
IND Number:	15770
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 both α and □β chains of a T cell receptor (TCR) that recognizes the DP0401/0402 restricted MAGE-A3 tumor antigen, which can be used to mediate genetic transfer of this TCR with high efficiency.
- In co-cultures with HLA-DP0401/0402 and MAGE-A3 double positive tumors, the anti-MAGE-A3- DP0401/0402 restricted (anti-MAGE-A3-DP4) TCR transduced T cells secreted significant amounts of IFN-γ(with high specificity.

Objectives:

- Primary objectives:
  - Determine a safe dose of the administration of autologous CD4 cells transduced with an anti-MAGE-A3-DP0401/0402 restricted (MAGE-A3-DP4) TCR and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen.
  - Determine if this approach will result in objective tumor regression in patients with metastatic cancer expressing MAGE-A3-DP4.
  - Determine the toxicity profile of this treatment regimen.

Eligibility:

Patients who are HLA-DP0401/0402 positive and 18 years of age or older must have:

- Metastatic cancer whose tumors express the MAGE-A3-DP4 antigen;
- Previously received and have been a non-responder to or recurred following at least one first line treatment for metastatic disease;

Patients may not have:

- Contraindications for high dose aldesleukin administration.

Design:

- PBMC obtained by leukapheresis will be enriched for CD4 cells and transduced with the retroviral vector supernatant encoding the anti-MAGE-A3-DP4 TCR.
- The study will begin in a standard phase 1 dose escalation. After the MTD cell dose has been determined, patients will be enrolled into the phase 2 portion of the trial at the MTD established during the phase 1 portion of the study. In the phase 2 portion, patients will be entered into two cohorts: cohort 1 will include patients with metastatic melanoma; cohort 2 will include patients with renal cancer and other types of metastatic cancer.
- Patients will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine followed by intravenous infusion of ex vivo tumor reactive, TCR gene-transduced PBMC plus IV aldesleukin.
- Patients will undergo complete evaluation of tumor response every 1-6 months until off study criteria are met.

- For each of the 2 strata evaluated in the phase 2 portion, the study will be conducted using a phase 2 optimal design where initially 21 evaluable patients will be enrolled. For each of these two arms of 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.
- For both strata, the objective will be to determine if the treatment regimen is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

- Determine a safe dose of the administration of autologous CD4 cells transduced with an anti-MAGE-A3-DP0401/0402 restricted (MAGE-A3-DP4) TCR and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen.
- Determine if this approach will result in objective tumor regression in patients with metastatic cancer expressing MAGE-A3-DP4.
- Determine the toxicity profile of this treatment regimen.

1.1.2 Secondary Objective

- Determine the in vivo survival of TCR 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. The goal of this protocol is to transfer MAGE-A3 reactive TCR genes 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 that have used HLA-Class I restricted TCRs except that in this trial we will use an HLA-Class II (DP0401/0402) restricted TCR reactive with a MAGE-A3 epitope expressed on the tumors of patients with melanoma as well as patients with common epithelial malignancies.

1.2.1 Surgery Branch Trials of Cell Transfer Therapy Using Transduction of Anti-Tumor Antigen TCR Genes into PBL

We have studied approaches to transduce genes encoding antigen specific TCRs genes into PBL as a method to generate large numbers of reactive anti-cancer T cells.

In earlier studies we treated 24 patients with metastatic melanoma using autologous PBL transduced with high-avidity MART-1 F5 TCR following a non-myeolablative chemotherapy [1, 2]. Six patients (25%) achieved an objective partial response though 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. We have also conducted a clinical trial with a TCR that recognizes the gp100:154-162 melanoma peptide[2]. This TCR was raised in an HLA-A2 transgenic mouse immunized with this peptide. We treated 21 patients with metastatic melanoma using autologous PBL transduced with this gp100 TCR following a non-myeloablative chemotherapy. Four patients (19%) 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 the trial. We are no longer using these TCRs in clinical studies.

At the time of this protocol, we have performed several additional studies utilizing peripheral blood lymphocytes transduced with either TCR or chimeric antigen receptors (CAR) genes targeting putative tumor antigens following a non-myeloablative chemotherapy regimen. In protocol 08-C-0121, 10 0f 19 patients (53%) with metastatic melanoma treated with autologous PBL genetically engineered to express an anti-NY-ESO-1 TCR experienced objective responses including four patients with complete responses, three of which are ongoing from 24 to 50 plus months[3]. Similarly, 15 patients with metastatic synovial cell sarcoma also received autologous genetically engineered cells expressing the anti-NY-ESO-1 TCR and 10 (67%) experienced objective regressions including one ongoing complete regression at five months[3]. In study 09-C-0082, we have recently treated 12 patients with autologous PBL transduced with genes encoding an anti-CD19 CAR. Four of these patients have ongoing complete regressions from 4 to 19 plus months and five additional patients have ongoing partial responses at 2 to 20 plus months. In both of these protocols only patients that have been heavily pre-treated with standard therapy have been admitted (early results published in [4, 5].

Most toxicities observed in these studies were expected toxicities of the chemotherapy and aldesleukin administration. However, in 2 studies, we have observed serious adverse events related to the transduced cells. 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[6]. In 09-C-0047 (anti-CEA TCR transduced PBL), all three treated patients experienced diarrhea, and colitis and one patient experienced an objective response of liver metastases[7]. All gastrointestinal side effects resolved in these patients 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.

1.2.2 MAGE-A3 as a target for cell transfer clinical studies

Because of our success using the anti-NY-ESO-1 TCR we have explored targeting other cancer-testes antigens. Cancer testis antigens (CTA) are proteins, which are normally only expressed in the placenta and in non-MHC expressing germ cells of testis, yet are aberrantly expressed in many tumors; thus CTA may represent ideal targets for tumor immunotherapy. More than 110 CTA genes or gene families have been identified that are expressed in multiple tumor types [8-10]. These proteins are being vigorously pursued as targets for therapeutic cancer vaccines, and TCR based adoptive immunotherapy [11-13]. In theory, targeting T cells against tumor associated CT antigens might selectively eliminate tumor cells while avoiding toxicity to normal tissue.

Since the identification of the first human MAGE CT gene in 1991, the number of MAGE family genes has grown to over 25 members [14, 15]. MAGE-A is a multigene family consisting of 12 homologous genes, MAGE-A1 to A12 located at chromosome Xq28[10]. Genomic clustering, restricted expression pattern and single exon open reading frames of the MAGE genes are consistent with the possibility that these genes evolved from retrotransposition and subsequent duplication[16]. The precise function and biological role of MAGE proteins are not completely elucidated. However, members of MAGE-A, B and C proteins have been implicated in the suppression of p53-dependent apoptosis [17, 18] and MAGE-A3 has been attributed to mediate fibronectin-controlled tumor progression and metastasis[19]. Expression of CTAs including MAGE genes in tumor cells has been attributed to global DNA demethylation and other mechanisms that normally silences these genes in somatic cells[20]. MAGE-A3 is one of the more frequently expressed CT antigens in human tumors, including melanoma[21], non-small cell lung carcinoma[22], head and neck squamous cell carcinoma[23], pancreatic cancer[24], hepatocellular carcinoma[25], non-Hodgkin lymphoma[26], and multiple myeloma[27]. The expression of MAGE-A3 has been shown to be higher in more advanced stages of disease, and is associated with poor disease prognosis [28-30]. Several antigenic peptides that bind to HLA class I or class II molecules on tumor cells have been reported [31-37]. Because of its high expression in a wide array of tumor types, MAGE-A3 was chosen as the target for cancer immunotherapy.

A high avidity Class I restricted TCR against MAGE-A3 was isolated using a transgenic mouse model that expressed the human HLA-A*0201 molecule. A retrovirus encoding the alpha and beta chains of this TCR that recognized the Class I restricted MAGE-A3 epitope (aa112-120) was constructed. Nine cancer patients were treated with adoptive cell therapy using autologous anti-MAGE-A3 T-cell receptor (TCR) engineered T cells. Five patients experienced clinical regression of their cancers including 2 on-going responders. However, postinfusion, 3 patients experienced mental status changes, and 2 patients lapsed into comas and subsequently died[38]. The TCR used in this study recognized epitopes in MAGE-A3/A9/A12. Molecular assays of human brain samples using real-time quantitative-polymerase chain reaction, Nanostring quantitation, and deep-sequencing indicated that MAGE-A12 was expressed in human brain and our TCR recognized an epitope in MAGE-A12 that was similar to the MAGE-A3 epitope being targeted. This previously unrecognized expression of MAGE-A12 in human brain was possibly the initiating event of a TCR-mediated inflammatory response that resulted in neuronal cell destruction. There was no evidence of MAGE-A3 expressed in the brain. In a protocol using a TCR that recognized HLA-A1 Class I restricted epitope from MAGE-A3, cardiac toxicity was seen that was attributed to changes introduced into the binding regions of the[39] TCR, which added new specificities resulting in cardiac rejection. These changes were thought to be the cause of the off target toxicity. This study will use an unmodified human receptor and thus we do not expect to see the same off target toxicities.

However, other MAGE-A3 epitopes may be good candidates for tumor immunotherapy because of the high percentage of tumors that express MAGE genes, (for example >60% of melanomas and >50% of NSCLC) and large numbers of patients can be eligible for treatment. Finally MAGE-A3 has not been found to be expressed in any normal tissue except testis and this may limit the risk of on-target toxicity to normal tissue.

1.2.3 Rationale for Targeting a Class II Restricted Epitope of MAGE-A3 recognized by CD4 T Cells

The ability of $CD8^+$ T cells that have been genetically modified to express TCRs that recognize HLA class I restricted antigens to mediate tumor regression has now been demonstrated in a series of clinical trials that target the melanocyte differentiation antigens (MDAs) MART-1 and gp100 [38, 40], as well as the cancer germ-line antigens MAGE-A3[2] and NY-ESO-1[3]. Nevertheless, durable complete tumor regressions were observed in only a relatively small percentage of patients treated in these trials. Multiple factors may be responsible for the lack of durable responses seen in these patients, including insufficient persistence of transferred cells, heterogeneity of antigen and HLA class I expression, as well as the presence of host factors that suppress the response of adoptively transferred T cells. In addition, $CD4^+$ T helper cells have been found to play a role in mediating anti-tumor immunity in murine tumor model systems, raising the possibility that lack of sufficient T cell help may impair the effectiveness of human therapies that have focused on the use of class I restricted TCRs. Tumor-reactive $CD4^+$ T cells that recognize the murine leukemia virus epitopes [41, 42] as well as the TRP-1 melanocyte differentiation antigen[43] have been shown to be effective in mediating the regression of established murine tumors. Additional findings indicate that $CD4^+$ T cells can enhance and maintain *in vivo* responses of tumor-reactive $CD8^+$ T cells[44, 45].

A variety of HLA class II restricted antigens expressed on human tumor cells have been identified, providing an opportunity to evaluate the effectiveness of CD4+ T cells in mediating tumor regression in patients. Multiple epitopes derived from MDAs, including MART-1[46], tyrosinase[47], gp100 [47-49] and TRP-1 [50, 51] have been shown to be recognized by human $CD4^+$ tumor reactive T cells. In addition, HLA class II restricted epitopes derived from multiple cancer germ-line gene products, including MAGE-A3 [32, 34, 52, 53], MAGE-A1[54], NY-ESO-1 [55, 56], SSX-2[57] and SSX-4[58], have been identified by stimulating T cells *in vitro* with either recombinant proteins or peptides derived from these products. In the only clinical trial to specifically target an HLA class II restricted antigen, an autologous $CD4^+$ T cell clone that recognized an HLA-DP*04-restricted epitope of NY-ESO-1 mediated tumor regression in a patient with melanoma[59]. This finding provided an impetus to further investigate the role of $CD4^+$ tumor reactive T cells in mediating tumor regression.

We thus focused on the isolation of class II restricted TCRs directed against the MAGE-A3 cancer germ-line antigens which is expressed in a variety of tumor histologies [60, 61]. In previous studies aimed at identifying HLA class II restricted MAGE-A3 T cell epitopes, PBMC were stimulated with autologous dendritic cells (DCs) that had been pulsed with recombinant MAGE-A3 protein[34]. A single $CD4^+$ T cell clone was identified in this study, clone 22, that recognized autologous EBV B cells that were either pulsed with recombinant MAGE-A3 protein or transduced with a recombinant retroviral construct encoding the MAGE-A3 protein. Further analysis lead to the identification of a peptide corresponding to amino acids 243-258 of the MAGE-A3 protein, KKLLTQHFVQENYLEY (MAGE-A3:243-258), that was recognized in association with two HLA class II allele DPβ1 alleles, *0401 and *0402. The DPβ1*0401 allele is expressed by approximately 70% of Caucasians, whereas the closely related DPβ1*0402 allele, which differs from the 0401 allele by four amino acids, is expressed by approximately 20% of Caucasians. Further analysis indicated that the minimal epitope recognized by clone 22 corresponded to the 12-mer TQHFVQENYLEY (MAGE-A3:247-258). Targets cells expressing DPβ1*0401 that were either pulsed with the MAGE-A3:247-258 peptide or transduced with the

MAGE-A3 gene were strongly recognized by clone 22, whereas targets expressing the DPβ1*0402 allele were recognized to a lower but nevertheless significant extent. Subsequently, a patient clinical trial was carried out involving immunization of patients with MAGE-A3 protein in combination with a group of HLA class I and class II restricted peptides that included the MAGE-A3:243-258 HLA-DPβ1*04 restricted peptide that were selected on the basis of the patients' HLA haplotype[62]. Although objective responses were not observed in this trial, subsequent *in vitro* studies were carried out to evaluate the phenotype and function of T cell clones from HLA-DPβ1*0401 or 0402$^+$ patients that were isolated from the peripheral blood of immunized patients using a HLA-DP*04 tetramer containing the MAGE-A3:243-258 peptide. This procedure lead to the isolation of CD4$^+$ MAGE-A3:243-258 reactive T effector clones that released IFN-γ and TNF-α following antigen stimulation, as well as T cell clones that appeared to represent CD4$^+$ regulatory T cells (CD4$^+$ Tregs) that recognized the same T cell epitope. These MAGE-A3 reactive CD4$^+$ Treg clones failed to release effector cytokines following specific antigen stimulation, but were capable of suppressing effector T cell responses in response to stimulation with the MAGE-A3:243-258 peptide.

Given the high prevalence of the HLA-DP*04 HLA class II allele in the patient population, as well as the high frequency of MAGE-A3$^+$ tumors in patients with melanoma as well as other malignancies, we attempted to identify potent TCRs directed against the MAGE-A3:243-258 HLA-DP*04 restricted epitope. Initially, we isolated the endogenous TCR alpha and beta chains expressed by two of the MAGE-A3:243-258-reactive effector CD4$^+$ T cell clones, clones R12C9 and 12C7, as well as a CD4$^+$ Treg clone reactive with this epitope, each of which was characterized in the MAGE-A3 cancer vaccine trial study described above (kindly provided by Dr. Pierre van der Bruggen). Following the cloning of the TCR alpha and beta chains isolated from the three MAGE-A3 reactive clones they were then joined with the P2A self-cleaving peptide sequence and cloned in the MSGV1 retroviral vector. Three patient PBMC that had been stimulated with OKT3 were then transduced with transient retroviral supernatants on day two and enriched for CD4$^+$ T cells on day seven, and assayed for their ability to recognize target cells on day 12. Analysis of the levels of transduction carried out by staining transduced cells with anti-Vβ22, Vβ6.7 and Vβ12 which detect the 6F9, R12C9 and 12A7 TCR (all kindly provided by Dr. Pierre van der Bruggen) indicated that the levels of transduction were similar, ranging between 25 and 35% (**Figure *1***a). The results of co-culture assays indicated that T cells transduced with either the 6F9, R12C9 or the 12C7 TCR strongly recognized HLA-DP*0401$^+$ target cells pulsed with the MAGE-A3:243-258 peptide (**Figure *1***b). In contrast, CD4$^+$ T cells transduced with the 6F9 but not the R12C9 or 12C7 TCRs recognized HLA DP*0401$^+$ 293-CIITA cells transfected with genes encoding MAGE-A3 or MAGE-A6, but not MAGE-A1 or A12 (**Figure *1***b). Comparison of amino acid sequences of the corresponding regions of the MAGE family members indicated that MAGE-A3 and MAGE-A6 only differed at one position (residue 249), whereas the other MAGE family members differed from MAGE-A3 at 2 (MAGE-A12) or 3 (MAGE-A1) positions (**Table *1***a). In addition, CD4$^+$ T cells transduced with the 6F9 TCR but not the R12C9 or 12C7 TCRs recognized the MAGE-A3$^+$/HLA-DP*0401$^+$ melanoma cell line 1359 mel-CIITA but failed to recognize the MAGE-A3$^+$/HLA-DP*0401$^-$ melanoma cell line 624 mel-CIITA. CD4$^+$ T cells transduced with the R12C9 TCR failed to recognize either of the tested melanoma cell lines, whereas cells transduced with the 12C7 TCR weakly recognized 624 mel-CIITA but not 1359 mel-CIITA cells (**Figure *1***b). Titration of the MAGE-A3:243-258 peptide indicated that CD4$^+$ T cells transduced with the 6F9 and R12C9 TCRs released

comparable levels of IFN-γ in response to targets pulsed with a minimum of between 0.001 and 0.01 mg/mL of the MAGE-A3:243-258 peptide, whereas cells transduced with the 12C7 TCR appeared to release significantly less IFN-γ than those transduced with the other two TCRs (**Figure *1***c). These results indicated that while $CD4^+$ T cells transduced with any of the three MAGE-A3 reactive TCRs 6F9, R12C9 and 12C7 TCR recognized peptide pulsed target cells, only cells transduced with the 6F9 TCR recognized transfected target cells as well as $MAGE\text{-}A3^+$ and $HLA\text{-}DP*04^+$ tumor cells.

The 6F9 TCR was further evaluated by determining the reactivity of $CD4^+$ and $CD8^+$ T cells separated from two patient PBMCs against a panel of tumor cell lines. Five melanoma cell lines that expressed MAGE-A3 and HLA-DP*0401, as well as the non-small cell lung carcinoma cell line H1299 NSCLC-CIITA were recognized by transduced $CD4^+$ and $CD8^+$ T cells, although $CD4^+$ T cells secreted higher amounts of cytokine in response to tumor targets than transduced $CD8^+$ T cells (**Figure 2A**). Prior studies have demonstrated higher reactivity of human TCRs in which sequences encoding the human TCR alpha and beta chain constant regions had been replaced with those encoding the corresponding murine constant regions[63]. The effects of replacing the constant regions of the 6F9 TCR alpha and beta chains with murine sequences was then evaluated by constructing a retroviral vector encoding the murine constant regions, designated 6F9mc. Comparisons of responses of CD4+ T cells transduced with the normal 6F9 TCR with those transduced with the 6F9mc TCR indicated that the murine constant regions resulted in between two and five-fold enhancement in the response of transduced T cells against the seven $MAGE\text{-}A3^+$ and $HLA\text{-}DP*0401^+$ targets that were evaluated (**Figure 2B**). In addition, the response of $CD8^+$ T cells transduced with the 6F9mc were enhanced by between two and ten-fold above those seen in cells transduced with the native 6F9 TCR. The responses of $CD8^+$ T cells transduced with the 6F9mc were generally lower than $CD4^+$ T cells transduced with this TCR, although comparable cytokine responses were observed in responses to some tumor targets (**Figure 2C**).

In order to evaluate the fine specificity of antigen recognition mediated by cells transduced with the 6F9 and 6F9mc TCR, $HLA\text{-}DP*0401^+$ target cells were pulsed with truncations of the MAGE-A3:243-258 peptide and related peptides from MAGE family members. Analysis of the response to truncated MAGE-A3 peptides from two cultures of transduced $CD4^+$ PBMC indicated that the 11-mer peptide QHFVQENYLEY corresponding to amino acids 248-258 of the MAGE-A3 protein represented the minimal peptide that elicited a response comparable to that elicited by the MAGE-A3:243-258 peptide (**Table 1B**) that was nearly identical to the minimal epitope that was previously described but that lacked the amino terminal threonine residue present in that peptide[34]. The MAGE-A3:243-258 peptide was predicted using an epitope prediction algorithm to possess a high affinity for HLA-DP*0401, and in addition, recognition of the truncated MAGE-A3 peptides appeared to correlate with T cell recognition (**Table 1B**). Significant recognition of the MAGE-A6:248-258 peptide that contained a single substitution of tyrosine for histidine at position 249, was seen but minimal reactivity was observed against additional members of the MAGE family of gene products that possessed between two and five differences from the MAGE-A3:248-258 peptide. A BLAST search of the NCBI database revealed that the most closely related peptide in the human genome was derived from the protein necdin. This peptide, which possessed five differences from the MAGE-A3:248-258 peptide, was also not recognized by T cells transduced with the 6F9 or 6F9mc TCR. These findings indicate that the 6F9 TCR possesses a high degree of specificity for the MAGE-

A3:248-258 peptide and suggest that T cells transduced with this TCR may possess little or no cross-reactivity with peptides derived from additional human proteins.

To evaluate the anti-MAGE-A3 DP4 TCR at clinical scale, CliniMACs clinical-scale cell separation apparatus was used to deplete $CD8^+$ lymphocytes from PBL. CD8-depleted PBL were stimulated for 2 days with OKT-3 antibody and then transduced twice by anti-MAGE-A3 DP4 TCR retrovirus. Flow cytometric analysis of transduced PBL showed that 92.0% of $CD4^+$ T cells were transduced with this TCR vector, detected by anti-mouse TCRβ antibody (**Figure 3**). In addition, 96.1% of transduced PBL were $CD4^+$ T cells, with less than 0.1% $CD8^+$ T cells. To assess the specific recognition of tumor cells, TCR-engineered $CD4^+$ T cells were co-cultured with HLA-$DP0401^+$ MAGE-$A3^+$ target cells or MAGE-A3 peptide-pulsed cells. High levels of IFN-γ were observed from the co-culture wells with specific target cells but not negative controls (**Figure 4A**). Furthermore, TCR-engineered $CD4^+$ T cells were rapidly expanded one time to achieve sufficient cell numbers for patient infusion, and the same biological assays were performed. Similar results were obtained, with 92.6% of transduced T cells and 99.2% of $CD4^+$ T cells (**Figure 3**). Specific release of IFN-γ from TCR-engineered $CD4^+$ T cells was also observed (**Figure 4B**).

1.2.4 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 as described below. The non-myeloablative chemotherapy and the administration of high-dose aldesleukin have expected toxicities which are discussed in Section 6 of this study. The nonmyeloablative chemotherapy used in this protocol has been administered to over 300 patients and all have reconstituted their hematopoietic systems.

In other protocols we have administered over 3 X 10^{11} TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. 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, WAS and X-CGD 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 locally advanced refractory/recurrent cancer that expresses MAGE-A3 as assessed by one of the following methods: RT-PCR on tumor tissue defined as 30,000 copies of MAGE-A3 per 10^6 GAPDH copies, or by immunohistochemistry of

resected tissue defined as 10% or greater of tumor cells being 2-3+ for MAGE-A3, or serum antibody reactive with MAGE-A3. Metastatic cancer diagnosis will be confirmed by the Laboratory of Pathology at the NCI.

b. Patients must have previously received prior first line standard therapy (or effective salvage chemotherapy regimens) for their disease, if known to be effective for that disease, and have been either non-responders (progressive disease) or have recurred.
c. Patients must be HLA-DP4 positive.
d. Patients with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for 1 month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.
e. Greater than or equal to 18 years of age and less than or equal to age 70.
f. Ability of subject to understand and the willingness to sign the Informed Consent Document
g. Willing to sign a durable power of attorney
h. Clinical performance status of ECOG 0 or 1
i. 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.
j. Serology:
    - 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.)
    - 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.
k. Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.
l. Hematology
    - Absolute neutrophil count greater than $1000/mm^3$ without the support of filgrastim
    - WBC $\geq 3000/mm^3$
    - Platelet count $\geq 100{,}000/mm^3$
    - Hemoglobin > 8.0 g/dL
m. Chemistry:
    - Serum ALT/AST $\leq$ to 2.5 times the upper limit of normal
    - Serum creatinine $\leq$ to 1.6 mg/dL
    - Total bilirubin $\leq$ to 1.5 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
n. 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). Patients must have progressing disease after prior treatment. Note: Patients who have previously received ipilimumab and have documented GI toxicity must have a normal colonoscopy with normal colonic biopsies.
o. Subjects must be co-enrolled in protocol 03-C-0277.

2.1.2 Exclusion Criteria

a. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.
b. Active systemic infections (e.g.: requiring anti-infective treatment), coagulation disorders or any other active major medical illnesses.
c. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
d. 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).
e. Concurrent systemic steroid therapy.
f. History of severe immediate hypersensitivity reaction to any of the agents used in this study.
g. History of any cardiac events including coronary revascularization or ischemic symptoms.
h. Documented LVEF of less than or equal to 45% testing is required in patients who are
    - ≥ 65 years' old
    - Clinically significant atrial and or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block or have a history of ischemic heart disease, or chest pain.
i. 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
j. 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 Prior to Enrollment

a) HIV antibody titer and HBsAg determination, anti HCV (may be performed within 3 months of chemotherapy start date).
b) Confirmation of HLA-DP4 positivity. (Note: Testing is permitted to be conducted at any time prior to enrollment.)
c) Confirmation of the diagnosis of cancer and MAGE A3 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 for patients with a prolonged history of cigarette smoking (20 pk/year of smoking within the past 2 years) or symptoms of respiratory dysfunction.
b) Cardiac Evaluation (stress thallium, echocardiogram, MUGA etc.,) for patients who are greater than or equal to age 60, or 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 LEVF 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, obesity).

2.2.3 Within 4 Weeks Prior to Enrollment:

a. Complete history and physical examination, including weight, vital signs, and noting in 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 tests:
   - Chemistries: Creatinine, ALT/GPT, AST/GOT, and Total Bilirubin
   - CBC with differential and platelet count
b. Urinalysis; urine culture, if indicated

2.2.5 Within 7 Days Prior to Enrollment:

- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- ECOG performance status of 0 or 1

2.3 REGISTRATION AND TREATMENT ASSIGNMENT PROCEDURES

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) prior to transduction of PBL cells (either fresh or cryopreserved samples), 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.

2.3.3 Treatment Assignment Procedures

Cohorts

Number	Name	Description

1	MAGE-A3 (Phase 1)	MAGE-A3-expressing metastatic or locally advanced refractory/recurrent cancer
2	Melanoma (Phase 2, Cohort I)	Metastatic melanoma
3	Renal and Other (Phase 2, Cohort 2)	Renal cancer and other types of metastatic cancer

Arms

Number	Name	Description
1	Phase 1 Experimental Therapy	Non-myeloablative lymphodepleting preparative regimen of cyclophosphamide and fludarabine + Anti-MAGE-A3-DP4 TCR PBL + high-dose aldesleukin
2	Phase 2 Experimental Therapy	Non-myeloablative lymphodepleting preparative regimen of cyclophosphamide and fludarabine + Anti-MAGE-A3-DP4 TCR PBL + high-dose aldesleukin

Cohort and Arm Assignment

- Subjects in Cohort 1 will be directly assigned to Arm 1.
- Subjects in Cohort 2 will either be assigned to Arm 1 or Arm 2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Performed on 03-C-0277

PBMC will be obtained by leukapheresis (approximately 1 X 10^{10} cells). $CD4^+$ cells will be enriched on Miltenyi columns as described in the IND and 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 X 10^7 to 5 X 10^8 cells to supernatant containing the anti-MAGE-DP4 retroviral vector. The CD4 enriched cells will then be further expanded in vitro prior to cell infusion. Successful TCR gene transfer will be determined by FACS analysis for the TCR protein and anti-tumor reactivity will be tested by cytokine release as measured on peptide pulsed $DP4^+$ cells. Successful TCR gene transfer for each transduced PBL population will be defined as >10% TCR positive cells and for biological activity, gamma-interferon secretion must be at least 200pg/ml, and > 2 times background.

3.1.2 Treatment Phase

Patients will receive no other experimental agents while on this protocol. Patients will receive the standard NCI Surgery Branch non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed by IV infusion of anti-MAGE-A3-DP4 TCR engineered PBL and aldesleukin. All 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. Patients may undergo a second treatment as described in Section **3.3**.

3.1.3 Dose Limiting Toxicity:

Dose-limiting toxicity is defined as follows: All grade 3 and greater toxicities with the exception of:

- Myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Aldesleukin expected toxicities as defined in **Appendix 2** and **Appendix 3**.
- Expected chemotherapy toxicities as defined in the Pharmaceutical Information section.
- 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 sequela 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

Patients who develop toxicity due to study agents will be followed until toxicity resolves to grade 2 or less, regardless of disease progression.

3.1.4 Phase I - Dose Escalation

Initially, the protocol will enroll 1 patient in each dose level unless a patient experiences a dose limiting toxicity (DLT). The total number of anti-MAGE -A3-DP4 engineered PBL cells transferred for each dose level will be:

Dose Level	**Dose**
Level 1	1.0×10^7 cells
Level 2	3.0×10^7 cells
Level 3	1.0×10^8 cells
Level 4	3.0×10^8 cells
Level 5	1.0×10^9 cells
Level 6	3.0×10^9 cells
Level 7	1.0×10^{10} cells
Level 8	3.0×10^{10} cells
Level 9	1.0×10^{11} cells and up to 2.0×10^{11} cells

In each dose level, if a patient experiences a DLT, a total of six patients will be treated at that dose to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher dose level. If a dose level with 2 or more DLTs in 3-6 patients has been identified, patients will be accrued at the next-lowest dose level, for a total of 6. If a dose limiting toxicity occurs in the first dose level, that dose level will be expanded to n=6 patients. If two DLTs occur in the first dose level, the study may be amended to treat patients at lower doses. If escalation is

permitted until Dose Level 9, it will be expanded to 6 patients unless it has 2 or more with DLTs before the 6th patient has been evaluated.

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.

A two-week safety assessment period following cell administration will be conducted between each dose level and before enrollment into the phase 2 portion of the protocol.

3.1.5 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 MAGE-A3-DP4 TCR transduced PBL.

The phase 2 portion of the protocol will proceed using from 10^9 cells up to the MTD as determined in the phase 1 portion of the study.

In the phase 2 portion of this study, patients will be entered into two cohorts based on histology: cohort 1 will include patients with metastatic melanoma; cohort 2 will include patients with renal cancer and other types of metastatic cancer.

3.1.6 Protocol Stopping Rules

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met, and discussions will be had with the FDA or NIH IRB regarding protocol revisions if applicable:

- If 1 or more treatment related death occur due to the cell infusion, we will promptly discuss this with the NIH IRB and the FDA.
- 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 one of the first three 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.
- During the phase 2 portion of the study - Once five or more patients have been enrolled, and if 20% or more patients cumulatively enrolled have developed a DLT, as described in Section **3.1.1**.

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. Chemotherapy infusions maybe slowed or delayed as medically indicated. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated.)

DAYS -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 <1 ml/kg/hr or if body weight >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 **Appendix 1**.

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) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Appendix 1**.

DAYS -7 to -3

Fludarabine 25 mg/m^2/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 **Appendix 1**. *(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 patient's PBL is 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 cell dose will be given 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.

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) 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. Doses 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 2**. Toxicities will be managed as outlined in **Appendix 3**. In addition, dosing may be held or stopped at the discretion of the treating investigator. (**Appendix 4** 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 (one 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 via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.
- Aldesleukin as described above.

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

- Beginning on day 1 or 2, filgrastim may 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 x10^9/L X 3 days or > 5.0 x10^9/L.
- Aldesleukin 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^2)	X	X	X	X	X							
Anti-MAGE-A3-DP4 TCR PBL								X[1]				
Aldesleukin								X[2]	X	X	X	X
Filgrastim[3] (5 mcg/kg/day)									X	X	X	X
TMP/SMX[4] 160mg/800 mg (example)								X		X		X
Fluconazole[5] (400 mg po)								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]One to four days after the last dose of fludarabine

[2]Initiate within approximately 24 hours after cell infusion

[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive days or > 5x10^9/L.

[4]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

[5]Continue until ANC > 1000/mm^3

[6]In patients positive for HSV or VZV continue until CD4 > 200 X 2

3.3 ON-STUDY EVALUATIONS

Note: Please refer to section 5 for research evaluations.

3.3.1 Within 14 days prior to starting the preparative regimen

- Apheresis as indicated
- Baseline blood test
    - 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
    - PT/PTT
    - TBNK
    - Thyroid Panel
    - Urinalysis
- Anti CMV antibody titer, HSV and VZV serology, and EBV panel. (may be performed within 3 months of chemotherapy)
- Chest x-ray
- EKG

3.3.2 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)

3.3.3 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
- Once total lymphocyte count is greater than 200/mm3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). **Please refer to section 5 for additional post cell infusion evaluations.**

3.3.4 During Hospitalization:

- **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
- Troponin levels on days 4 and 7 and as clinically indicated
- Other tests will be performed as clinically indicated.
- Vital signs and weight will be monitored as clinically indicated.

3.4 RETREATMENT

Patients experiencing a partial or complete response may receive a second treatment when progression by RECIST criteria is documented after evaluation by the principal investigator. Patients will be retreated at the currently enrolling dose level but will not count towards establishing the MTD. Patients who develop grade 3 or grade 4 toxicity due to cell infusion will not be retreated. Patients must continue to meet the original eligibility criteria to be considered for retreatment. Toxicity related to cyclophosphamide, fludarabine, or aldeskeukin should be stable and resolved to less than grade 1 prior to retreatment. Retreatment benefits and risks will be carefully explained to the patient. A maximum of 1 retreatment course may occur.

3.5 POST TREATMENT EVALUATION

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

3.5.1 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

3.5.2

At each scheduled 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

- PT/PTT
- Urinalysis as needed
- Thyroid panel as clinically indicated
- TBNK, until CD4 > 200 X 2
- Toxicity assessment, including a review of systems.
- Imaging (CT, MRI, and/or PET) as performed at baseline.
- A 5liter apheresis may be performed. If a patient is unable to undergo apheresis, approximately 96 ml of blood may be obtained at the first follow up visit. Subsequently, 60 ml of blood will be obtained at follow up visits 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 gene transduced cells (See Section **5.1.5**)
- 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 e-mail contacts. 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.

3.6 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, effort must be made to have all subjects complete an evaluation safety visit approximately 6 weeks (± 2 weeks) following administration of the cell product (at the first follow-up evaluation).

3.6.1 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 withdraw from active therapy
- Investigator discretion
- Positive pregnancy test

3.6.2 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
- Progressive disease, unless the patient is eligible for a second treatment
- 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 maybe followed on Protocol 09-C-0161 "Follow up Protocol for Subjects Previously Enrolled in Surgery Branch Studies".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 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 or 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^2 IV every 12 hrs 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 has 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 the CD4 count is greater than 200 for 2 consecutive measures.

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^3. 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.

4.1.4 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/mm3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

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^3. 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 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 antiemetics 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

5.1 CORRELATIVE STUDIES FOR RESEARCH:

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.

5.2 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.

5.3 SAMPLES SENT TO 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.

5.3.1 Prior to chemotherapy administration:

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

5.3.2 Prior to cell infusion (one 8ml SST):

- Baseline blood samples for cytokine analysis. – Figg's lab

5.3.3 Post cell infusion evaluations:

Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count 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 (8 ml each) – SB's lab
- 1 SST tube (8 ml) – Figg's lab

5.4 IMMUNOLOGICAL TESTING:

- Apheresis may be performed prior to and 4-6 weeks (+/- 2 weeks) following the administration of the cell product. 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 1) cryopreserved for immunological monitoring of cell function and 2) 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 MAGE-A3/12 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.

5.5 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.

- 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 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 envelope gene and are performed under contract by the Indiana University Vector Production Facility or other qualified testing facilities. The results of these tests are maintained by the contractor performing the RCR tests and by the Surgery Branch research team.

5.6 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 collected (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).

5.7 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR DR. FIGG'S LAB

5.7.1 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.).

5.7.2 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 (C3D) 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 adverse events (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 AEs until their first week 6 follow-up evaluation or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** 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.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and 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 Recording:

Patients will be receiving multiple agents which include commercially available agents (fludarabine, cyclophosphamide 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 PLAN

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. In addition to a baseline scan, confirmatory scans should also be obtained approximately 4 (not less than 4) weeks 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 cycle 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 cycle 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 cycle 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:

- By chest x-ray: ≥20 mm;
- By CT scan:
  - Scan slice thickness 5 mm or under as ≥10 mm with CT scan
  - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

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 ≥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 ≥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:

a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b) 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.
c) 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[1]

- 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[2]

- 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

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[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

6.3.5 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).

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
- A persistent or significant disability/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 Problem

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 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to the time of the first follow-up evaluation (6 weeks (± 2 weeks) following the administration of the cell product), the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 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:

- Deaths (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

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

7.3.1 Waivier 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 hypoxia, dyspnea, 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.

7.3.2 Reporting Pregnancy

7.3.2.1 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 one 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.

7.3.2.2 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.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 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 Anti-MAGE-A3-DP4 TCR 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-MAGE-A3-DP4 TCR 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.

7.4.2 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, the 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.

7.4.2.1 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 for each trial:

- 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.

7.4.2.2 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.

7.5 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 dose level enrollment and dose escalation if applicable 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 or a lead associate 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 a CCR 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

Following an initial portion of the study in which the safety of cells used in this protocol will be determined, the primary objective of this trial is to determine whether the combination of high dose aldesleukin, lymphocyte-depleting chemotherapy, and an infusion of anti-MAGE-A3-DP4-TCR-gene engineered lymphocytes is able to be associated with a modest fraction of patients that can experience a clinical response (PR +CR) to therapy. A secondary objective is to have sufficient patients in order to do exploratory evaluations of survival of cells.

The study will begin by evaluating the safety of escalating doses of cells from 10^7 to 10^{11}. Once a safe dose has been confirmed, patients will be enrolled into the phase 2 portion of the trial using the cell dose found to be safe in the first phase of this protocol.

Following the determination of a safe cell dose, the following will describe the phase 2 portion of the study.

Patients who express MAGE-A3-DP4 will be enrolled into individual strata depending on their specific histology. This stratification is being used to separate patients who have been shown historically to be aldesleukin sensitive (melanoma) from those who have not. In each case, the MAGE-A3-DP4 expression is expected to be so dominant in regulating potential for response that differences in clinical response according to histology will not be expected but will be evaluated in an exploratory manner to help assess this, in a pilot sense.

For each of the 2 strata, the study will be conducted using a phase 2 optimal design (Simon R, Controlled Clinical Trials 10:1-10, 1989). For both strata, the objective will be to determine if the combination of high dose aldesleukin, lymphocyte depleting chemotherapy, and anti-MAGE-A3-DP4-TCR-gene engineered lymphocytes is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

In patients in each of the two strata (melanoma vs. other histologies or renal carcinoma), 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 breast cancer or other 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.

The dose escalation portion of the study may require 6 patients per cohort. For purposes of sample size estimation, we will assume that as few as 9 and no more than 25 patients will be required to perform the initial safety evaluation. In order to complete the dose escalation phase and both phase 2 cohorts, a total of up to 25+82=107 patients may be required (25 + 2 strata with a maximum of 41 apiece). Up to 6 patients enrolled at the MTD will count towards the accrual in

the appropriate phase 2 strata if they are evaluable for response and if they would be fully eligible for enrollment in the phase 2 portion of the trial. Provided that about 4-5 patients per month will be able to be enrolled onto this trial, approximately 2 years may be needed to accrual the maximum number of required patients. However, as adequate responses to proceed to the second stage of accrual may not occur, the trial may end up accruing as few as 9 +42=57 patients if no arm demonstrates sufficient patients to accrue a second stage.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADAs #02716 and #03168) with Kite Pharma, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT 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 10.5), all subjects ≥ age 18 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 MAS 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/Discomforts

The experimental treatment has a chance to provide clinical benefit though it is not known if it will do so. The risks in this treatment are detailed in section 11. The success of this effort cannot be predicted at this time.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have metastatic cancer, which is refractory to standard therapy and limited life expectancies, the potential benefit is thought to outweigh the potential risks.

10.6 Consent Process and Documentation

If the patient has a tumor that is found to be HLA-DP4 positive and MAGE-A3 positive by immunohistochemistry, the patient is consented on protocol 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 Principal Investigator, associate investigator, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 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

11.1 INVESTIGATIONAL INVESTIGATION

11.1.1 Cell Preparation (ANTI-MAGE-A3-DP4 TRANSDUCED PBL)

The procedure for expanding the human PBL and the certificate of analysis 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 certificate of analysis is included in Appendix 5. The PBL will be transduced with retroviral supernatant containing the alpha chain and beta chain genes of the Anti-MAGE-A3-DP4.

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-Mage-A3-DP4 Gene

The retroviral vector supernatant [PG13-MAGE-A3 TCR encoding a T cell receptor directed against MAGE-A3-DP4, was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector, PG13-MAGE-A3 6F9mC TCR (B8), consists of 7,382 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, alpha chain and beta chain genes of the anti-MAGE-A3-DP4 TCR. The alpha and beta chains are linked by a P2A peptide. For clone selection, the physical titer will be determined by RNA dot blot. The physical titer of the clinical vector will be measured in transducing units per mL following a titration on human PBL according to sponsor protocols. The supernatant has been stored at the SBVPF upon the completion of production at –80 °C. A portion of the supernatant will be shipped on dry ice and stored –80 °C at Fisher Bioservices, Rockville, MD. This storage facility is equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in in vitro transduction. There will be no re-use of the same unit of supernatant 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://bmbl.od.nih.gov/sect3bsl2.htm.

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

How Supplied: Interleukin-2 (aldesleukin) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (–1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). 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 RIL-2. 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 dosage will be calculated based on total body weight. 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 2** and **Appendix 3**. 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 2**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 3**.

11.1.3 Fludarabine

(Please refer to package insert for complete product information)

Description: Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased 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 **Appendix 1**.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. 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 alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the NIH 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 **Appendix 1**.

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

11.2 SUPPORTIVE MEDICATIONS

11.2.1 Mesna

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

(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 **Appendix 1**. 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 on 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 (± 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 is 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 (± 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. Valcyclovir 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, pruritis, 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 pruritis. Consult the package insert for a complete list of all side effects.

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13 FIGURES, TABLES & APPENDICES

Figure 1

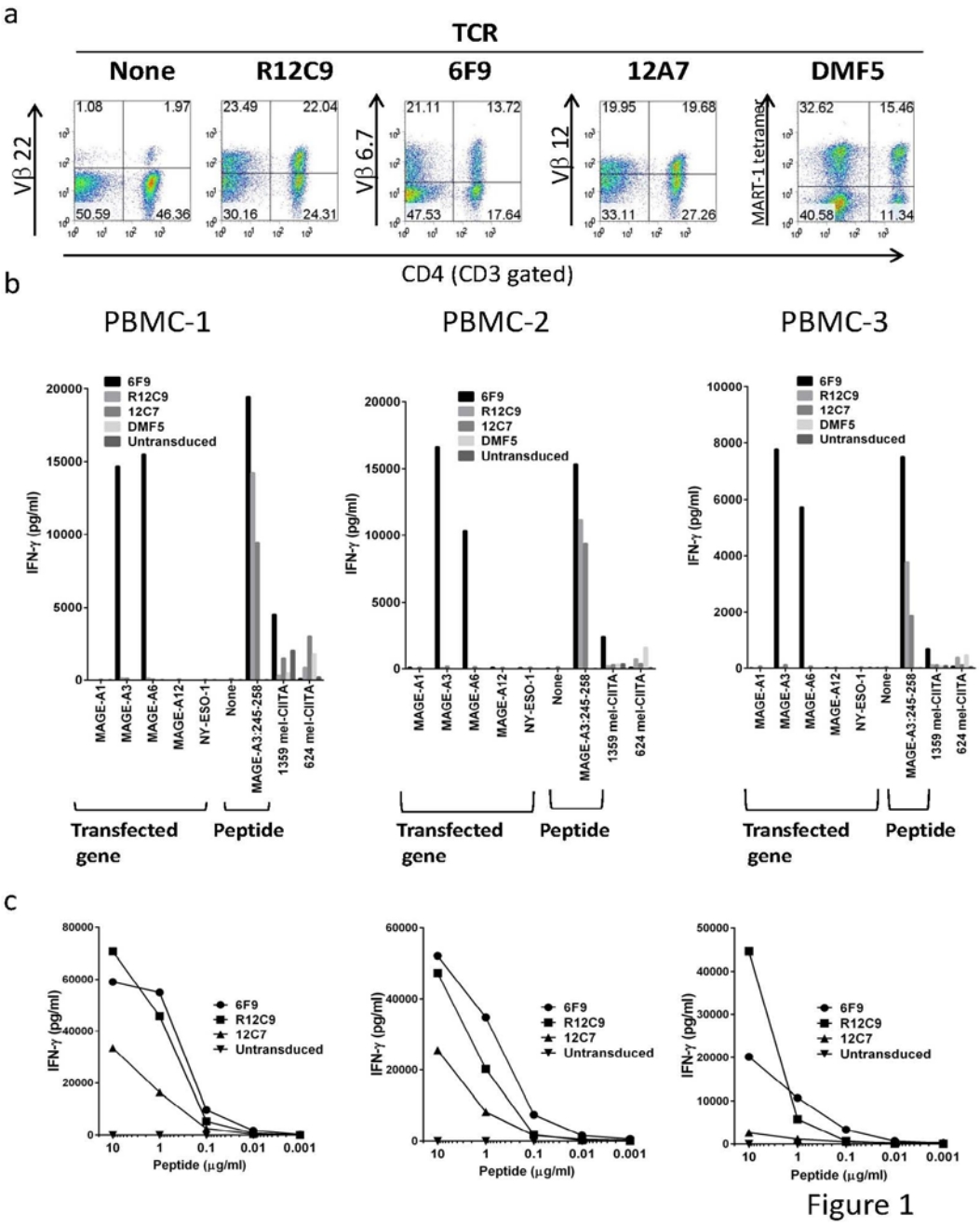


Figure 1

Figure 1. Comparison of reactivity of T cells transduced with three TCR that recognize the MAGE-A3:245-258 HLA class II restricted epitope **(a)** PBMC from three melanoma patients were stimulated with OKT3 and transduced on day 2, and evaluated six days later for expression of the transduced TCRs using the appropriate anti-TCR Vβ antibodies. Un-transduced cells incubated with the anti-Vβ22 antibody demonstrated relatively low levels of endogenous cell surface expression of this TCR. **(b)** $CD4^+$ T cells from the three patient PBMC that had been transduced with the 6F9, R12C9 and 12C7 TCRs were isolated by using a CD4 T lymphocyte enrichment kit (Life Technologies, Grand Island, NY). The isolated T cells (10^5) were cultured with either HLA-DP*0401^+ 293-CITTA cells (10^5) that were transiently transfected with plasmid constructed encoding individual MAGE family members, 293-CIITA cells that were pulsed with the 10 μg/mL of the MAGE-A3:245-258 peptide. In addition, transduced $CD4^+$ T cells were cultured with 10^5 1359 mel-CIITA cells, which express MAGE-A3 as well as HLA-DP*0401, or 624 mel-CIITA cells, which express MAGE-A3 but do not express HLA-DP*0401 or 0402. Following an overnight co-culture, the levels of soluble IFN-γ were evaluated by ELISA. **(c)** $CD4^+$ T cells transduced with the three MAGE-A3 TCRs (10^5) were incubated with 293-CIITA cells (10^5) that were pulsed with the indicated concentrations of the MAGE-A3:245-258 peptide for two hours at 37^0C. Following the incubation, the target cells were washed twice and co-cultured overnight with $CD4^+$ T cells from PBMC 1, and the release of soluble IFN-γ measured by ELISA.

Figure 2

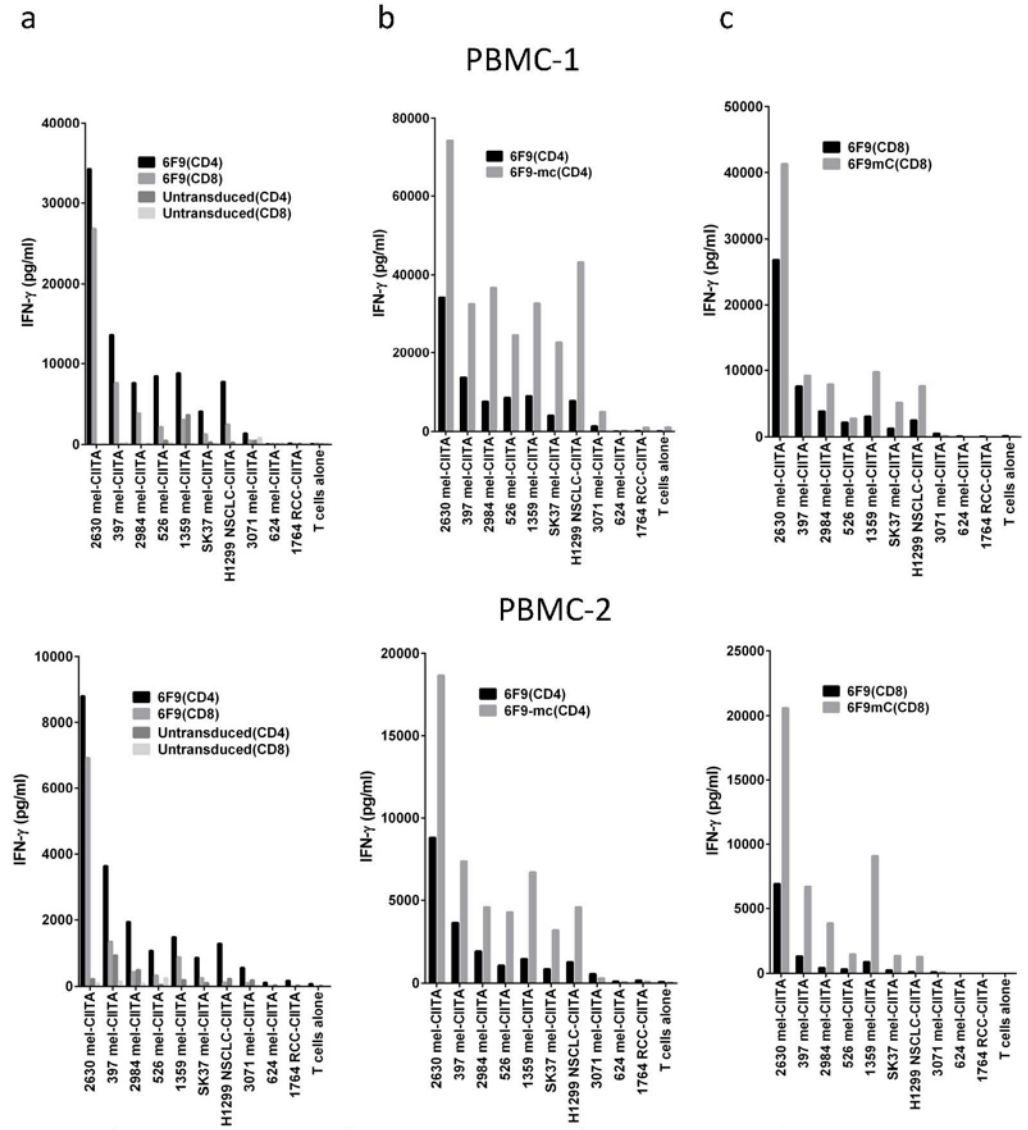


Figure 2

Figure 2. Comparison of responses of $CD4^+$ and $CD8^+$ T cells transduced with the 6F9 TCR or the 6F9 TCR containing murine constant region sequences (6F9mc). **(a)** Evaluation of responses of $CD4^+$ and $CD8^+$ T cells isolated from two patient PBMC against MAGE-$A3^+$ and MAGE-$A3^-$ tumor cells that express HLA-DP*0401, 0402, or lack expression of either HLA-DP allele. Targets include 8 melanoma cell lines (2630 mel-CIITA, 397 mel-CIITA,2984-CIITA, 526 mel-CIITA, 1359 mel-CIITA, SK37 mel-CIITA, 3071 mel-CIITA, and 624 mel-CIITA), one non-small cell lung carcinoma (H1299 NSCLC-CIITA), and one renal cancer cell line (1764 RCC-CIITA). **(b)** Evaluation of responses of $CD4^+$ T cells that were transduced with either the 6F9 or the 6F9mc TCR against tumor targets. **(c)** Evaluation of responses of $CD8^+$ T cells that were transduced with either the 6F9 or the 6F9mc TCR against tumor targets. Co-cultures were carried out as described in Figure 1.

Figure 3

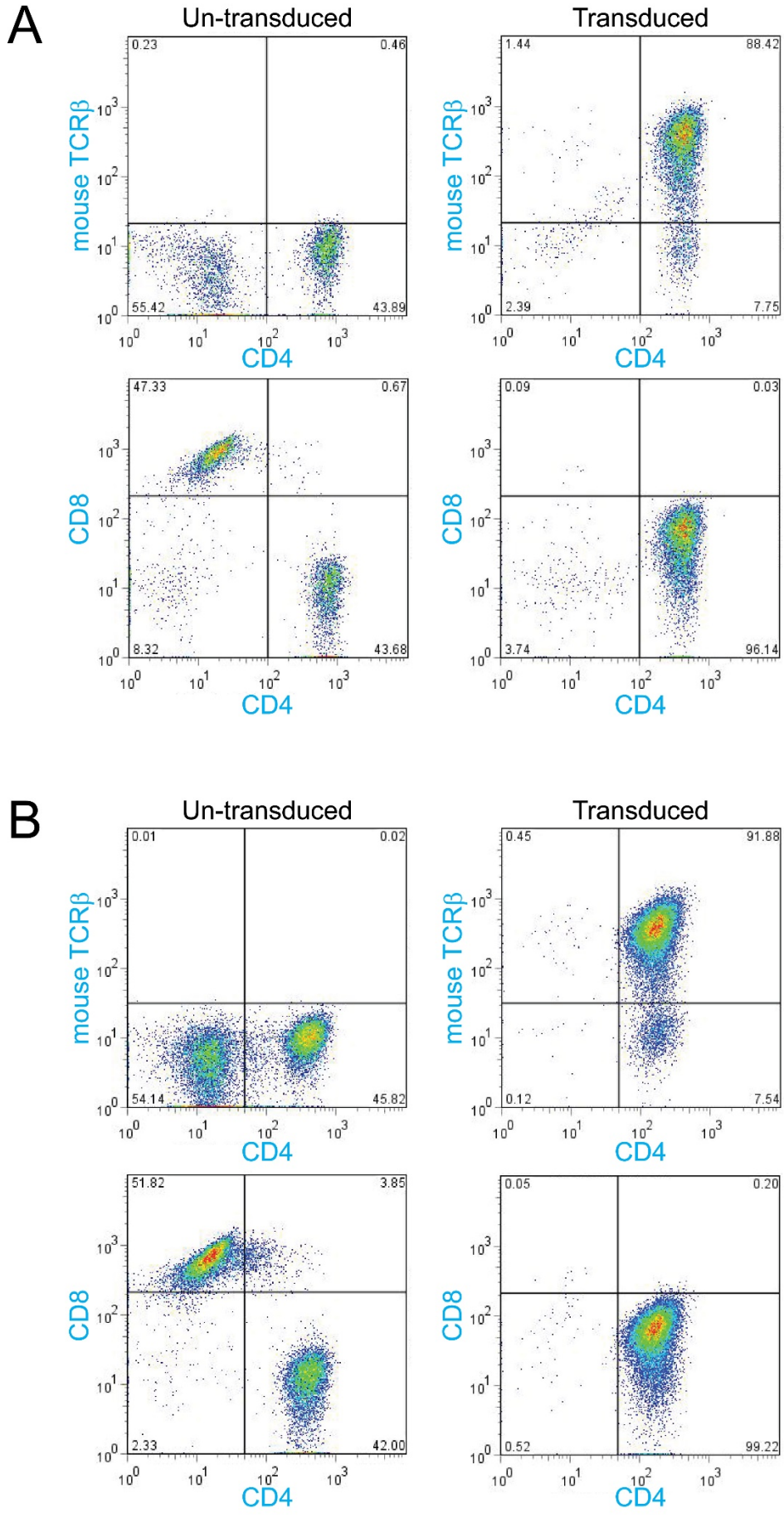
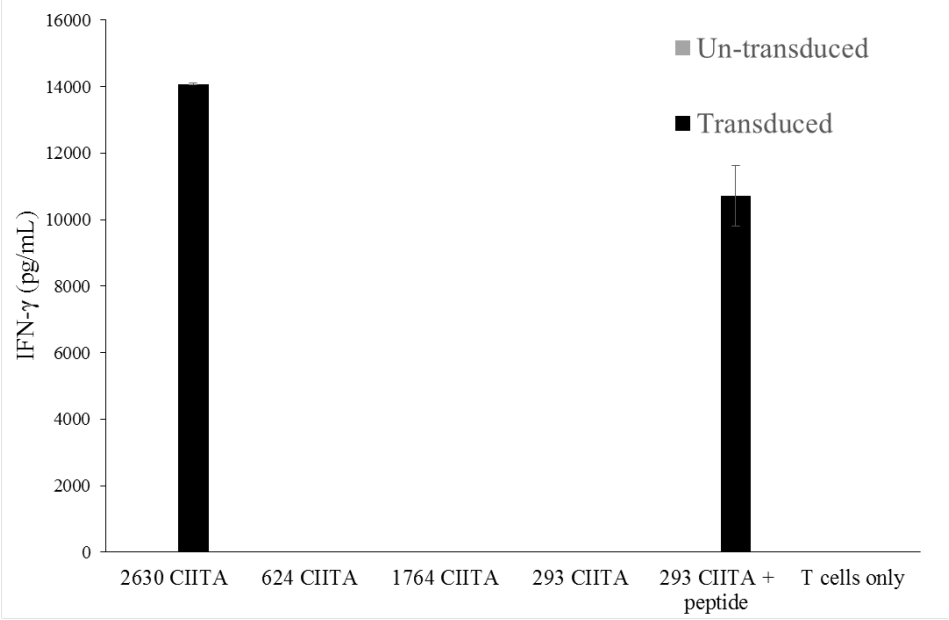

A
Un-transduced
Transduced
mouse TCRβ
CD4
CD8
0.23
0.46
55.42
43.89
1.44
88.42
2.39
7.75
47.33
0.67
8.32
43.68
0.09
0.03
3.74
96.14
B
Un-transduced
Transduced
0.01
0.02
54.14
45.82
0.45
91.88
0.12
7.54
51.82
3.85
2.33
42.00
0.05
0.20
0.52
99.22

Figure 3. Flow cytometric analysis of T cells for patient infusion. Peripheral blood lymphocytes were $CD8^+$ T cells depleted on day 0 and transduced with MAGE-A3 DP4 TCR on day 2 and 3. Cells were subjected to flow cytometric analysis on day 9 (A). Cells on day 13 after rapid expansion were also subjected to flow cytometric analysis (B). Unseparated, un-transduced lymphocytes functioned as the negative control. Representative data from one of the three different donors are shown.

Figure 4

A


16000
14000
12000
10000
8000
6000
4000
2000
0
IFN-γ (pg/mL)
Un-transduced
Transduced
2630 CIITA
624 CIITA
1764 CIITA
293 CIITA
293 CIITA + peptide
T cells only

B

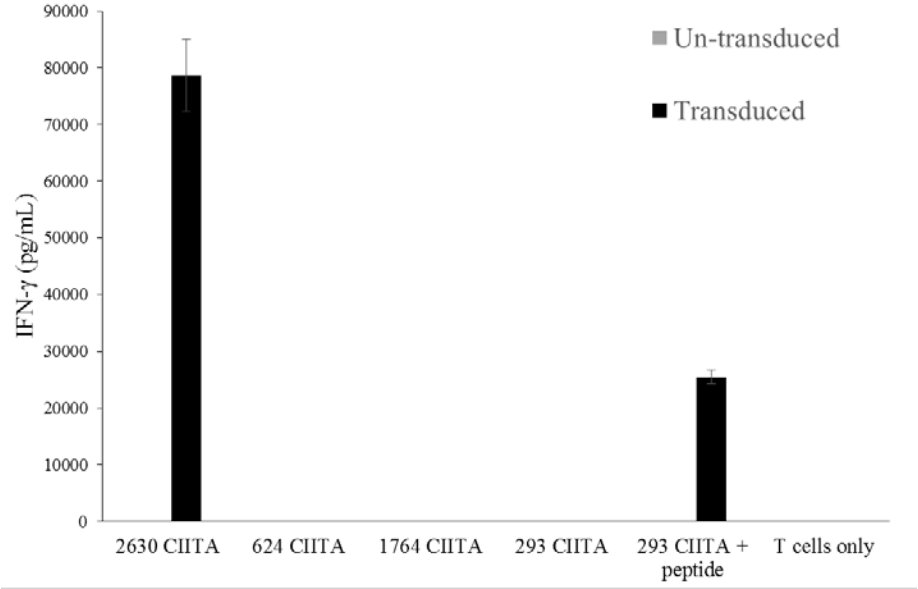

90000
80000
70000
60000
50000
40000
30000
20000
10000
0
IFN-γ (pg/mL)
Un-transduced
Transduced
2630 CIITA
624 CIITA
1764 CIITA
293 CIITA
293 CIITA + peptide
T cells only

Figure 4. Recognition of MAGE-A3^{+}, DP0401^{+} tumor cell line and MAGE-A3 DP4 peptide-pulsed cells. On day 10 after CD8^{+} T cell depletion (A) or day 13 after rapid expansion (B), MAGE-A3 DP4 TCR-transduced CD4^{+} T cells were co-cultured for 16 hr with tumor target cell lines (2630 CIITA: MAGE-A3^{+}, DP0401^{+}; 624 CIITA: MAGE-A3^{+}, DP0401^{-}; 1764 CIITA: MAGE-A3^{-}, DP0401^{+}) or MAGE-A3 DP4 peptide-pulsed 293 CIITA cells. The secretion of IFN-gamma was determined by ELISA. Representative data from one of the three different donors are shown.

Table 1

Table 1. Evaluation of response to peptides related to MAGE-A3:243-258

a

	Amino acids 243-258
MAGE-A3	KKLLTQHFVQENYLEY
MAGE-A6	KKLLTQYFVQENYLEY
MAGE-A1	KKLLTQDLVQEKYLEY
MAGE-A12	KKLLTQDLVQENYLEY

b

		PBMC-1* transduced with:			PBMC-2 transduced with:			
Gene (position)	Amino Acid Sequence	6F9	6F9mc	None	6F9	6F9mc	None	Predicted affinity(nM)
		IFN-γ (pg/ml)						
MAGE-A3:243-258	KKLLTQHFVQENYLEY	10,220	15,210	33	10,350	17,520	45	3
MAGE-A3:243-256	KKLLTQHFVQENYL	1,018	1,815	72	1,670	2,490	78	323
MAGE-A3:243-255	KKLLTQHFVQENY	76	137	29	111	117	71	378
MAGE-A3:243-254	KKLLTQHFVQEN	28	0	67	30	39	78	466
MAGE-A3:243-253	KKLLTQHFVQE	0	40	38	30	45	90	2444
MAGE-A3:245-258	LLTQHFVQENYLEY	9,290	14,970	84	8,920	17,820	74	3
MAGE-A3:246-258	LTQHFVQENYLEY	7,140	12,700	56	9,200	16,170	76	3
MAGE-A3:247-258	TQHFVQENYLEY	6,710	10,600	30	6,810	13,280	41	3
MAGE-A3:248-258	QHFVQENYLEY	6,220	9,000	52	7,400	8,700	56	4
MAGE-A3:249-258	HFVQENYLEY	669	1,643	57	922	2,034	66	5
MAGE-A6:248-258	QYFVQENYLEY	6,440	11,800	54	13,200	8,370	127	3
MAGE-A2/A12:248-258	QDLVQENYLEY	33	66	49	37	56	65	59
MAGE-A4/A9:249-259	QDWVQENYLEY	0	23	32	22	26	62	92
MAGE-A8:251-261	QEWVQENYLEY	43	58	79	39	41	55	87
MAGE-A1/B4:241-251	QDLVQEKYLEY	129	126	55	108	84	53	16
MAGE-B2:250-260	KDLVQEKYLEY	0	0	43	7	20	38	16
MAGE-B10:250-260	KDLVKENYLEY	22	18	69	28	34	66	105
MAGE-B16:252-262	KDFVKEKYLEY	0	27	16	11	28	42	3
MAGE-C1:113-123	KVWVQEHYLEY	9	0	30	25	27	30	35
MAGE-D4:300-315	RKLITDDFVKQKYLEY	193	234	81	194	268	80	6
MAGE-D2:413-428	KKLITDEFVKQKYLDY	82	43	56	226	223	71	8
MAGE-L2:582-597	KKLITEVFVRQKYLEY	45	56	58	78	107	114	6
MAGE-G1:220-235	KKLITEDFVRQRYLEY	0	29	68	25	33	62	3
Necdin:237-247	EEFVQMNYLKY	0	22	59	21	32	83	13
No peptide		0	5	58	15	25	59	

***CD4$^+$ T cells isolated from two patient PBMC by negative selection were transduced with either the 6F9 TCR, the 6F9mc TCR, or were un-transduced and assayed 10 days following OKT3 stimulation for their response to 293-CIITA cells that were pulsed with 10 μg/ml of the indicated peptides, as described in Figure 1.**

Appendix 1

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:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight

   Male = 50 kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of 5'10'' male

   50 + 2.3 (10) = 73 kg

   Female = 45.5 kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of 5'3'' female

   45.5 + 2.3 (3) = 57 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.

Appendix 2

Adverse Events Occurring In ≥10% Of Patients Treated With Aldesleukin (N=525)[1]

Body System	% Patients	Body System	% Patients
Body as a Whole		*Metabolic and Nutritional Disorders*	
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
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital*	
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.

[1]Source: Proleukin® Prescribing Information – June 2007

Appendix 3

Expected IL-2 Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg 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 4

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	652*
Number of Courses	236	85	210	35	30	348	95	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
Arrythmias	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^3)								
<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 5

Certificate of Analysis

Infused T cells transduced with MAGE-A3 DP0401/0402 TCR genes.

Date of preparation of final product:

Patient:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests Performed by*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%			
Total viable cell number[1]	visual microscopic count	$\geq$1 x10^7			
Tumor reactivity[2]	γ-IFN release vs. peptide pulsed 293-CIITA cells	>200 pg/mL, and > 2 times background			
TCR expression[2]	FACS analysis of the transduced cells	PBL, >10%			
Microbiological studies	gram stain[1,3,]	no micro-organisms seen			
	aerobic culture[3,4]	no growth			
	fungal culture[3,4]	no growth			
	anaerobic culture[3,4]	no growth			
	mycoplasma test[5]	negative			
Endotoxin	limulus assay[1]	≤ 5 E.U./kg			
RCR	S+L- Assay[4] RCR-PCR[6]	negative			

[1] Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.
[2] Performed 2-10 post transduction. Results are available at the time of infusion.
[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.
[4] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.
[5] Performed 2-10 days prior to infusion. Results are available at the time of infusion.
[6] Performed on sample approximately 1-4 days prior to infusion. Results are available at the time of infusion.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____
Qualified Clinical or Laboratory Supervisor